HANDBOOK OF MEAT, POULTRY & SEAFOOD QUALITY

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Associate Editors

Terri Boylston — Gertie Hollig
Feng Chen — Chris R. Kerth
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Blackwell Publishing

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Preface

The quality of a food is defined from two perspectives—scientific status and consumer preferences. Scientific factors affecting the quality of a food include: composition, spoilage, colorants, additives, nutrients, flavorants, functional ingredients (affecting health), contamination, general safety, etc. Consumer preferences are linked directly to the human senses—sight, touch, smell, taste, and mouthfeel. Visual factors refer to color, moisture, overall appearance, etc. Tactile factors refer to sliminess, elasticity, softness, hardness, etc. Factors responsible for taste and smell cover many specific chemicals. Mouthfeel refers to texture, softness, tenderness, chewy sensation, and so on. In the last 10 years or so, food quality has been defined by most professionals to include "health" and "safety." The nutrition and safety of foods has always been important, especially since the seventies. The word "health" now includes manipulating certain chemical components in food to increase food's positive impact on our health. "Safety" now refers to a whole spectrum of new legal or recommended requirements for both fresh and processed foods. These requirements are designed to exclude or prevent undesirable agents (biological, chemical, physical, environmental, and extraneous) in our foods.

For ease of reference, we can consider that the quality of a food is the composite picture of many factors. In the last five to ten years, many professional reference books have become available that explore the relationship between such factors and food quality. This book discusses the quality factors of muscle foods (meat, poultry, and seafood). Each professional reference treatise has its characteristics and the users determine which one best suits their purpose. From that perspective, we will describe the major features of our book.

This book provides an initial discussion of basic scientific factors responsible for the quality of muscle foods, with a specific emphasis on sensory attributes and flavors. The remaining sections discuss factors affecting the quality of beef, pork, poultry, and seafood. Under each muscle food, some or all of the following factors affecting the quality will be discussed—additives, aroma, color, contaminants, flavors, microbiology, moisture, mouthfeel, nutrition, packaging, safety, sensory attributes, shelf-life, stability, tainting, texture, and water-activity. Each muscle food discussed may be fresh, frozen, or processed.

This work is the result of the combined efforts of more than 60 professionals from industry, government, and academia worldwide. They represent more than 16 countries with diverse expertise and background in the quality of muscle foods. An international editorial team of 9 members from four countries led these experts. Each contributor or editor was responsible for researching and reviewing subjects of immense depth, breadth, and complexity. Care and attention were paramount to ensure technical accuracy for each topic. It is our sincere hope and expectation that it will serve as an essential reference on the quality of muscle foods for all professionals in government, industry, and academia.

The editorial team wishes to thank all the contributors for sharing their expertise throughout our journey. We also thank the reviewers for giving their valuable comments on how to improve the contents of each chapter. All these professionals are the ones who made this book possible. We trust that you will benefit from the fruits of their labor.

This book is relevant to many professionals in industry, government, and academia and will be most appreciated by the following users:

- All libraries.
- Research units in government, industry, and academia specializing in one or more food quality factors (color, flavor, microbiology, packaging, sensory attributes, and so on).
- Academic institutions: food science, food technology, food engineering, animal science, poultry science, cereal science, marine science, etc.
- Food industries of commodities covered.
- Individuals with expertise in any of the food quality factors discussed in the book.

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

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Handbook of Meat, Poultry and Seafood Quality

Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

Part I
General Food Quality Factors

Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

1
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Y. H. Hui

Introduction
Biology and Genetics
Nutrition
Flavors and Aroma
Color
Microbiology and Safety
Processing
Sensory Attributes and the Consumer
Government Standards and Specifications
Summary

INTRODUCTION

The quality of a food is defined from two perspectives: scientific status and consumer preferences. Scientific factors affecting the quality of a food include composition, spoilage, colorants, additives, nutrients, flavorants, functional ingredients (affecting health), contamination, general safety, etc. Consumer preferences are linked directly to the human senses such as sight, touch, smell, taste, and mouthfeel. Visual factors include color, moisture, overall appearance, etc. Tactile factors include sliminess, elasticity, softness, hardness, etc. Factors responsible for taste and smell cover many specific chemicals. Mouthfeel refers to texture, softness, tenderness, chewy sensation, and so on. In the last 10 years or so, food quality has been defined by most professionals to include "health" and "safety." The nutrition and safety of foods have always been important, especially so since the 1970s. The word "health" now includes manipulating certain chemical components in food to increase the positive impact of food on our health. "Safety" now refers to a whole spectrum of new legal or recommended requirements for both fresh and processed foods. These requirements are designed to exclude or prevent undesirable agents (biological, chemical, physical, environmental, and extraneous) in our foods. For ease of reference, we can consider that the quality of muscle foods (meat, poultry, and seafood) is the composite picture of many factors, and this chapter provides a brief mention of some of them.

BIOLOGY AND GENETICS

Obviously, the quality of any muscle food depends first and foremost on the genetics and biology of the animal. The beef from a young animal is more tender than that from an old animal. Due primarily to biological reasons, muscle from some parts of beef cattle is tastier and more tender than those from another part. Chickens are more tender than turkey. White meat is biologically different from dark meat. Of course, the preference of a consumer varies with regard to the two different kinds of meat. Saltwater fish is different from freshwater fish. Some fish have more bones than others. Western consumers prefer fish with fewer bones while most often the opposite is true for Asians.

NUTRITION

Recently, the nutrition of food has reached an all-time high as far as its impact on our health is concerned. There is no doubt the majority of Americans

consider a quality food as one with high nutritional value. Some salient points follow:

1. Meat and poultry are nutritious because of their high source of protein, vitamins, and minerals.
2. The high content of fat and cholesterol in land muscle foods is undesirable. Thus, "lean" is in.
3. Fish and shellfish are an important part of a healthy diet. Fish and shellfish contain high-quality protein and other essential nutrients, are low in saturated fat, and contain omega-3 fatty acids. A well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children's proper growth and development.

FLAVORS AND AROMA

One major reason, among many, that we like to eat is because food tastes good, which equates to flavor and aroma. Extensive research over the past 25 to 30 years has identified more than 1,000 flavor compounds in meats. However, a single compound or group of compounds responsible for "meaty flavor" has not and perhaps never will be identified due to the overall complexity of meat flavor. Meat flavor is dependent on the pool of flavor precursors in the meat tissue and the chemical reactions that occur during processing. Processing and subsequent storage contribute to the development of the characteristic flavors of meats. Because the precise flavor precursors vary between and within species, beef, pork, lamb, and poultry each have distinctive flavor characteristics. The quality of meat and poultry is to a large extent defined by its flavor and aroma.

In general, fresh saltwater fish are almost odorless because they contain a small quantity of volatiles while freshwater fish give off pyrrolidine and other earthy-odor compounds.

The compounds responsible for the development of flavor during seafood cooking can be classified in two groups. One, which represents the pleasant cucumber/green, almond/nutty, and potato aroma notes, consists of highly volatile, low molecular weight compounds belonging to various chemical classes such as aldehydes, ketones, alcohols, esters, nitrogen, phenols, and sulfur-containing compounds. The second is due to water soluble, low molecular weight free amino acids (taurine, glutamic acid, glycine), nucleotides (purine derivatives), organic acids (lactic acid), and inorganic salts (Na, K, Cl).

Biogenic amines are nitrogen-containing compounds, which are present at very low levels in fresh fish. However, during storage and deterioration, biogenic amines can be produced by amino acid decarboxylation from bacterial enzymes. Among biogenic amines formed, putrescine and cadaverine have a putrid flavor while histamine and phenylethylamine have a pungent and fishy flavor, respectively. Biogenic amines are thermally stable and, therefore, have been used as indices to determine fish freshness. Volatile amines such as trimethylamine (TMA) or dimethylamine (DMA) are formed from trimethylamine oxide (TMAO), and these compounds also serve as a quality index for marine fish.

COLOR

The first impression that a consumer receives concerning a food product is established visually, and among the properties observed are color, form, and surface characteristics.

Color is the main aspect that defines a food's quality, and a product may be rejected simply because of its color, even before other properties, such as aroma, texture, and taste, can be evaluated. This is why the appearance (optical properties, physical form, and presentation) of meat and poultry products at the point of sale is of such importance for the industry. Regarding the specific characteristics that contribute to the physical appearance of meat and poultry, color is the quality that most influences consumer choice.

Food technologists have a special interest in the color of food for several reasons. First, because of the need to maintain a uniform color throughout processing; second, to prevent any external or internal agent from acting on the product during processing, storage, and display; third, to improve or optimize a product's color and appearance; and, last, to attempt to bring the product's color into line with what the consumer expects.

Put simply, the color of meat is determined by the pigments present. These can be classified into the following four types:

- Biological (carotenes and haemopigments), which are accumulated or synthesized in the organism antemortem

- Pigments produced as a result of damage during manipulation or inadequate processing conditions
- Pigments produced postmortem (through enzymatic or nonenzymatic reactions)
- Those resulting from the addition of natural or artificial colorants

As a quality parameter, color has been widely studied in fresh meat and cooked products. Dry-cured meat products have received less attention because in this type of product, color formation takes place during the different processing stages. Recently, new haempigment has been identified in this type of product.

From a practical point of view, color plays a fundamental role in the animal production sector, especially in meat production (primarily beef and poultry,) since in many countries of the European Union, paleness receives a wholesale premium.

MICROBIOLOGY AND SAFETY

All foods contain microorganisms, some beneficial to and some with potential harm for mankind. With muscle foods, the beneficial ones are responsible for fermented meat and fish. Those potential pathogens are of concern. In the last 25 years, government records show that pathogenic organisms in meat, poultry, and seafood have been responsible for many deaths and injuries. Also, marine toxins pose big threats to our well-being considering that most of us enjoy eating fish and shellfish. It is not surprising that a quality muscle food must also be a safe one.

In view of potential hazards from the consumption of muscle foods, state and federal agencies have developed and implemented stringent safety requirements in the processing of meat, poultry, and seafood.

PROCESSING

The quality of any muscle food is obviously affected by the way it is processed.

Why do we want to process food? At present, there are many modern reasons why foods are processed, e.g., adding value to a food, improving the visual appeal, convenience. However, traditionally, the single most important reason that we wish to process food is to make them last longer without spoiling. Probably the oldest methods of achieving this goal are the salting of meat and fish, fermenting of milk, and pickling of vegetables.

Foods are made from natural materials, and like any living matter, will deteriorate in time. The deterioration of food, or food spoilage, is the natural way of recycling, restoring carbon, phosphorus, and nitrogenous matters to the good earth. However, putrefaction (spoilage) will modify the quality of foods resulting in poor appearance (discoloration), offensive smell, and inferior taste. Food spoilage can be caused by a number of factors, chiefly by biological factors, but also by chemical and physical factors. Consumption of spoiled foods can cause sickness and even death. There is no doubt none of us consider spoiled foods as having quality.

Selected examples will illustrate how food processing can affect the quality of a food product:

- Heat application. All of us know that overheating tender meat and chicken usually means toughness. The same is especially true for seafood.
- Heat removal or cold preservation. Freezing is a good example. Most of us are familiar with freezer-burn of meat, chicken, fish, shellfish, or other products left in the freezer over extended periods of time.
- Evaporation and dehydration. Food drying has been popular since the beginning of time. Destruction of nutrients, especially vitamins, is one drawback to this method of preservation.
- Fermentation. In general, of meat, poultry, and fish products, fermented meat such as sausages is most popular. The quality of a sausage is to a large extent determined by the consumer, e.g., dry, sweet, salty, and pickled. Each method affects the quality in terms of nutrients, hardness, tenderness, and flavor.
- New technology. There are numerous new technologies in food processing such as irradiation, microwaving, and ohmic heating. Each method affects the quality of a food in various ways.

The finished product requires packaging. The obvious reason for packaging a food product, muscle foods or other, is to protect the food so it will not be exposed to the elements until it is ready to be prepared and consumed. The quality and shelf life of a

food, especially a muscle food, depends very much on the way it is packaged.

SENSORY ATTRIBUTES AND THE CONSUMER

The sensory attributes of muscle foods are related to the senses of taste, smell, sight, feel, and sound. Of all the foods consumed, muscle foods have the lowest tolerance for complete sensorial acceptability. A muscle food is either acceptable or unacceptable with little in between. Predominately, the consumer visually assesses the color and surface texture of the muscle. The preparation technique of consumer choice is utilized, thereby altering the sensory attributes (usually completely). The consumer cooks or prepares the muscle food as they prefer, changing the surface color, appearance, and texture. The internal altering of texture and flavor is a result of the preparation or cooking process as well. This will vary depending on the many methods applied. For instance, the muscle may be grilled, baked, broiled, or otherwise prepared, all with different fluctuating end results. Consumption of muscle foods is one of the most pleasurable eating experiences. The satiety value applied by the consumption of a muscle food is great when comparing the satisfying effect of foods in general. This is why the sensorial properties of muscle foods can be viewed as often more important than that of other foods.

GOVERNMENT STANDARDS AND SPECIFICATIONS

The technical information in this book is applicable to food scientists and technologists worldwide. However, users from the United States will be very interested in the current government standards and specifications for muscle foods (meat, poultry, and seafood) since such documents usually include quality factors. Since many countries use the United States as an example in formulating their standards and specifications for muscle foods, scientists, technologists, and engineers from the international community may also benefit from information included in the appendix.

SUMMARY

This chapter provides a short introduction to the factors affecting the quality of foods, especially muscle foods. More details on most of the factors will be provided throughout the book.

Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

2
Hazard Analysis and Critical Control Points and Muscle Food Safety in the United States

Y. H. Hui

Introduction
Current Good Manufacturing Practice Regulations
Hazard Analysis Critical Control Points Regulations or Programs
- What is HACCP?
- The Need for HACCP
- Advantages and Plans
- Hazard Analysis
- The HACCP Plan
  - The Contents of the HACCP Plan
  - Signing and Dating the HACCP Plan
- Sanitation
- Implementation

References

INTRODUCTION

Nearly 25 years ago, the United States Food and Drug Administration (FDA) started the approach of using umbrella regulations to help the food industries to produce wholesome food as required by the Federal Food, Drug, Cosmetic Act (The Act). In 1986, the FDA promulgated the first umbrella regulations under the title of Good Manufacturing Practice Regulations (GMPR). Since then, many aspects of the regulations have been revised.

Traditionally, industry and regulators have depended on spot-checks of manufacturing conditions and random sampling of final products to ensure safe food. The Current Good Manufacturing Practice Regulations (CGMPR) form the basis on which the FDA will inform a food manufacturer about deficiencies in its operations. This approach, however, tends to be reactive, rather than preventive, and can definitely be improved.

For more than 35 years, FDA has been regulating the low-acid canned food (LACF) industries with a special set of regulations, many of which are preventive in nature. This action aims at preventing botulism. In the last 35 years, threats from other biological pathogens have increased tremendously. Between 1980 and 1995, the FDA studied the approach of using Hazard Analysis and Critical Control Points (HACCP) programs.

For this approach, FDA uses the LACF regulations as a partial guide. Since 1995, the FDA has issued HACCP regulations (HACCPR) for the manufacture or production of seafood, among others.

In the last two decades, increasing death and injuries associated with contaminated meat and poultry have prompted new safety measures for these two muscle foods. Currently, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) have issued regulations implementing HACCP for the processing of meat and poultry.

CURRENT GOOD MANUFACTURING PRACTICE REGULATIONS

The Current Good Manufacturing Practice Regulations (CGMPR) cover the topics listed in Table 2.1.

These regulations cover essential practices to prevent food from being contaminated with biological, chemical and physical hazards, and foreign objects, such as the following:

Personnel: Use a hair net.
Plants and grounds: Use proper containers and locations for garbage.
Sanitation operations: Keep processed ingredients away from raw ingredients.
Sanitary facilities and controls: Maintain rest rooms and remove water that collects on the floor of processing areas.
Equipment and utensils: Clean vats daily.
Warehouse and distribution: Reduce the presence of rodents; do not transport food ingredients in a truck that has not been sanitized after transporting pesticides.

It is obvious that a careful food processor will become familiar with these regulations to make sure that their products are safe for public consumption. With this understanding, this chapter will not provide more details on this topic. Rather, our discussion will concentrate on HACCP because one of its objectives is to make sure that food processors implement CGMPR.

HAZARD ANALYSIS CRITICAL CONTROL POINTS REGULATIONS OR PROGRAMS

In 1997, the FDA adopted a food safety program that was developed nearly 30 years ago for astronauts and is now applying it to seafood, and fruit and vegetable juices. The agency intends to eventually use it for much of the U.S. food supply. The program for the astronauts focuses on preventing hazards that could cause food-borne illnesses by applying science-based controls, from raw material to finished products. The FDA's new system will do the same.

Many principles of this new system now called (HACCP) are already in place in the FDA-regulated LACF industry. Since 1997, the FDA has mandated HACCP for the processing of seafood, among others. The FDA has also incorporated HACCP into its *Food Code*, a document that gives guidance to and serves as model legislation for state and territorial agencies that license and inspect food service establishments, retail food stores, and food vending operations in the United States.

Table 2.1. Current good manufacturing practices regulations as stated in 21 CFR 110 (Title 21, United States Code of Federal Regulations, Part 110).

21 CFR 110.3	Definitions.
21 CFR 110.5	Current good manufacturing practice.
21 CFR 110.10	Personnel.
21 CFR 110.19	Exclusions.
21 CFR 110.20	Plant and grounds.
21 CFR 110.35	Sanitary operations.
21 CFR 110.37	Sanitary facilities and controls.
21 CFR 110.40	Equipment and utensils.
21 CFR 110.80	Processes and controls.
21 CFR 110.93	Warehousing and distribution.

The USDA has developed HACCP programs for meat, poultry, and other land muscle foods. It is important to realize that the underlying principles are the same, no matter what the manufacturing process. The same principles apply to the processing of meat, poultry, and seafood. The details vary. The discussion in this chapter will concentrate on the principles, citing specific examples for meat, poultry, and seafood.

Please note that the word "shall" in a legal document means mandatory and is used routinely in USDA FDA regulations published in the U.S. In this chapter, the words "should" and "must" are used to make for smoother reading. However, this in no way diminishes the legal impact of the original regulations.

WHAT IS HACCP?

HACCP involves the following seven principles:

1. Analyze hazards. Potential hazards associated with a food and measures to control those hazards are identified. The hazard could be biological, such as a microbe; chemical, such as a toxin; or physical, such as ground glass or metal fragments.
2. Identify critical control points. These are points in a food's production—from its raw state through processing and shipping to consumption by the consumer—at which the potential hazard can be controlled or eliminated. Examples are cooking, cooling, packaging, and metal detection.

3. Establish preventive measures with critical limits for each control point. For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.
4. Establish procedures to monitor the critical control points. Such procedures might include determining how and by whom cooking time and temperature should be monitored.
5. Establish corrective actions to be taken when monitoring shows that a critical limit has not been met—for example, reprocessing or disposing of food if the minimum cooking temperature is not met.
6. Establish procedures to verify that the system is working properly—for example, testing time and temperature recording devices to verify that a cooking unit is working properly.
7. Establish effective record keeping to document the HACCP system. This would include records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potential problems.

Each of these principles must be backed by sound scientific knowledge such as published microbiological studies on time and temperature factors for controlling food-borne pathogens.

The Need for HACCP

New challenges to the U.S. food supply have prompted the USDA and FDA to consider adopting an HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. There also is increasing public health concern about chemical contamination of food, for example, the effects of lead in food on the nervous system.

Another important factor is that the size of the food industry and the diversity of products and processes have grown tremendously, in the amount of domestic food manufactured and the number and kinds of foods imported. At the same time, federal, state, and local agencies have the same limited level of resources to ensure food safety. The need for HACCP in the United States, particularly in the MUSCLE food industries, is further fueled by the growing trend in international trade for worldwide equivalence of food products and the Codex Alimentarius Commission's adoption of HACCP as the international standard for food safety.

Advantages and Plans

HACCP offers a number of advantages over previous systems. Most importantly, HACCP:

1. focuses on identifying and preventing hazards from contaminating food.
2. is based on sound science.
3. permits more efficient and effective government oversight, primarily because the record keeping allows investigators to see how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day.
4. places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.
5. helps food companies compete more effectively in the world market.
6. reduces barriers to international trade.

The seven steps used in HACCP plan development follow:

1. Preliminary Steps
    a. General information
    b. Describe the food
    c. Describe the method of distribution and storage
    d. Identify the intended use and consumer
    e. Develop a flow diagram
2. Hazard Analysis Worksheet
    a. Set up the Hazard Analysis Worksheet
    b. Identify the potential species-related hazards
    c. Identify the potential process-related hazards
    d. Complete the Hazard Analysis Worksheet
    e. Understand the potential hazard
    f. Determine if the potential hazard is significant
    g. Identify the critical control points (CCP)
3. HACCP Plan Form
    a. Complete the HACCP Plan Form
    b. Set the critical limits (CL)

4. Establish Monitoring Procedures
   a. What
   b. How
   c. Frequency
   d. Who
5. Establish Corrective Action Procedures
6. Establish a Record Keeping System
7. Establish Verification Procedures

It is important to remember that apart from HACCPR promulgated for seafood and juices, the implementation of HACCP by other categories of food processing is voluntary. However, the FDA and various types of food processors are working together so that eventually HACCPR will become available for many other food processing systems under FDA jurisdiction. Using the HACCPR for seafood processing as a guide, the following discussion for an HACCP plan applies to all categories of food products being processed in the United States.

Hazard Analysis

Every processor should conduct a hazard analysis to determine whether there are food safety hazards that are reasonably likely to occur for each kind of product processed by that processor and to identify the preventive measures that the processor can apply to control those hazards. Such food safety hazards can be introduced both within and outside the processing plant environment, including food safety hazards that can occur before, during, and after harvest. A food safety hazard that is reasonably likely to occur is one for which a prudent processor would establish controls because experience, illness data, scientific reports, or other information provide a basis to conclude that there is a reasonable possibility that it will occur in the particular type of product being processed in the absence of those controls.

The HACCP Plan

Every processor should have and implement a written HACCP plan whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur. An HACCP plan should be specific to the following:

1. Each location where products are processed by that processor.
2. Each kind of product processed by the processor.

The plan may group kinds of products together, or group kinds of production methods together, if the food safety hazards, CCPs, CLs, and procedures that are required to be identified and performed are identical for all products so grouped or for all production methods so grouped.

The Contents of the HACCP Plan

The HACCP plan should, at a minimum:

> List the food safety hazards that are reasonably likely to occur, as identified, and that thus must be controlled for each product. Consideration should be given to whether any food safety hazards are reasonably likely to occur as a result of the following: natural toxins; microbiological contamination; chemical contamination; pesticides; drug residues; decomposition in products where a food safety hazard has been associated with decomposition; parasites, where the processor has knowledge that the parasite-containing product will be consumed without a process sufficient to kill the parasites; unapproved use of direct or indirect food or color additives; and physical hazards;
>
> List the critical control points for each of the identified food safety hazards, including as appropriate: critical control points designed to control food safety hazards that could be introduced in the processing plant environment; and critical control points designed to control food safety hazards introduced outside the processing plant environment, including food safety hazards that occur before, during, and after harvest;
>
> List the critical limits that must be met at each of the critical control points;
>
> List the procedures, and frequency thereof, that will be used to monitor each of the critical control points to ensure compliance with the critical limits;
>
> Include any corrective action plans that have been developed to be followed in response to deviations from critical limits at critical control points;
>
> List the verification procedures, and frequency thereof, that the processor will use;
>
> Provide for a record keeping system that documents the monitoring of the critical control points. The records should contain the actual values and observations obtained during monitoring.

Signing and Dating the HACCP Plan

The HACCP plan should be signed and dated either by the most responsible individual on site at the processing facility or by a higher-level official of the

processor. This signature should signify that the HACCP plan has been accepted for implementation by the firm.

It should be signed and dated upon initial acceptance; upon any modification; and upon verification of the plan.

Sanitation

Sanitation controls (3) may be included in the HACCP plan. However, to the extent that they are otherwise monitored, they need not be included in the HACCP plan.

Implementation

This book is not the proper forum to discuss in detail the implementation of HACCPR. Readers interested in additional information on HACCP should visit the FDA HACCP website http://vm.cfsan.fda.gov/ that lists all of the currently available documents on the subject.

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Part II
Sensory Attributes of Muscle Foods

Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

3
History, Background, and Objectives of Sensory Evaluation in Muscle Foods

M. W. Schilling

Introduction
History of Muscle Foods
History, Background, and Development of Sensory Evaluation
Objectives
Sensory Evaluation Specific to Muscle Foods
Relationship of Consumer Panels to Trained Panels
Quality Characteristics
Conclusion and Future of Sensory Evaluation
References

INTRODUCTION

Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (Anonymous 1975). This definition verifies that sensory evaluation encompasses not only taste but all of the senses (Stone and Sidel 1993). Sensory evaluation of food products has been conducted as long as human beings have been evaluating the goodness and badness of food (Meilgaard and others 1991) and is currently crucial in the field of food science for two separate but important purposes. First, consumer testing is crucial since acceptability is the number one determinant of whether a consumer will purchase a product (Ramirez and others 2001). Second, objective quality measurements must be performed in order to relate the consumer's acceptance of a product to laboratory data. Muñoz and Chambers (1993) reported that relating consumer data to laboratory data (instrumental and/or trained panelists) addresses the limitations of consumer panels. This is because only consumers can provide information on a product's acceptance or its perceived integrated attributes, and only laboratory methods can provide the technical, precise, and reliable information researchers need regarding product attributes.

The principles of sensory evaluation originated from physiology and psychology, and sensory science is currently an interdisciplinary science including these fields along with food science, biochemistry, statistics, nutrition, and others. Because of the utilization of live human panelists, sensory experimentation is much more complex than research where instrumentation is used and requires much greater care in experimental design and interpretation of results. Meilgaard and others (1991) reported that we perceive attributes of a food item in the order of appearance, odor, texture, and flavor. Appearance is generally the only attribute on which consumers base a decision to purchase or consume a food product and includes but is not exclusive of color, size, shape, and surface texture. Odor refers to the sensation that occurs when volatiles from a product enter the nasal passage and are perceived by the olfactometry system, and these sensations are most often associated with the formulation of compounds during the cooking process. Texture can be defined as the sensory and functional manifestation of the structural and

mechanical properties of foods, detected through the senses of vision, hearing, touch, and kinesthetics (Szczesniak 1963) and consists of hardness, cohesiveness, adhesiveness, denseness, springiness, perception of particles, and perception of water. These attributes are perceived by sensors in the mouth both before and after chewing and have been described extensively (Brandt and others 1963; Szczesniak 1963; Szczesniak and others 1975). Flavor includes aromatics, tastes, and chemical feelings. Aromatics consist of olfactometry perceptions caused by volatile substances released from a product in the mouth. Tastes consist of salty, sweet, sour, and bitter perceptions caused by soluble substances in the mouth, and chemical feelings include astringency, spice heat, cooling, and metallic flavor. Flavor is crucial in the acceptance of food, and the use of flavor-related words is a very important aspect of marketing (Amerine and others 1965). The National Research Council (1988) reported that the three most important factors that consumers look for in meat products are nutrition, price, and taste. However, if taste is not acceptable, then nutrition and price are irrelevant.

Stone and others (1991) have reported that thousands of new products are unsuccessfully introduced to the retail market every year. With the continued influx of new products into the market, sensory evaluation has become increasingly important to food companies in the creation of new food products as well as in determining the quality attributes of products within a competition category. Marketing and sensory evaluation departments must work together to bring the appropriate new product to market. Both groups have equally important roles that must be understood by each other as well as by upper management to perform the appropriate market research, trained panel sensory evaluations, and consumer testing. This book will provide an in-depth look at the sensory characteristics of all muscle foods from both a trained panel and a consumer panel perspective through discussing the important sensory attributes as well as the sensory methods utilized to evaluate beef, pork, poultry, seafood, processed muscle foods, as well as lamb, venison, bison, and equine.

HISTORY OF MUSCLE FOODS

Muscle foods or meats can be defined as flesh from animals that is suitable for consumption (Kinsman and others 1994), and first became a steady food source in the diet between 10,000 and 16,000 years ago as animals were domesticated and people became less nomadic in nature (Kinsman 1994). Refer-ences have been made in the *Iliad* and in the Old Testament to meat consumption that has been carbon dated back greater than 3,000 years, and the meat industry in the United States evolved from Columbus and Cortez bringing cattle, hogs, and sheep to North America in 1493 and 1519, respectively. The history of the meat industry will not be discussed in this chapter, but an informative perspective on the history of this exciting industry can be found in Kinsman (1994). The seafood industry also has an exciting history in which large human populations tended to settle near seas or large river systems where fish and shellfish were readily abundant as food. References to fishing have also been made in the Old and New Testament of the Bible carbon dating back thousands of years. As time progressed, larger fish were desired and fishermen built boats to travel further from shore. Quite often fishing was the reason for discovering new lands, and the excellent fishing possibilities in the North Atlantic Ocean lured fishermen to North America from Europe leading to commercial fishing becoming the first industry in the New World (Martin 1990). The history of this diverse industry will not be discussed in detail, but there is an excellent account of this history in Martin (1990).

U.S. meat and poultry products consumption has fluctuated between 87 and 100 kilograms (kg) per capita consumption between 1980 and 2001, respectively (American Meat Institute [AMI] 2003), and seafood products consumption has held steady at 5 to 7 kg per capita between 1960 and 2002 (AMI 1993; NMFS 2002). This demonstrates the staying power of meat products in the food industry. Meals will continue to be planned around meat products for two reasons. First, muscle foods are the most naturally occurring nutrient-dense foodstuff (Kinsman 1994). Second, meat products are of vital importance in providing high quality proteins, minerals, vitamins, and a high satiety value (Price and Schweigert 1987). With the high percentage of two-parent wage earners, there will be a greater need and demand for more table-ready and microwaveable foods that require minimum preparation time (Kinsman 1994). This will lead to an increased importance of sensory testing since all meat products

of these types must be evaluated for sensory quality (appearance, odor, texture, and flavor).

HISTORY, BACKGROUND, AND DEVELOPMENT OF SENSORY EVALUATION

Much literature has been reported pertaining to the evolution of sensory evaluation into the complex science that it is currently. Likewise, the practice of using a trained panel to relate product attributes to consumer acceptability in the successful development of food products is well documented. The principal sources behind the science of sensory evaluation as it is studied today are psychology, physiology, sociology, and statistics (Peryam 1990). The psychophysical roots for sensory evaluation can be traced to the work of Weber, a German physiologist (Boring 1950), but it was Fechner, a German psychologist, who built on the observations of Weber in order to demonstrate a link between the physical and psychological worlds. Weber found that difference thresholds, the extent of changes in stimuli necessary to produce noticeable differences, increase in proportion to the initial perceived stimulus intensity at which they are measured. Fechner then utilized the findings of Weber to demonstrate that perceived magnitude may be related to the change in intensity and absolute intensity of a sensation. The combined efforts of these two scientists laid much of the groundwork during the late nineteenth century for the field of psychometrics that deals with the mathematical explanation of psychological phenomena.

The development of statistical methods (during the nineteenth century) that are currently utilized in sensory evaluation was also essential in the foundation of this discipline. When sensory evaluation started to show life in the 1930s, many statistical procedures including analysis of variance were already in place to describe the variation in perception and behavior of people evaluating food products (Peryam 1990). The evolution of sensory science as it is practiced today originated in the 1930s around the time of the organization of the Institute of Food Technologists. During this time, sensory information was largely confined to recording opinions of one or two experts evaluating the quality of a specific commodity in order to provide quality control for their organization's products (Pangborn 1964). The problem with this type of sensory evaluation was that it did not necessarily reflect consumer attitudes. In a regional environment, utilizing one or two experts was extremely helpful in determining product quality, but Hinreiner (1956) stated that certain values on scorecards can become fixed in the expert's minds as acceptable to consumers that do not necessarily reflect consumer attitudes. Stone and Sidel (1993) stated that though this approach is common in quality control, its prevalence in sensory evaluation reflects a basic lack of understanding of human behavior or the wistful desire of some to reduce response behavior to some simplistic level. Platt (1931) recommended that critical experts be eliminated, and that judges be selected for participation on sensory panels on the basis of being able to predict public preference. Platt understood the importance of meeting the needs and wants of the consumer, but the consumer testing and trained panel testing necessary to accomplish this goal was not yet available to the industry.

Dove (1947) reported that food acceptance research was a result of rationing of foods to the troops in World War II. Rations were tested for quality specifications on a nutritional basis. However, the soldier-consumer refused to eat some rations, which ended up in storage dumps. This occurrence led to an official directive to determine causes of unacceptance. These food products had been tested for nutrition, tenderness, viscosity, compression, flavor, and quality, or had been tested by scorecard ratings. These methods had all been applied, and the food was still rejected. In this objective approach, measurements are directed toward the food. However, the subjective test deals with an individual's physiological or psychological response. These occurrences led to an impetus in sensory evaluation development through the U.S. Army Quartermaster Food and Container Institute, an organization that supported research in the acceptance of food products consumed by the armed forces (Peryam 1990). During this time period, the U.S. Army Quarter-master Food and Container Institute made many great contributions to sensory evaluation research. The most well-known contribution was the "invention" of the 9-point hedonic scale (Peryam and Pilgrim 1957). Another outstanding contribution of the Institute was undoubtedly the collaboration between psychologists, food technologists, and statisticians. This multidisciplinary collaboration is a good model for the scientists that should be working together to

perform sensory evaluation on all foods, including muscle foods. The importance of sensory acceptance was then quickly forgotten by the federal government as they initiated their "War on Hunger" and "Food from the Sea" programs (Stone and Sidel 1993) in which the government's intention was to feed starving and malnourished people even though no research was performed on whether the products being provided were acceptable to the targeted groups.

During a similar time period, many developments were occurring in the private sector relating to the use of both affective testing as well as trained panelists to explain the quality of food products. In the late 1940s, the Kroger company started performing affective consumer tests by sending samples to housewives (Peryam 1990). This innovative idea was very informal but provided an impetus toward consumer tests that are utilized today. The duo-trio tests and triangle tests were also developed during the 1930s and 1940s by Seagram Distillers and researchers in Europe (Peryam 1990) in order to maintain uniform quality in standard products. This was the beginning of the development of trained panel methods that are currently used. In the 1950s, the flavor profile analysis was first developed by Arthur D. Little in Cambridge, Massachusetts, to provide information about the complexities of perception of flavor characteristics and their relation to the physical components of food products (Cairncross and Sjostrom 1950). This methodology led others to introduce texture profile analysis (Brandt and others 1963; Szczesniak 1963) and quantitative descriptive analysis (Stone and others 1974), respectively. Cross and others (1978) adapted this methodology into a descriptive analysis method that is now commonly utilized in muscle foods (AMSA 1995).

During this time period, former departments of dairy science, meat science, and other food products merged into departments of food science and technology, and researchers began to relate sensory evaluation or subjective testing to objective tests such as the Instron and gas chromatograph (GC) (Pangborn 1989). Academic endeavors in evaluating sensory properties of foods date back to the mid-1930s. The University of California at Davis has contributed greatly to the scientific community from an academic viewpoint. Maynard Amerine and his associates were followed by Rose Marie Pangborn and her coworkers utilizing the best techniques available and developing new techniques and effective variations of tests (Peryam 1990). Currently, sensory evaluation is taught in connection with many food science and nutrition curricula, and many universities now offer a Ph.D. in sensory science with an array of multidisciplinary courses. Pangborn (1989) stated that the industrial demand for well-trained sensory professionals at the B.S., M.S., and Ph.D. levels greatly exceeds the supply, a statement that is still true today. Another development that has made a huge impact on sensory science in recent years (1969–1988) is the development of journals such as *Journal of Texture Studies*, *Chemical Senses*, *Journal of Food Quality*, *Appetite*, *Journal of Sensory Studies*, and *Food Quality Preference* (Pangborn 1989). These journals have improved the level of applied sensory science by providing both increased avenues for scientists to share their findings and method developments with the scientific community. Sensory evaluation has now evolved into testing that can use complicated multivariate analysis as well as mathematical modeling and other statistical analyses to evaluate data. This has allowed for excellent connections between trained sensory panels and consumer data. However, no matter how complicated the analysis, it is crucial that the sensory design be set up appropriately, or the data will be meaningless. This is of increased importance when dealing with human subjects. Human panelists are more sensitive than instruments but are also more variable.

OBJECTIVES

The objectives of sensory evaluation can be divided into two categories. The first can be viewed as the purpose of sensory evaluation. This purpose is to determine product quality and ultimately provide the consumer with the products that they desire. The second category can be viewed as the actual objectives that must be followed in conducting sensory testing that are both specific and nonspecific to muscle foods.

Determining Quality and Consumer Acceptability

The objective of providing the consumer with desired products requires two kinds of information—sensory descriptive and preference (quality) judg-

ments. The former are usually obtained from a trained panel and the latter are obtained from appropriately recruited and qualified consumers (Stone and others 1993). These two goals can be broken down further into four types of tests, each with their own objective. These tests include affective, discriminative, descriptive, and quality tests (Sidel and others 1981). Affective tests are generally utilized to indicate preference or acceptance of products through selecting, ranking, or scoring samples by panelists that represent the target consumer population. Discrimination tests are utilized to test whether samples are different from one another and should usually be run with trained panels. Some discrimination tests such as triangle tests can also be utilized in panel selection for descriptive tests. Descriptive tests describe sensory properties and measure the perceived intensity of those properties. The two most popular descriptive methods include classical and modified flavor profile (Cairncross and Sjostrom 1950), texture profile (Brandt and others 1963), and quantitative descriptive analysis (Stone and others 1974). Cross and others (1978) later adapted these methods in the creation of a descriptive attribute panel intended for the evaluation of meat products. The Spectrum method published by Meilgaard and others (1991) further enhances descriptive testing by adapting the test to the product being evaluated through the use of reference points. These are indicated by intensities of descriptors in specific commercially available foods. This scale prevents panelists from avoiding the ends of the scale, which is a major problem with fixed scales. The fourth type of testing utilized is quality testing in which one or two experts are utilized to test a product to determine if it meets quality specifications. This method can work well when it is solely used to see if a product meets specifications, but it should not be assumed that these judgments by trained experts directly relate to consumer preference or acceptance of food products. Typically, physical and chemical instrumental measurements should also be incorporated in testing to relate instrumental measurements of quality to sensory perceptions of quality. Therefore, it can be stated that the ultimate objective of sensory evaluation is to predict consumer acceptability, but this cannot be done without trained sensory panels and instrumental measures to relate to preference testing. This objective can be accomplished through different means, but the company that has this capability and is able to exploit this knowledge has achieved a major accomplishment (Stone and Sidel 1993).

Practical Objectives of Sensory Evaluation

In dealing with a subject's sensory responses, there is a three-step mechanism (Meilgaard and others 1991). First, a sensation results from a stimulus that is detected by a sense organ and travels to the brain as a nerve signal. The brain is then utilized to transpose the sensations into perceptions, and lastly, a response is formulated based on the subject's perceptions (Carlson 1998). This causes much greater variability when utilizing humans as instruments since this three-step process exists in comparison to a one-step process that exists in other instrumental tests. The complexity of sensory evaluation makes it imperative that a specific process be taken in the design of sensory evaluation experiments. Pangborn (1979) states that there are three common problems to sensory evaluation that include lack of test objective, adherence to a single test method regardless of application, and improper subject selection procedures. These three details must be addressed in all sensory analyses. Erhardt (1978) reports that there is a specific role of the sensory analyst that can be broken down into seven tasks. The following is a paraphrased version of these seven steps that are discussed in detail in that paper. The first step in conducting a sensory experiment is to determine the project objective. The next step is to determine the test objective to assure that the resulting data will be relevant to the overall objective of the sensory project. The third step for the analyst is to screen samples. This allows the analyst to become familiar with the responses that might be expected, minimizes the evaluation of obviously different samples, and helps the analyst to design the test. The next step for the sensory analyst is to design the test. The correct time to consider experimental design and statistical analyses is after a test is planned and not at the conclusion of the work. The next step is to conduct the test. When conducting a test, it is imperative that the procedures are strictly adhered to in order to prevent nontest variables from influencing panelist responses and/or perception. The last two responsibilities of the sensory analyst are to analyze data according to the

predetermined statistical analyses and to report the results to the entity that requested the research. The analyst must make it clear to the requester what test results mean, the conclusion that can be drawn from the results, and the next step to take based on the initial project objective.

SENSORY EVALUATION SPECIFIC TO MUSCLE FOODS

Sensory evaluation as it is currently practiced for muscle foods is documented by the American Meat Science Association (AMSA 1995). Through the use of sophisticated panel training and method selection, sensory evaluation can provide accurate and repeatable data. Sensory factors in meat include tenderness, juiciness, flavor and aroma, and color (Cross 1987). Cross and others (1978) originated the most commonly utilized method for descriptive analysis in the testing of meat products. This is the most referenced method for descriptive testing in muscle foods, thus implying that it is also the most utilized method. Consumer testing of meat products is generally performed with affective tests of acceptance or preference that are utilized for all food products. Those tests that were termed consumer guidance tests in Griffin (1999) should be utilized along with market research tests in the development of food products in the industry. There is a clear distinction between the two types of testing, and it is the sensory scientist's responsibility to help upper management understand this in order to provide products to the consumer that will be successful for the company (Griffin 1999).

Trained Panels

Trained panels are utilized to provide accurate and repeatable data pertaining to the quality of meat products. Cross and others (1978) reported four steps that should be taken in the selection of a trained panel including recruitment, screening, training, and performance evaluation. A sensory study should only be initiated after all of these steps have been taken (AMSA 1995; Cross and others 1978). Trained tests in muscle foods include ranking and scaling of samples, magnitude estimation, and descriptive sensory analysis. The second of these methods has limitless applications. It has been utilized to relate to physical and chemical analyses, product formulations, preferences, and other kinds of consumer measures of concepts, pricing, and so forth (Stone and Sidel 1998). Descriptive analyses are very important to the meat industry since they are useful in investigating treatment differences, monitoring ingredient process control criteria, and defining sensory properties of a target product (Bett 1993).

Consumer Panels

Kauffman (1993) stated that meat quality includes seven variables: wholesomeness, nutrition, processing yield, convenience, consistency, appearance, and palatability. Palatability has five components: tenderness, texture, juiciness, and flavor (odor and taste) (Kauffman and others 1990). Booth (1990) stated that people eat foods they like, including meat, and sensory properties impact those likes. However, Logue and Smith (1986) reported that liking fresh meat was not related to liking fish, and neither of those was related to liking restructured meat products. This demonstrates that consumers expect and emphasize different sensory characteristics for various meat products (Chambers and Bowers 1993). For example, consumer studies have revealed that tenderness is the most important attribute of beef (AMSA 1978) and chicken, but this attribute is not as important in other species since it is not as variable. This has led to researchers determining the relationship between consumer acceptability and objective measurements of tenderness in meat products (Lyon and others 1990; Schilling and others 2003). These factors are measured objectively, but the most important perception is evaluated by sensory panels. Since meat cookery has a significant influence on sensory characteristics, its selection is an integral part of sensory evaluation (Cross 1987). The sensory properties that consumers want depend on species as well as whether the food is being purchased, stored, cooked, or eaten (Chambers and Bowers 1993). To fully understand consumer acceptability testing in meat products, it must be understood what attributes are important to consumers for each particular study. Objectives will often be similar when testing different muscle food products, but quality attributes that are important to consumers may differ among different muscle food products.

RELATIONSHIP OF CONSUMER PANELS TO TRAINED PANELS

Elrod (1978) reported that many companies are structured so that trained panels are utilized to design products and then the marketing department is responsible for consumer testing if it is performed. To produce the best possible product, it is imperative for marketing and research and development departments to relate all market research, consumer testing, and trained sensory evaluation. Muñoz and Chambers (1993) describe a model for relating descriptive analysis techniques and objective testing to consumer acceptability. These authors report that this approach can provide the following useful pieces of information. First, actionable product guidance will be provided that is based on attributes for product formulation and reformulation to achieve high consumer acceptance. Second, the attributes that affect consumer acceptability can be determined. Third, laboratory data can be used to predict consumer response and determine its usefulness in explaining consumer responses. Fourth, appropriate marketing terms can be identified that coincide with consumers desires, and lastly, it allows researchers to interpret and understand consumer terminology.

QUALITY CHARACTERISTICS

In general, it has been reported that tenderness, a component of texture, is the most important attribute of fresh meat products, and has thus been studied more with consumers than other properties. This may be in part due to the broad range in tenderness of products not seen in other quality characteristics. Cross and Stanfield (1976) and Diamant and others (1976) have reported that tenderness was the most important attribute in determining acceptability in restructured beef steaks and cooked pork chops, respectively. If a product is not tender, it is automatically deemed unacceptable. Textural characteristics of muscle food products are often evaluated using trained texture profile analyses. This methodology is most often utilized in processed meat products and the characteristics studied are generally hardness, springiness, chewiness, gumminess, and cohesiveness.

Color is one of the most important characteristics of meat since it is the primary attribute by which both fresh and cured meats are judged by the consumer before purchase (Fox 1987). Two common examples of this are the preference of purchasing beef that is cherry red in color and the lack of consumer acceptability for pork and poultry products that are pale in color (Kropf 1980).

Flavor is a very important sensory attribute in muscle foods, but this attribute cannot be explained well by consumers since their vocabulary is insufficient to describe the complex flavors found in most meat products (Chambers and Bowers 1993). For this reason, flavor intensity and off-flavor are usually the only flavor characteristics determined in consumer studies. However, these variables may not be well understood by consumers. Chambers and others (1992) concluded that off-flavor as described by consumers was characterized as soapy by a descriptive panel. This reveals that the consumers were not really distinguishing off-flavors at all. They were really distinguishing the soapy flavor that can be a function of phosphate addition in processed meat products. In trained panels, descriptive analysis procedures have been utilized to accurately characterize meat flavor from different species as well as processed meat products. Volatiles extracted from meat products that are responsible for these flavors have been characterized utilizing gas chromatography (GC), GC-mass spectrometry (GC-MS), and GC-olfactometry (GC-O).

Sensory evaluation is commonly utilized in determining the shelf life of muscle food products. Both analytical and affective testing can be effective in determining shelf life, and the simultaneous use of both tests allows the best determination of how long the product will have acceptable quality (Dethmers 1979). This testing will allow for determinations of expiration, freshness or quality assurance, and pack dates. A product's shelf-life is determined by bacterial or enzymatic spoilage, loss of aesthetic qualities, physical changes such as moisture evaporation, chemical reactions such as oxidation, contamination from storage environment, loss of nutritive value, and interactions between product and package conditions (IFT 1974). Sensory evaluation will allow for determination of manufacturing, packaging, and storing conditions that will minimize these deteriorations from occurring. Such shelf life determinations follow a similar pattern to the relationships between consumer and trained sensory panels as described by Muñoz and Chambers (1993).

Novel Uses of Statistical Methods

Four novel methods that have come to fruition over the last 10 to 15 years include response surface methodology, principal components analysis, principal factor analysis, and logistic regression. The potential for the relation of trained sensory panels to objective measurements and consumer acceptability is very exciting. Also, the possibility for building flavor languages for food products based on brand, region, or other appropriate attributes will allow for the determination of consumers' desires in regions as well as allow for increased communication among researchers working in different parts of the country. Novel approaches to statistical design of sensory experiments and analysis of sensory data are being developed or adapted to this discipline all of the time. These novel statistical methods are valuable tools, but they should never take the place of common sense and clearly understanding the objective of the study. Utilizing computers has also increased the number of possibilities available for analyzing sensory data. This is excellent in that it makes it much easier to run complicated regression and multivariate analyses. However, utilizing computer programs can also be dangerous since they will give you incorrect results if you do not understand the experimental design and/or the computer program. Substitution of statistical programs for practical knowledge of sensory evaluation and data analysis is a danger that must be avoided. The possibilities and applications of logistic regression and various multivariate analyses are limitless in their application as far as relating trained data to consumer data, in determining shelf life, and in product development. Proper use of these experimental designs and statistical packages will help contribute to a company providing products that are desired by consumers.

CONCLUSION AND FUTURE OF SENSORY EVALUATION

As food technology becomes more complex, the basics of sensory evaluation need to be remembered. The goal of sensory evaluation is to explain the consumer acceptability of food products. This can only be done through utilization of the four sensory methodologies listed in this chapter and working together with the market research department to make sure that the appropriate questions and problems are being answered and solved. Education on appropriate utilization of sensory analysis must be continued. It is clear that most companies are utilizing sensory analysis, but quite often, the wrong methods are being utilized for the stated objectives of the studies (Stone and Sidel 1993). This book will provide a basic understanding of sensory properties and evaluation as they relate to muscle foods.

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4
Chemical and Biochemical Aspects of Color in Muscle Foods

J. A. Pérez-Alvarez and J. Fernández-López

General Aspects of Muscle-Based Food Color
Chemical and Biochemical Aspects of Muscle-Based Food Color
Carotenes
Hemoproteins
Structure of Myoglobin
Chemical Properties of Myoglobin
Cytochromes
Color Characteristics of Blood
Fat Color
Alterations in Muscle-Based Food Color
Pink Color of Uncured Meat Products
Melanosis
Fish Skin Discoloration
Premature Browning
Color and Shelf Life of Muscle-Based Foods
Microorganisms and Muscle-Based Food Color
References

GENERAL ASPECTS OF MUSCLE-BASED FOOD COLOR

The first impression that a consumer receives concerning a food product is established visually, and among the properties observed are color, form, and surface characteristics. Color is the main aspect that defines a food's quality, and a product may be rejected simply because of its color, even before other properties, such as aroma, texture, and taste, can be evaluated. This is why the appearance (optical properties, physical form, and presentation) of muscle-based products at the sales point is of such importance (Lanari et al. 2002).

Regarding the specific characteristics that contribute to the physical appearance of meat, color is the quality that most influences consumer choice (Krammer 1994). The relationship between meat color and quality has been the subject of study since the 1950s, indeed, since Urbain (1952) described how consumers had learned through experience that the color of fresh meat is bright red, and any deviation from this color (nonuniform or anomalous coloring) is unacceptable (Diestre 1992). The color of fresh meat and associated adipose tissue is, then, of great importance for its commercial acceptability, especially in the cases of beef and lamb (Cornforth 1994) and in certain countries, for example, the United States and Canada, and there have been many studies to identify the factors controlling its stability. Adams and Huffman (1972) affirmed that consumers relate the color of meat to its freshness. In poultry, the consumers of many countries also associate meat color with the way in which the animal was raised (intensive or extensive) and fed (cereals, animal feed, etc.).

Color as a quality factor for meat can be appreciated in different ways in different countries; for example, in Denmark, pork meat color holds fifth place among qualities that affect consumers' purchase decisions (Bryhni et al. 2002). The sensorial quality, especially color and appearance (Brewer and Mckeith 1999), of meat can be affected by both internal and external factors. In the case of internal factors, in fish, for example, a particular problem that has been encountered in rearing some Pargus species is the darkening of the body after the capture of wild fish and during farming. During farming and marketing, the skin color (silver-red) turns dark gray

(especially the tail and fins) (Kentouri et al. 1995, Lin et al. 1998). In the case of farmed salmon, too, feeding fish with carotenoid pigments is regarded as the most important management practice for marketing (Moe 1990) because without them, flesh and skin color would be less visually attractive, and therefore would be less valued as a food (Baker 2002).

Food technologists, especially those concerned with the meat industry, have a special interest in the color of food for several reasons—first, because of the need to maintain a uniform color throughout processing; second, to prevent any external or internal agent from acting on the product during its processing, storage, and display; third, to improve or optimize a product's color and appearance; and, lastly, to attempt to bring the product's color into line with what the consumer expects. Put simply, the color of meat is determined by the pigments present in it. These can be classified into four types: (1) biological pigments (carotenes and hemopigments), which are accumulated or synthesized in the organism antemortem (Lanari et al. 2002); (2) pigments produced as a result of damage during manipulation or inadequate processing conditions; (3) pigments produced postmortem (through enzymatic or nonenzymatic reactions) (Montero et al. 2001, Klomklao et al. 2006); and (4) pigments resulting from the addition of natural or artificial colorants (Fernández-López et al. 2002).

As a quality parameter, color has been widely studied in fresh meat (MacDougall 1982, Cassens et al. 1995, Faustman et al. 1996) and cooked products (Anderson et al. 1990, Fernández-Ginés et al. 2003, Fernández-López et al. 2003). However, dry-cured meat products have received less attention (Pérez-Alvarez 1996, Pagán-Moreno et al. 1998, Aleson et al. 2003) because in this type of product, color formation takes place during the different processing stages (Pérez-Alvarez et al. 1997, Fernández-López et al. 2000); recently, a new heme pigment has been identified in this type of product (Parolari et al. 2003, Wakamatsu et al. 2004a,b). From a practical point of view, color plays a fundamental role in the animal production sector, especially in meat production (beef and poultry, basically) (Zhou et al. 1993, Esteve 1994, Verdoes et al. 1999, Irie 2001), since in many countries of the European Union (e.g., Spain and Holland) paleness receives a wholesale premium.

For fish, skin and flesh discoloration is a very important problem, especially in highly appreciated species. Since the skin and flesh color must be very vivid, many efforts have been directed at improving color, mainly through dietary control (carotene-enriched diets) (Fujita et al. 1983, Mori 1993). Without these pigments, the aquaculture industry would find it hard to undertake the production of some species because fish demand is driven through consumer demand for quality products (Baker 2002). In fish, consumer preference is often influenced by body pigmentation. Fish flesh color is an important quality parameter for most farmed fish, especially with salmonids (salmon, rainbow trout), (Francis 1995, Hyun et al. 1999), in which the pink or red color of fillets is an important feature (Sigurgisladottir et al. 1994, Sigurgisladottir et al. 1997). For example, a uniform red color in rainbow trout is considered to indicate a high-quality product and is a reason for its acceptability, while for the tuna fish industry, it is very important to avoid discoloration in fresh and processed meat and to increase its shelf life (Goodrick et al. 1991, Tze et al. 2001). Fish nutrition has an important impact on several parameters that directly influence the quality of fish, some of which are color and appearance. The color of salmonid flesh is one of the most important quality parameters because consumers have a preference for red- or pink-colored products in the case of salmonids. This is the reason for using carotenoids in aquaculture.

CHEMICAL AND BIOCHEMICAL ASPECTS OF MUSCLE-BASED FOOD COLOR

Of the major components of meat, proteins are the most important since they are only provided by essential amino acids, which are very important for the organism's correct functioning; proteins also make a technological contribution during processing, and some are responsible for such important attributes as color. These are the so-called chromoproteins, and they are mainly composed of a porphyrinic group conjugated with a transition metal, principally iron metalloporphyrin, which forms conjugation complexes (heme groups) (Whitaker 1972) that are responsible for color. However, carotenes and carotenoproteins (organic compounds with isoprenoid-type conjugated systems) exist alongside chromoproteins and also play an important part in meat color. There are also some enzymatic systems whose coenzymes

or prosthetic groups possess chromophoric properties (peroxidases, cytochromes, and flavins) (Faustman et al. 1996). However, their contribution to meat color is slight. Below, the principal characteristics of the major compounds that impart color to meat are described.

CAROTENES

Carotenes are responsible for the color of beef fat, poultry meat and skin, fish, and shellfish; in the last two cases, these are of great economic importance. The color of the fat is also important in carcass grading. Furthermore, carotenoids can be used as muscle-based food coloring agents (Verdoes et al. 1999). An important factor to be taken into account with these compounds is that they not synthesized by the live animal but are obtained by assimilation (Pérez-Alvarez et al. 2000), for instance, in the diet. Salmonids, for example, obtain carotenes in the wild in their preys, but in intensive fish culture, carotenoids must be added to the diet. Farmed fish, especially colored fish (salmon and rainbow trout, for example), are now a major industry. For example, Norway exports a great part of its salmon production. Carotenoid pigments have been used in aquafeed for many years in order to impart the desired flesh color in farmed salmonids (Baker 2002). Astaxanthin has been the main flesh-coloring pigment of choice in most trout and salmon farming industries. The type of carotene used in animal feed is very important because the fish farmer may find that pigmentation takes on a heterogeneous appearance, which is contrary to general consumer acceptance (Yanar et al. 2006). The preferred pigments used in the Canadian aquaculture industry are synthetic canthaxanthin (Cx) and synthetic astaxanthin (Ax) (Higgs et al. 1995). In fats, the fatty acid composition can affect their color. When the ratio of *cis*-monounsaturated to saturated fatty acids is high, the fat exhibits a greater yellow color (Zhou et al. 1993). In the case of the carotenes present in fish tissues, these come from the ingestion of zooplankton, algae, and crustacean wastes (Ostemeyer and Schmidt 2004), and the levels are sometimes very high. This is possible because fish have the capacity to transport and deposit this pigment to specific sites in their muscles (Baker 2002). The deposition of Ax is higher in dark muscle than in light muscle (Ingemansson et al. 1993). The shells of many crustaceans, for example, lobster *(Panilurus argus),* also contain these compounds. Carotenoids have been extracted from crustacean wastes with organic solvents, but in many of the methods pigment degradation occurs (Charest et al. 2001).

The pigments responsible for color in fish, particularly salmonids (trout and salmon, among others), are Ax and Cx, although they are also present in tunids and are one of the most important natural pigments of marine origin. In the case of shellfish, their color depends on the so-called carotenoproteins, which are proteins with a prostetic group that may contain various types of carotene (Minguez-Mosquera 1997), which are themselves water soluble (Shahidi and Matusalach-Brown 1998). Henmi and coworkers (1990a) reported that carotenoid-protein interaction in the salmon muscle is weak, and that Ax and Cx have a *trans* configuration *in vivo.* Henmi and coworkers (1990b) also reported that the actomyosins from salmonids showed a higher affinity for ketocarotenoids than those of other fish, except common mackerel. These authors also described correlations between the surface hydrophobicity of actomyosins and the combination of Ax and/or canthaxanthin with actomyosins. From a chemical point of view, astaxanthin or canthaxanthin bind via a beta-ionone ring to a hydrophobic binding site on actomyosin; the hydroxyl and keto end groups of the beta-end group of carotenoids intensify binding to actomyosin. Salmon actomyosin forms complexes with free Ax, astaxanthin monoester, canthaxanthin, echinenone, zeaxanthin, and beta-carotene, but not astaxanthin diester (in which a long-chain fatty acid residue may cause steric hindrance). The lipids in the actomyosin complex have no effect on the binding of carotenoids (Henmi et al. 1989). They are distributed in different amounts in the flesh, head, and carapace of crustaceans, for example, astaxanthin and its esters are the major carotenoids found in the extracts from different species of shrimp (*Penaeus monodon, Penaeus indicus, Metapenaeus dobsonii, Parapen aeopsis stylifera*) (Sachindra et al. 2005a), but there are different types of carotene, depending on whether the crustaceans are marine or fresh water (Sachindra et al. 2005b). Another difference is that the concentration of unsaturated fatty acids in its carotenoid extracts was found to be higher than that of saturated fatty acids. In raw muscle, the main carotenoid concentration was strongly correlated with some color

attributes (hue, chroma, and lightness) (Choubert et al. 1992). Torrisen and coworkers (1989) reported that a level of 4 milligrams per kilogram (mg/kg) in fish fillets is regarded as a minimum acceptable carotenoid concentration in marketable-farmed salmon. Sex also affects carotene concentration: female muscles, which contain much more carotenoid, are more strongly colored than male muscles (Norris and Cunningham 2004).

As suggested by Torrisen and coworkers (1989), the rate of carotenoid deposition in salmonids is curvilinear throughout the life of the fish. As the growth rate is obviously under strong genetic control, the genetic correlation between the growth rate and color is high. It must be taken into account that carotenoids migrate from the muscle to the gonads. Carotene type deposition in salmonid species differs; for example, Ax is more efficiently deposited than Cx in rainbow trout (Store-bakken and Choubert 1991, Torrissen 1986), but this pattern is not the same for Atlantic salmon. These differences may be due to genetic background and/or environment (Baker 2002). Choubert et al. (1997) reported that in rainbow trout there is an unequal distribution of carotenoids so that the color of the muscle lightens from the head toward the tail and from the midline of the fish toward the dorsal and ventral external area of the fish.

From a chemical point of view, carotenoids are organic molecules that contain a conjugated carbon-carbon double bond system, which is responsible for their color. But this can be a problem during processing, because a high number of conjugated double bonds may be subject to oxidation, which can lead to discoloration of the carotenoids (Liaaen-Jensen 1971, Choubert and Baccaunaud 2006). As carotenoids are lipid soluble compounds, it might be thought that increasing dietary fat would increase carotene absorption and deposition, but this is not necessarily the case for all salmonids. The retention of carotenoids in the flesh is relatively poor, with only 10–18% of pigment obtained from the diet being retained (Nickell and Bromage 1998). Astaxanthin can be found in its free, mono-, or diesterified forms. In processed shrimps, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the principal fatty acids esterified with the portion of astaxanthin linked to chitin in the carapace (Guillou et al. 1995). β-carotene and Ax are fat-soluble pigments found in squid oil. However, technological processes, such as refining, can remove Ax completely (Hwei and Min 1994).

In fish-derived products, the carotene content has previously been used as a quality parameter on its own; however, it has been demonstrated that this is not appropriate, and that other characteristics may influence color (Little et al. 1979). The carotene content and its influence on color is perhaps one of the characteristics that has received most attention (Swatland 1995). In the case of meat, especially beef, an excess of carotenes may actually lower the quality (Irie 2001), as occurs sometimes when classifying carcasses. The Japanese system for beef carcass classification identifies acceptable fats as white, slightly off-white, or slightly reddish white in color, while pink-yellowish and dark yellow are unacceptable (Irie 2001). It is precisely the carotenes that are responsible for these last two colorations. However, in other animal species, such as chicken (Castaneda et al. 2005), the opposite effect is observed, since a high carotene (xantophile) concentration is much appreciated by consumers (Esteve 1994), yellow being associated with traditional or "home-reared" feeding (Pérez-Alvarez et al. 2000). The use of the carotenoid canthaxanthin as a coloring agent in poultry feeds is designed to result in the desired coloration of poultry meat skins. The carotenoids used include citranaxanthin, capsanthin, and capsorubin, but Cx shows superior pigmenting properties and stability during processing and storage (Blanch 1999). To improve its color and brilliance, 0.004–0.04 weight percent (wt%) proanthocyanidin is added to fish feed containing carotenoids (Sakiura 2001). For rainbow trout carotenoid concentrations could be 10.7 or 73 parts per million (ppm) Cx, or 47 or 53 ppm Ax.

HEMOPROTEINS

Of the hemoproteins present, postmortem in the muscle, myoglobin (Mb) is the one mainly responsible for color, since hemoglobin (Hb) arises from the red cells that are not eliminated during the bleeding process and are retained in the vascular system, basically in the capillaries (incomplete exsanguination; the average amount of blood remaining in meat joints is 0.3%) (Warris and Rodes 1977). However, the contribution of red cells to color does not usually exceed 5% (Swatland 1995). There is wide variation in the amounts of hemoglobin from muscle tissue of

bled and unbled fish. Myoglobin content is minimal compared with the hemoglobin content in fish light muscle and white fish whole muscle. Hemoglobin made up 65 and 56% by weight of the total heme protein in dark muscle from unbled and bled fish, respectively (Richards and Hultin 2002). Myoglobin, on average, represents 1.5% by weight of the proteins of the skeletal muscle, while Hb represents about 0.5%, the same as the cytochromes and flavoproteins combined. Myoglobin is an intracellular (sarcoplasmic) pigment apparently distributed uniformly within muscles (Ledwar 1992, Kanner 1994). It is red in color and water soluble, and it is found in the red fibers of both vertebrates and invertebrates (Knipe 1993, Park and Morrisey 1994), where it fulfills the physiological role of intervening in the oxidative phosphorylization chain in the muscle (Moss 1992).

Structure of Myoglobin

Structurally, Mb can be described as a monomeric globular protein with a very compact, well-ordered structure that is specifically, almost triangularly, folded and bound to a heme group (Whitaker 1972). It is structurally composed of two groups: a proteinaceous group and a heme group. The protein group has only one polypeptidic chain composed of 140–160 amino acid residues, measuring 3.6 nanometer (nm) and weighing 16,900 Daltons in vertebrates (Lehningher 1981). It is composed of eight relatively straight segments (where 70% of the amino acids are found), separated by curvatures caused by the incorporation into the chain of proline and other amino acids that do not form alpha-helices (such as serine and isoleukin). Each segment is composed of a portion of alpha-helix, the largest of 23 amino acids and the shortest of seven amino acids, all dextrogyrating. Myoglobin's high helicoidal content (forming an ellipsoid of 44 × 44 × 25 Å) and lack of disulphide bonds (there is no cysteine) make it an atypical globular protein. The absence of these groups makes the molecule highly stable (Whitaker 1972). Although the three-dimensional structure seems irregular and asymmetric, it is not totally anarchic, and all the molecules of Mb have the same conformation. One very important aspect of the protein part of Mb is its lack of color. However, the variations presented by its primary structure and the amino acid composition of the different animal and fish species destined for human consumption are the cause of the different colorations of meat and their stability when the meats are displayed in the same retail illumination conditions (Lorient 1982, Lee et al. 2003). The heme group of Mb (as in Hb and other proteins) is, as mentioned above, a metalloporphyrin. These molecules are characterized by their high degree of coloration as a result of their conjugated cyclic tetrapyrrolic structure (Kalyanasundaram 1992). The heme group is composed of a complex, organic annular structure, protoporphyrin, to which an iron atom in ferrous state is united (Fe II). This atom has six coordination bonds, four with the flat protoporphyrin molecule (forming a flat square complex) and two perpendicular to it. The sixth bond is open and acts as a binding site for the oxygen molecule.

Protoporphyrin is a system with a voluminous flat ring composed of four pyrrolic units connected by methyl bridges (=C-). The Fe atom, with a coordination number of 6, lies at the center of the tetrapyrrol ring and is complexed to four pyrrolic nitrogens. The heme group is complexed to the polypeptidic chain (globin) through a specific histidine residue (imadazolic ring) occupying the fifth position of the Fe atom (Davidson and Henry 1978). The heme group is bound to the molecule by hydrogen bridges, which are formed between the propyonic acid side chains and other side chains. Other aromatic rings exist near, and almost parallel to the heme group, which may also form pi (π) bonds (Stauton-West et al. 1969).

The Hb contains a porphyrinic heme group identical to that of Mb and equally capable of undergoing reversible oxygenation and deoxygenation. Indeed, is functionally and structurally paired with Mb, and its molecular weight is four times greater since it contains four peptidic chains and four heme groups. The Hb, like Mb, has its fifth ligand occupied by the imidazol group of a histidine residue, while the sixth ligand may or may not be occupied. It should be mentioned that positions 5 and 6 of other hemoproteins (cytochromes) are occupied by R groups of specific amino acid residues of the proteins and therefore cannot bind to oxygen (O_2), carbon monoxide (CO), or cyanide (CN-), except a_3, which, in its biological role, usually binds to oxygen.

One of the main differences between fish and mammalian Mb is that fish Mb have two distinct endothermic peaks, indicating multiple states of

structural unfolding, whereas mammalian Mb followed a two-state unfolding process. Changes in alpha-helix content and tryptophan fluorescence intensity with temperature are greater for fish Mb than for mammalian Mb. Fish Mb shows labile structural folding, suggesting greater susceptibility to heat denaturation than that of mammalian Mb (Saksit et al. 1996).

The helical contents of frozen-thawed Mb were practically the same as those of unfrozen Mb, regardless of pH. Frozen-thawed Mb showed a higher autoxidation rate than unfrozen Mb. During freezing and thawing, Mb suffered some conformational changes in the nonhelical region, resulting in a higher susceptibility to both unfolding and autoxidation (Chow et al. 1989). In tuna fish, Mb stability followed the order bluefin tuna *(Thunnus thynnus)* > yellowfin tuna *(Thunnus albacares)* > bigeye tuna *(Thunnus obesus);* autoxidation rates were in the reverse order. The pH dependency of Mb from skipjack tuna *(Katsuwonus pelamis)* and mackerel *(Scomber scombrus)* were similar. Lower Mb stability was associated with higher autoxidation rates (Chow 1991).

Chemical Properties of Myoglobin

The chemical properties of Mb center on its ability to form ionic and covalent groups with other molecules. Its interaction with several gases and water depends on the oxidation state of the Fe of the heme group (Fox 1966), since this may be in either its ferrous (Fe II) or its ferric (Fe III) state. Upon oxidation, the Fe of the heme group takes on a positive charge (Kanner 1994) and, typically, binds with negatively charged ligands, such as nitrites, the agents responsible for the nitrosation reactions in cured meat products.

When the sixth coordination ligand is free Mb is usually denominated deoxymyoglobin (DMb), which is purple in color. However, when this site is occupied by oxygen, the oxygen and the Mb form a noncovalent complex, denominated oxymyoglobin (OMb), which is cherry or bright red (Lanari and Cassens 1991). When the oxidation state of the iron atom is modified to the ferric state and the sixth position is occupied by a molecule of water, the Mb is denominated metmyoglobin (MMb), which is brown. There are several possible causes for MMb generation, and these may include the ways in which tunids, meat, and meat products are obtained, transformed, or stored (MacDougall 1982, Lee et al. 2003, Mancini et al. 2003). Among the most important factors are low pH, the presence of ions, and high temperatures during processing (Osborn et al. 2003); the growth and/or formation of metabolites from the microbiota (Renerre 1990); the activity of endogenous reducing enzymes (Arihara et al. 1995, Osborn et al. 2003); and the levels of endogenous (Lanari et al. 2002) or exogenous antioxidants, such as ascorbic acid or its salts, tocopherols (Irie et al. 1999), or plant extracts (Xin and Shun 1993, Fernández-López et al. 2003, Sánchez-Escalante et al. 2003). The pH, which may be altered depending on postslaughter metabolism and on ingredient addition, can affect the stability of the central iron atom in myoglobin and hemoglobin. At high pH, the heme iron is predominantly in the Fe^{2+} state; low pH accelerates Fe^{2+} conversion to Fe^{3+} (Zhu and Brewer 2002, 2003). While oxygen can bind to Fe^{2+} only, many other ligands (CN, nitric oxide [NO], CO) can bind to either Fe^{2+} or Fe^{3+} so producing a variety of colors. This change in the oxidation state of the heme group will result in the group being unable to bind with the oxygen molecule (Arihara et al. 1995). DMb is able to react with other molecules to form colored complexes, many of which are of great economic relevance for the meat industry. The most characteristic example is the reaction of DMb with nitrite, since its incorporation generates a series of compounds with distinctive colors: red in dry-cured meat products or pink in heat-treated products. The products resulting from the incorporation of nitrite are denominated cured, and such products are of enormous economic importance worldwide (Pérez-Alvarez 1996). The reaction mechanism is based on the propensity of nitric oxide (NO, generated in the reaction of nitrite in acid medium, readily gives up electrons) to form strong coordinated covalent bonds; it forms an iron complex with the DMb heme group independent of the oxidation state of the heme structure. The compound formed after the nitrification reaction is denominated nitrosomyoglobin (NOMb). As mentioned above, the presence of reducing agents such as hydrogen sulfide acid (H_2S) and ascorbates lead to the formation of undesirable pigments in both meat and meat products. These green pigments are called sulphomyoglobin (SMb) and colemyoglobin (ColeMb), respectively, and are formed as a result of bacterial activity and an excess of reducing agents in the medium. The formation of

SMb is reversible, but that of ColeMb is an irreversible mechanism, since it is rapidly oxidized between pH 5 and 7, releasing the different parts of the Mb (globin, iron, and the tetrapyrrolic ring).

From a chemical point of view, it should be borne in mind that the color of Mb, and therefore of the meat or meat products, not only depends on the molecule that occupies the sixth coordination site, but also on the oxidation state of the iron atom (ferrous or ferric), the type of bond formed between the ligand and the heme group (coordinated covalent, ionic, or none), and the state of the protein (native or denatured form), not to mention the state of the porphyrin of the heme group (intact, substituted, or degraded) (Pérez-Alvarez 1996).

During the heat treatment of fish flesh, the aggregation of denatured fish proteins is generally accompanied by changes in light-scattering intensity. Results demonstrate changes in relative light-scattering intensity can be used for studying structural unfolding and aggregation of proteins under thermal denaturation (Saksit et al. 1998). When fatty fish meat like *Trachurus japonicus* was heat treated, the MMb content increased linearly, and the percentages of denatured myoglobin and apomyoglobin increased rapidly when mince was exposed to heat, but when the temperature reached 60°C the linearity was broken. The results indicated that MMb color stability was higher than that of Mb and that the thermal stability of heme was higher than that of apomyoglobin (Hui et al. 1998). Both Mb and ferrous iron accelerated the lipid oxidation of cooked, water-extracted fish meat. EDTA (ethylenediaminetetraacetic acid) inhibited the lipid oxidation accelerated by ferrous iron, but not that accelerated by Mb. Also, with cooked, nonextracted mackerel meat, EDTA noticeably inhibited lipid oxidation. Nonheme iron catalysis seemed to be related in part to lipid oxidation in cooked mackerel meat. The addition of nitrite in combination with ascorbate resulted in a marked inhibition of lipid oxidation in the cooked mackerel meat. From these results, it was postulated that nitric oxide ferrohemochromogen, formed from added nitrite and Mb (present in the mackerel meat) in the presence of a reducing agent, possesses an antioxidant activity, which is attributable in part to its function as a metal chelator (Ohshima et al. 1988).

Tuna fish meat color can be improved when the flesh is treated or packaged with a modified atmosphere in which CO is included. Normally, the rate of penetration of CO or carbon dioxide (CO_2) in fish meat such as tuna, cod, or salmon, under different packaging conditions, is measured by monitoring pressure changes in a closed constant volume chamber with constant volume and temperature. Alternatively, however, the specific absorption spectrum of carboxymyoglobin (MbCO), within the visible range, can be obtained and used as an indicator of MbCO formation. Mb extracts from tuna muscle treated with CO exhibited higher absorbance at 570 than at 580 nm. Therefore, the relationship between absorbance at 570 nm and absorbance at 580 nm could be used to determine the extent of CO penetration of tuna steaks placed in a modified atmosphere in which CO was included. The penetration of CO into tuna muscle was very slow. After approximately 1–4 hours, CO had penetrated 2–4 mm under the surface, and after 8 hours, CO had penetrated 4–6 mm (Chau et al. 1997).

In products with added nitrite or nitrate the complex nitrosylmyoglobin (MbFe[II]NO) is the main contributor to the characteristic color of cooked cured ham, and brine-cured and dry-cured meat products. Meat and meat products without nitrite/nitrate addition will normally attain a dull brown color or a gray color in heated products, which influences consumer acceptance negatively (Adamsen et al. 2005). In dry-cured meat products such as Parma ham produced without nitrite or nitrate addition, the characteristic bright red color (Wakamatsu et al. 2004a) is caused by Zn-protoporphyrin IX (ZPP) complex, a heme derivative. Adamsen et al. (2005) showed that the use of nitrite as a curing ingredient inhibits the formation of Zn-pp. In the same work the author described that this color compound is present in other meat products like Iberian ham, although in a lower concentration.

Virgili et al. (1999) reported that this color may be due to the action of low-molecular weight compounds containing electron-donating atoms, formed during maturation, in particular basic peptides or amino acids resulting from an external proteolysis, which may play a role as Fe ligands in Mb. Wakamatsu and coworkers (2004b) reported that anaerobic conditions favor the formation of Zn-pp and that endogenous enzymes as well as microorganisms may also be involved. There are several hypotheses that try to explain the formation of this compound. Wakamatsu et al. (2004b) described

three possible substitution patterns: (i) a nonenzymatic reaction in which Zn(II) substitutes Fe(II) under anaerobic conditions, with concomitant dissociation of the heme; (ii) a bacterial enzymatic reaction, whereby bacterial growths naturally degrade the meat proteins including the pigment; or (iii) an enzymatic reaction where an endogenous ferrochelatase interchanges the two metals. However, Adamsen et al. (2005) described this process as having the three following mechanisms to explain the metal substation: (i) a nonenzymatic enzymatic reaction driven by binding of iron in the high chloride meat matrix; (ii) a bacterial enzymatic reaction; or (iii) an endogenous enzymatic reaction.

Also spectroscopic studies of Parma ham during processing revealed a gradual transformation of muscle myoglobin, initiated by salting and continuing during aging. Using electron spin resonance spectroscopy, Moller and coworkers (2003) have shown that the Parma ham pigment is different from MbFe(II)NO and is not a nitric oxide complex such as that found in brine-cured ham and Spanish Serrano hams. These authors also establish that the heme moiety is present in the acetone-water extract and that Parma ham pigment is gradually transformed from a myoglobin derivative into a nonprotein heme complex, which is thermally stable in an acetone-water solution. Adamsen et al. (2003) also demonstrated that the heme moieties of Parma ham pigments have antioxidative properties. Pigments became increasingly lipophilic during processing, suggesting that a combination of drying and maturing yields a stable red color (Parolari et al. 2003).

Cytochromes

Cytochromes are metalloproteins with a prostetic heme group, whose putative role in meat coloration is undergoing revision (Boyle et al. 1994, Faustman et al. 1996). Initially, they were not thought to play a very important role (Ledwar 1984). These compounds are found in low concentrations in the skeletal muscle, and in poultry, they do not represent more than 4.23% of the total hemeoproteins present (Pikul et al. 1986). It has now been shown that the role of cytochrome (especially its concentration) in poultry meat color is fundamental, when the animal has been previously exposed to stress (Ngoka and Froning 1982, Pikul et al. 1986). Cytochromes are most concentrated in cardiac muscle so that when this organ is included in meat products, heart contribution to color, not to mention the reactions that take place during elaboration processes, must be taken into consideration (Pérez-Alvarez et al. 2000).

COLOR CHARACTERISTICS OF BLOOD

Animal blood is little used in the food industry because of the dark color it imparts to the products to which it is added. For solving the negative aspects of blood incorporation, specifically food color-related problems, several different processes and means have been employed, but they are not always completely satisfactory. The addition of 12% blood plasma to meat sausages leads to pale-colored products. Addition of discolored whole blood or globin (from which the hemoglobin's heme group has been eliminated) has also been used to address color problems. Natural red pigments can be obtained from blood without using coloring agents such as nitrous acid salts; these pigments have zinc protoporphyrin as the metalloporphyrin moiety and can be used to produce favorably colored beef products, whale meat products, and fish products (including fish pastes) (Numata and Wakamatsu 2003). There was wide variation in amounts of haemoglobin extracted from the muscle tissue of bled and unbled fish, and the residual level in the muscle of bled fish was substantial. Myoglobin content was minimal as compared with hemoglobin content in mackerel light muscle and trout whole muscle. Hemoglobin made up 65 and 56% by weight of the total heme protein in dark muscle from unbled and bled mackerel, respectively. The blood-mediated lipid oxidation in fish muscle depends on various factors, including hemoglobin concentration, hemoglobin type, plasma volume, and erythrocyte integrity (Richards and Hultin 2002). The presence of blood, Hb, Mb, Fe^{+2}, Fe^{+3}, or Cu^{+2} can stimulate lipid oxidation in the fillets of icefish (Rehbein and Orlick 1990, Richards and Li 2004). Kanner and coworkers (1987) reported that hemoglobin, myoglobin, copper, and iron have the potential to promote lipid oxidation in muscle foods. Since iron can be released from hemoglobin during storage, it is difficult to ascertain whether the intact heme protein, dissociated heme, or released iron is responsible for the bulk of

lipid oxidation that occurs during storage. For this reason, Svingen and coworkers (1979) used the term low molecular weight iron instead of free iron since iron binds to other low molecular weight compounds to gain solubility and hence potential reactivity. Ferrous and ferric forms of iron can promote lipid oxidation processes (Gutteridge 1986, Tadolini and Hakim 1996). Iron shows a high reactivity with reactants such as hydrogen peroxide and lipid peroxides (Kanner and Harel 1987).

Mitochondria are a source of reactive oxygen species that could confound lipid oxidation reactions due to added hemoglobin. During fish processing (e.g., tuna fish), the loss of redness can be a good indicator that lipid oxidation processes mediated by hemoglobin (Hb) are progressing. Just after death, Hb in muscle tissue is primarily in the reduced state (i.e., oxyhemoglobin [oxyHb] and deoxyhemoglobin [deoxyHb]).

This mixture of oxyHb and deoxyHb has a red color. With increased postmortem aging, Hb autooxidizes to methemoglobin [metHb], a brown pigment. MetHb is considered more prooxidative than reduced Hb due to its less tightly bound heme group and its reactivity with hydrogen peroxide and lipid peroxides to form hypervalent Hb catalysts (Everse and Hsia 1997).

From a technological point of view, during meat or fish processing, rapid chilling may alter oxygen solubility in tissues resulting in less available oxygen to oxygenate either oxymyoglobin or hemoglobin. The conversion of oxymyoglobin to metmyoglobin, which is brown and unattractive, occurs under conditions of very low oxygen tension as well (Nicolalde et al. 2005).

Field and coworkers (1978) describe how bone marrow is high in hemoglobin, while muscle has a high myoglobin content. As with other meats, its color and hemoglobin stability depend on packaging and storage conditions. Good temperature control and modified atmosphere packaging (MAP) with high oxygen atmospheres (80%) are often used to extend both microbiological and color shelf life (Nicolalde et al. 2005).

FAT COLOR

From a technological point of view, fat fulfills several functions, although, regarding color, its principal role is in the brightness of meat products. Processes such as "afinado" during the elaboration of dry-cured ham involve temperatures at which fat melts so that it infiltrates the muscle mass and increases its brilliance (Sayas 1997). When the fat is finely chopped, it "dilutes" the red components of the color, thus decreasing the color intensity of the finished product (Pérez-Alvarez et al. 2000). However, fats do not play such an important role in fine pastes since, after emulsification, the fat is masked by the matrix effect of the emulsion so that it contributes very little to the final color. The color of fat basically depends on the feed that the live animal received (Esteve 1994, Irie 2001). In the case of chicken and ostrich, the fat has a "white" appearance (common in Europe) when the animal has been fed with "white" cereals or other ingredients not containing xanthophylls, since these are accumulated in subcutaneous fat and other fatty deposits. However, when the same species are fed maize (rich in xanthophylls), the fatty deposits take on a yellow color. Beef or veal fat, that is dark, hard (or soft), excessively bright, or shiny lowers the carcass and cut price. Fat with a yellowish color in healthy animals reflects a diet containing beta-carotene (Swatland 1988). While fat color evaluation has traditionally been a subjective process, modern methods include such techniques as optical fiber spectrophotometry (Irie 2001). Another factor influencing fat color is the concentration of the Hb retained in the capillaries of the adipose tissues (Swatland 1995). As in meat, the different states of Hb may influence the color of the meat cut. OMb is responsible for the yellowish appearance of fat, since it affects different color components (yellow-blue and red-green).

The different states of hemoglobin present in adipose tissue may react in a similar way to those in meat so that fat color should be measured as soon as possible to avoid possible color alterations. When the Hb in the adipose tissue reacts with nitrite incorporated in the form of salt, nitrosohemoglobin (NOHb), a pigment that imparts a pink color to fat, is generated. This phenomenon occurs principally in dry-cured meat products with a degree of anatomical integrity, such as dry-cured ham or shoulder (Sayas 1997). When fat color is measured, its composition should be borne in mind since its relation with fatty acids modifies its characteristics, making it more brilliant or duller in appearance. The fat con-

tent of the conjunctive tissue must also be borne in mind—collagen may present a glassy appearance because, at acidic pH, it is "swollen," imparting a transparent aspect to the product.

ALTERATIONS IN MUSCLE-BASED FOOD COLOR

The color of meat and meat products may be altered by several factors, including exposure to light (source and intensity), microbial growth, rancidity, and exposure to oxygen. Despite the different alterations in color that may take place, only a few have been studied; these include the pink color of boiled uncured products, premature browning, fish skin discoloration, and melanosis in crustaceans.

PINK COLOR OF UNCURED MEAT PRODUCTS

The normal color of a meat product that has been heat treated but not cured is "brown," although it has recently been observed that these products show an anomalous coloration (red or pink) (Hunt and Kropf 1987). This problem is of great economic importance in "grilled" products since this type of color is not considered desirable. This defect may occur both in meats with a high hemoprotein content, such as beef and lamb (red); and in those with a low hemoprotein concentration, including chicken and turkey (pink) (Conforth et al. 1986). One of the principal causes of this defect is the use of water rich in nitrates, which are reduced to nitrites by nitrate-reducing bacteria, which react with the Mb in meat to form NOMb (Nash et al. 1985). The same defect may occur in meat products containing paprika, which according to Fernández-López (1998), contains nitrates that, once incorporated in the product, may be similarly reduced by microorganisms. Conforth et al. (1991) mention that several nitrogen oxides may be generated in gas and electric ovens used for cooking ham and that these nitrogen oxides will react with the Mb to generate nitrosohemopigments. CO is also produced in ovens, which reacts with Mb during thermal treatment to form a pink-colored pigment, carboxyhemochrome. It has also been described how the use of adhesives formed from starchy substances produces the same undesirable pink color in cooked products (Scriven et al. 1987). The same anomalous pink color may be generated when the pH of the meat is high (because of the addition of egg albumin to the ingredients) (Froning et al. 1968) and when the cooking temperature during processing is too low. These conditions favor the development of a reducing environment that maintains the iron of the Mb in its ferrous form, imparting a reddish/pink color (as a function of the concentration of hemopigments) instead of the typical grayish brown color of heat-treated, uncured meat products.

Cooking uncured meat products, such as roast beef, at low temperatures (less than 60°C) may produce a reddish color inside the product, which some consumers may like. This internal coloring is not related to the formation of nitrosopigments, but results from the formation of OMb, a phenomenon that occurs because there exist in the muscle MMb-reducing enzymatic systems that are activated at temperatures below 60°C (Osborn et al. 2003). Microbial growth may also cause the formation of a pink color in cooked meats since these reduce the oxidoreduction potential of the product during their growth. This is important when the microorganisms that develop in the medium are anaerobes, since they may generate reducing substances that decrease the heme iron. When extracts of *Pseudomonas* cultures are applied, the MMb may be reduced to Mb (Faustman et al. 1990).

MELANOSIS

Melanosis, or blackspot, involving the appearance of a dark, even black, color, may develop postmortem in certain shellfish during chilled and frozen storage (Slattery et al. 1995). Melanosis is of huge economic importance since the coloration may suggest a priori in the eyes of the consumer that the product is in bad condition, despite the fact that the formation of the pigments responsible involves no health risk. Melanosis is an undesirable surface discoloration of such high value shellfish as lobsters that takes place immediately after harvesting since it starts with oxygen contact (López-Caballero et al. 2006). Blackspot is caused by enzymic formation of the precursors of phenolic pigments (Williams et al. 2003). Blackspot is a process regulated by a complex biochemical mechanism, whereby the phenols present in a food are oxidized to quinones in a series of enzymatic reactions caused by polyphenol oxidase (PPO) (Ogawa et al. 1984). This is followed by a polymerization reaction, which produces pig-

ments of a high molecular weight and dark color. Melanosis is produced in the exoskeleton of crustaceans, first in the head and gradually spreading toward the tail. Melanosis of shell and hyperdermal tissue in some shellfish, such as lobsters, has been related to stage of molt, since the molting fluid is considered to be the source of the natural activator(s) of pro-PPO.

Polyphenol oxidase (catechol oxidase) can be isolated from shellfish cuticle (Ali et al. 1994) and is still active during iced or refrigerated storage. Some authors have found a connection between melanosis and microbial growth in crustaceans. Thus, color formation (melanin) due to strains of *P. fragi* may occur if prawns are not properly chilled (Chinivasagam et al. 1998). In this respect, López-Caballero et al. (2006) reported that the presence of microorganisms (e.g., *Proteus* spp., *Pseudomonas*, etc.) and the H_2S produced reacted with metals of the lobster shell resulting in melanosis. Sulphites can be used to control the process (Ferrer et al. 1989, Gomez-Guillen et al. 2005), although their use is prohibited in many countries. It is well known that the inhibitory effect of blackspot is specific for each species, requiring adequate doses and formulations (Montero et al. 2001). The effective dose of 4-hexylresorcinol differs depending on the physiological state, season, method of application, etc., although the species being treated is one of the most important of these factors. Montero et al. (2004) and López-Caballero et al. (2006) found that, regardless of the season, a concentration of 0.25% 4-hexylresorcinol was effective in extending the shelf life of pink shrimp. Ficin (Taoukis et al. 1990) and 4-hexylresorcinol also functioned as a blackspot inhibitor, alone and in combination with L-lactic acid (Benner et al. 1994).

FISH SKIN DISCOLORATION

In fish and other vertebrates, in which the pigmentation of the skin can be changed by hormonal stimulation, the color of the background and illumination are determining factors for the intensity and/or the pattern of skin fish pigmentation (Sugimoto 1997, Duray et al. 1996, Crook 1997, Healey 1999, Papoutsoglou et al. 2000, Rotllant et al. 2003). In addition, temperature may also have an impact on color (Fernandez and Bagnara 1991). In some types of fish, especially those with a red skin, the color tone becomes dark immediately after killing, reducing the commercial value of the fish. Most of the color changes in fish are often related to stress. It is generally accepted that melanophores play an important role in the rapid color change of certain fish (Fujii 1969). These changes are related to hormonal (α-melanocyte-stimulating hormones) responses causing dispersion of the melanin granules in melanophores and are responsible for skin darkening (Green and Baker 1991, Lamers et al. 1992, Gröneveld et al. 1995, Arends et al. 2000, Burton and Vokey 2000).

In the case of Red Sea Bream, the rapid skin color changes after killing of cultured fish is thought to be mainly due to the rapid dispersion of chromatosomes in melanophores elicited by handling and killing stresses. Potassium ions through the noradrenaline pathway can induce aggregation of chromatosomes in melanophores (Kumazawa and Fujii 1984). In fish skin, besides melanophores there are other chromatophores, such as xanthophores and erythrophores, the latter mainly contributing to the red color of the fish skin.

PREMATURE BROWNING

Hard-to-cook patties show persistent internal red color and are associated with high pH (>6) raw meat. Pigment concentration affects red color intensity after cooking (residual undenatured myoglobin), so this phenomenon is often linked to high pH dark cutting meat from older animals. Premature browning is a condition in which ground beef (mince) looks well done at a lower than expected temperature (Warren et al. 1996). Premature browning (PMB) of ground beef is a condition in which myoglobin denaturation appears to occur on cooking at a temperature lower than expected; it may indicate falsely that an appropriate internal core temperature of 71°C has been achieved (Suman et al. 2004). The relationship between cooked color and internal temperature of beef muscle is inconsistent and depends on pH and animal maturity. Increasing the pH may be of benefit in preventing premature browning, but it may increase the incidence of red color in well-cooked meat (cooked over an internal temperature of 71.1°C) (Berry 1997). When pale, soft, exudative (PSE) meat was used in patty processing, patties containing OMb easily exhibited premature browning. One reason for this behavior is that the percentage of Mb denaturation increased as cooking temperature rose (Lien et al. 2002).

COLOR AND SHELF LIFE OF MUSCLE-BASED FOODS

Meat and meat products are susceptible to degradation during storage and throughout the retail process. In this respect, color is one of the most important quality attributes for indicating the state of preservation in meat. Any energy received by food can initiate its degradation, but the rate of any reaction depends on the exact composition of the product (Jensen et al. 1998), environmental factors (light, temperature, presence of oxygen), and the presence of additives. Transition metals such as copper and iron are very important in the oxidative/ antioxidative balance of meat. When the free ions of these two metals interact, they reduce the action of certain agents, such as cysteine, ascorbate, and alpha-tocopherol, oxidizing them and significantly reducing the antioxidant capacity in muscle (Zanardi et al. 1998). Traditionally, researchers have determined the discoloration of meat using as criterion the brown color of the product, calculated as percent MMb (Mancini et al. 2003). These authors demonstrated that in the estimation of the shelf life of beef or veal (considered as discoloration of the product), the diminution in the percent of OMb is a better tool than the increase in percentage of MMb. Occasionally, when the meat cut contains bone (especially in pork and beef), the hemopigments (mainly Hb) present in the medulla lose color because the erythrocytes are broken during cutting and accumulate on the surface of the bone hemoglobin. When exposed to light and air, the color of the Hb changes from the bright red (oxyhemoglobin [Ohb]), the characteristic of blood, to brown (methemoglobin [MHb]) or even black (Gill 1996). This discoloration basically takes place during long periods of storage, especially during shelf life display (Mancini et al. 2004). This characteristic is aggravated if the product is kept in a modified atmosphere rich in oxygen (Lanari et al. 1995). These authors also point out that the effect of bone marrow discoloration is minimized by the effect of bacterial growth in modified atmosphere packaging. As in the case of fresh meat, the shelf life of meat products is limited by discoloration (Mancini et al. 2004). This phenomenon is important in this type of product because they are normally displayed in illuminated cabinets. Consequently, the possibility of photooxidation of nitrosomyoglobin (NOMb) needs to be taken into account.

During this process, the molecule is activated because it absorbs light; this may subsequently deactivate the NOMb and give the free electrons to the oxygen to generate MMb and free nitrite. In model systems of NOMb photooxidation, the addition of solutions of dextrose, an important component of the salts used for curing cooked products and in meat emulsions, can diminish the effect of NOMb photooxidation. When a meat product is exposed to light or is stored in darkness, the use of ascorbic acid or its salts may help stabilize the product's color. Such behavior has been described both in model systems of NOMb (Walsh and Rose 1956) and in dry-cured meat products (e.g., longanizas, Spanish dry-fermented sausage). However, when sodium isoascorbate or erythorbate is used in longanizas production, color stability is much reduced during the retail process (Ruíz-Peluffo et al. 1994).

The discoloration of white meats such as turkey is characterized by color changes that go from pink-yellow to yellow-brown, while in veal and beef, the changes go from purple to grayish brown. In turkey, it has been demonstrated that the presence or absence of lipid oxidation depends on, among other things, the concentration of vitamin E in the tissues. The color and lipid oxidation are interrelated since it has been seen that lipid oxidation in red and white muscle depends on the predominant form of catalyzing iron, Mb, or free iron (Mercier et al. 1998). Compared with red meat, tuna flesh tends to undergo more rapid discoloration during the refrigerated storage. Discoloration due to the oxidation of Mb in red fish presented a problem, even at low temperatures. This low color stability might be related to the lower activity or poorer stability of MMb reductase in tuna flesh (Ching et al. 2000). Another reason for the low color stability is that aldehydes produced during lipid oxidation can accelerate tuna OMb oxidation *in vitro* (Lee et al. 2003). Tuna flesh could be immersed in an MMb reductase solution to extend the color stability of tuna fish. Also, the use of this enzyme can reduce MMb formation during refrigerated storage of tuna (Tze et al. 2001). Yellowtail *(Seriola quinqueradiata)* fillets stored in gas barrier film packs filled with nitrogen (N_2) and placed in cold storage at 0–5°C, stayed fresh for 4–7 days. N_2 or CO_2 packaging did not prevent discoloration in frozen tuna fillets; better results were achieved by thawing the frozen tuna meat in an O_2 atmosphere (Oka 1989). Packaging in atmospheres

containing 4 or 9% O_2 was inferior to packaging in air, as these atmospheres promoted MMb formation. Packaging in 70% O_2 maintained the fresh red color of tuna dorsal muscle for storage periods less than 3 days (Tanaka et al. 1996). To change the dark brown color to a bright red color, processors sometimes treat tuna with 100% carbon monoxide (CO) during modified atmosphere packaging. Since Mb can react with CO rapidly even at low CO concentrations (Chi et al. 2001), modified atmosphere packaging with 100% CO may result in high CO residues in the flesh, which may cause health problems.

MICROORGANISMS AND MUSCLE-BASED FOOD COLOR

Although the real limiting factor in the shelf life of fresh meat is the microbial load, consumers choose fresh meat according to its color. The bacterial load is usually the most important cause of discoloration in fresh meat and meat products (sausages and other cooked products), and slaughter, cutting, and packaging must be strictly controlled. Bacterial contamination decisively affects the biochemical mechanisms responsible for the deterioration of meat (Renerre 1990). Is it important to take into account that, just as with the bacterial load, the effect of discoloration on meat is more pronounced in meats that are more strongly pigmented (beef) than in less pigmented meats such as pork and chicken (Gobantes and Oliver 2000). Another variable affecting color stability in meat is the quantity of microorganisms present (Houben et al. 1998); concentrations in excess of 10^6/gram (g) have a strong effect. Although antioxidants, such as ascorbic acid, slow lipid oxidation and consequently improve color stability, these substances have little effect when bacterial growth is a problem (Zerby et al. 1999).

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5
Sensory: Human Biology and Physiology

Maria Aparecida A. P. Da Silva and Fernando Cendes

The Concept of Flavor
The Anatomy and Physiology of Human Olfaction
Odorant Receptors
Prediction of Odorants' Binding Sites and Three-Dimensional Structures
The Human Olfactory Code for Odor Discrimination
Molecular Theories of Olfaction
Neuroimaging and Neurophysiological Studies of Olfactory Events
References

Smell is a phylogenetically primitive form of sensation associated with brain regions that control both emotion and memory. The olfactory system of primates, including that of humans, is allometrically reduced in size in comparison with that of macrosmatic mammals, but appears to be similarly organized (Gloor 1997). In fact, olfaction has an important impact on social behaviors, even in humans (Kandel et al. 2000).

The phylogenetic history of olfaction suggests that this sense modality was singled out in early mammalian evolution to subserve the role of relating olfactory stimuli to experiences of great significance to the animal's behavior and survival as, for example, procreation and various forms of intercourse, and protection from poisons and dangerous predators. Olfactory stimuli quickly form strong associations that are very enduring. This role of olfaction may be less obvious in humans, but it survives (Gloor 1997).

In humans, odors are reputed to have a particularly potent ability to evoke old memories of events or situations that usually occurred in a socio-affective context of which that odor was a part. Memories evoked by such odors are said to have an immediacy and vividness that are rarely matched by those evoked by other sensory stimuli. Olfactory memories have some peculiarities that indeed set them apart from other types of memory. The usual testing of human memory, for example, visual memory, often relies on verbal cues. By contrast, olfactory memories are poorly described by verbal labels, even those that are familiar.

The neural systems controlling smell and taste are remarkably sensitive and highly influenced by learning (Stockhorst and Pietrowsky 2004). The association of all the events stimulated by aromas—memories, emotions, and learning—causes human beings to strongly react to odors. We are very particular about the aroma and flavor of our juice, wine, and even bubble gum. This explains the large amount of human and financial resources applied every year by the food industry to flavor chemistry research.

In addition, there has been an increased interest in understanding the role of olfactory dysfunction in several neurological disorders. Patients with temporal lobe epilepsy (TLE) not only have difficulty in odor discrimination and memory, but may also have olfactory hallucinations as part of their seizures; these are often described as unpleasant odors. Anosmia and other types of olfactory dysfunction are frequent and often severe in neurodegenerative diseases such as Parkinson's disease and Alzheimer-type dementia. In fact some authors indicate that the absence of olfactory dysfunction virtually excludes

the diagnosis of idiopathic Parkinson's disease (Hawkes 2003).

The last decade has brought exciting advances in the knowledge of mechanisms associated with the detection and processing of odors, although several aspects are still unknown. The aim of this chapter is to present current concepts and leading tendencies in research on odor detection and processing by humans, using a language easily understandable and hopefully enjoyable by flavor chemists.

THE CONCEPT OF FLAVOR

Flavor perception rises from the simultaneous stimulation of the sense of taste (gustation), the sense of smell (olfaction), and the trigeminal nerve (chemesthesis). It represents the sum of sensations generated by the *taste receptors* located in the mouth; by the olfactory receptors located in the nasal olfactory mucosa, and the trigeminal nerve, located in the human mouth and nose (Lawless and Heymann 1999, Kandel et al. 2000, Nelson and Cox 2000).

Thus, it is inadequate to use the word "taste" to signify "flavor" because flavor is the sum of numerous different sensations, such as:

1. The basic taste sensations: sweet, salty, sour, bitter and possibly umami generated by the stimulation of the taste receptors by soluble molecules released in the mouth during food mastication.
2. The thousands of different aroma sensations: toasted, floral, fruity, putrid, etc., provoked by volatile compounds that rise from the food during mastication and reach the olfactory receptors in the nose by a retro nasal pass way located in the back of the human mouth.
3. The perceptions of astringency, spice heat, temperature, etc., that stimulate the trigeminal nerve terminals present in the human mouth and nose.

According to the concepts covered so far, a full understating of flavor involves the knowledge of both the senses of taste and smell, as well as their interaction with the whole human nervous system. However, it is unquestionable that the largest contribution to a food flavor comes from the volatile compounds present in the product. This explains why flavor chemists usually aim to identify the volatile compounds rather than the nonvolatile molecules to understand a food flavor. Therefore, this chapter will focus on the sense of smell.

THE ANATOMY AND PHYSIOLOGY OF HUMAN OLFACTION

The olfactory epithelium is comprised of six morphologically and biochemically distinct cell types of which only one, the bipolar sensory receptor neuron, has a chemosensory function. The other cell types, mainly the supporting and the microvillar cells, have supporting functions like regulating the composition of the mucus, releasing xenobiotic-metabolizing enzyme, or supporting the regeneration of receptor cells (Kandel et al. 2000).

The initial events in olfactory perception occur in the sensory receptor neurons. They are located in the olfactory epithelium, a region about 5 square centimeters (cm^2) in size, in the back of the human nasal cavity (Figure 5.1). They have an average life span ranging from 30 to 60 days, being continuously replaced. The primary job of the olfactory neurons are to transform chemical energy from a volatile molecule into an electrical signal, also referred to as action potential, in a process known as transduction. Electrical signals generated by the olfactory receptors are transmitted to the brain through a bundle of neurons, which form the human nervous system. The brain interprets the signals as odor (Goldstein 1989, Kandel et al. 2000, Nelson and Cox 2000).

Each olfactory neuron possesses in one end, 5 to 20 cilia (0.1 to 0.2 micrometer [μm] diameter) projecting down out of the olfactory epithelium into a mucous layer, which is about 60 microns thick (Figure 5.1). The cilia have specific receptors known as odorant receptors (OR), where the odorant molecules interact and sensory transduction, the generation of electrical signal/action potential, occurs, beginning the process of odor perception. Each OR expresses only one OR gene, and until very recently, it was mostly reported by the scientific community the existence of about 1,000 different types of mammalian OR (Buck and Axel 1991, Ressler et al. 1993, Mombaerts et al. 1996, Kandel et al. 2000, Firestein 2001, Glusman et al. 2001, Yoshihiro et al. 2001, Young and Trask 2002, Nimura and Nei 2003, Man et al. 2004). In fact, Glusman and others (2001) estimated the existence of over 900 human OR genes and pseudogenes, but recent results reported by Malnic and others (2004) argue against the proposed number. The latter authors proposed the existence of 636 OR genes, 339 of which are intact and,

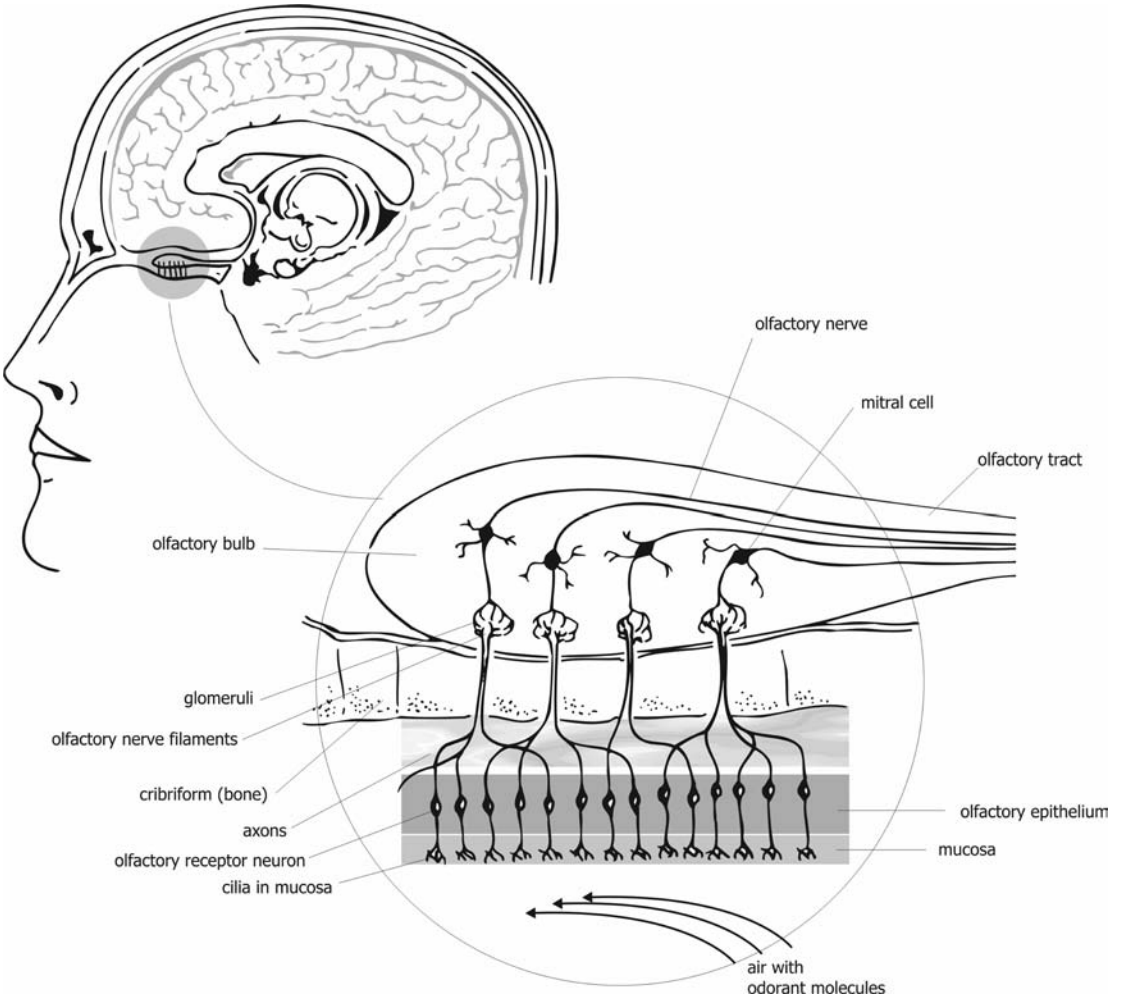

Figure 5.1. The human olfactory tract.

furthermore, likely to encode functional OR in the human nose. In agreement with Malnic and others (2004), the most recent publications are reporting the existence of roughly 350 functional human ORs (Friedrich, 2004).

The mucous secretion (Figure 5.1), which bathes the cilia, contains mucopolysaccharides, immunoglobulins, proteins such as lysozyme and several enzymes such as peptidases. It has been proposed that odorants first dissolve in the aqueous-lipid environment of the epithelium mucous and bind to proteins called odorant-binding proteins. These proteins facilitate the transfer of the odorant molecules to the ORs on the cilia of the olfactory sensory neurons. It has also been proposed that these proteins could contribute to the degradation of odorants that are already bound to the receptors, allowing other molecules to interact with them. Another proposed role for these proteins is to prevent excessive amounts of molecules from reaching the ORs. The mucous secretion is produced by supporting cells of the olfactory epithelium and by the Bowman's glands (Tegoni et al. 2000, Kandel et al. 2000, Nelson and Cox 2000, Leffingwell 2002, Young and Trask 2002).

Within the epithelium, each olfactory neuron possesses a single axon, which bundles together in groups of 10 to 100 to penetrate through the bone plate above the nasal cavity, reaching the olfactory bulbs, where they converge forming structures named glomerulus (Figure 5.1). The olfactory bulbs are paired structures located just above the nasal cavities, inside the cranium at the encephala base.

The human nasal epithelium contains about 10 million olfactory neurons spread out through four zones. Olfactory neurons expressing the same OR type, concentrate in one zone of the olfactory epithelium, but they are scattered throughout that zone, along with other neurons expressing different types of OR. However, axons from the same type of OR synapse onto a few glomeruli in the olfactory bulb (Figure 5.1). Thus, electrical signals generated by the same type of OR spread out throughout the olfactory epithelium, are summed up together at the glomeruli level. Physiologically, this convergence possibly increases the sensitivity of the signals sent to the brain. On the other hand, the dispersion of ORs throughout the olfactory epithelium helps to preserve one's sense of smell, if a portion of his/her epithelium is damaged by infection, accident, etc. It also increases the likelihood of odor compounds inhaled with whiffs of air interacting with the cognate receptors through their trajectory in the nasal cavity. Glomeruli that receive inputs from a specific type of OR are closely located in the olfactory bulb (Mombaerts et al. 1996, Kandel et al. 2000, Mombaerts 2001, Firestein 2001, Yoshihiro et al. 2001, Young and Trask 2002).

The glomerulus structures converge into the mitral and into the tufted cells (Figure 5.1). The axons of mitral and tufted cells project into the olfactory tract and then into the olfactory cortex without synapsing with the thalamus.

The primary olfactory cortex (Figure 5.2) is divided into five parts: (1) the anterior olfactory nucleus, which connects the two olfactory bulbs through a portion of the anterior commissure; (2) the olfactory tubercule; (3) the pyriform cortex, which is the main olfactory discrimination region; (4) the cortical nucleus of the amygdala; and (5) the entorhinal area, which in turn projects to the hippocampus (Kandel et al. 2000).

The pyriform cortex, also called the primary olfactory cortex (Figure 5.2), is an old and primitive structure, which is thought to be responsible for the perception and discrimination of odor quality. The sense of smell is unique among the sensory systems in that its central connections first project into the archicortex before reaching the thalamus and the neocortex. The fact that the sensory olfactory information reaches the primary olfactory cortex directly, without passing through the thalamus first, distinguishes the sense of smell from all the other sensory systems.

The primary olfactory cortex projects into the medial dorsal nucleus of the thalamus, hypothalamus, the nucleus basalis of Meynert, the hippocampus, the septal region, the substantia innominata, the mesencephalic reticular system and the orbitofrontal cortex, which are involved in the further processing of olfactory impulses (Figure 5.2). The amygdala, the hippocampus, and the hypothalamus are thought to be involved with the memories, and the motivational, the emotional and behavioral aspects of odors (Stockhorst and Pietrowsky 2004).

A number of studies indicate that the orbitofrontal cortex may be more important for higher olfactory functions than the "primary olfactory cortex" located in the medial part of the temporal lobe. However, although the orbitofrontal cortex may rank higher in the hierarchy of olfactory areas of the brain, it is absolutely dependent on the olfactory processing taking place in the medial temporal lobe areas (Gloor 1997).

ODORANT RECEPTORS

Figure 5.3 shows an illustration of an OR positioned in the cilia of an olfactory sensory receptor neuron. As can be seen, ORs are contained in the membranes of the olfactory neurons, which consist of fluid phospholipid bilayers of about 5 nm in thickness, and which include proteins and cholesterol in their structure, as well as phospholipids. These membranes are impermeable to large and charged ionic molecules.

Figure 5.3 also shows an OR at the membrane of an olfactory sensory receptor cell as proposed by Buck and Axel (1991). The portion of OR within the membrane consists of an amino acid sequence containing seven hydrophobic regions. This structure is known as a transmembranal 7-helical receptor protein, for obvious reasons. Four of the helices are nearly perpendicular to the membrane, and the other three are tilted, overlapping in the projection.

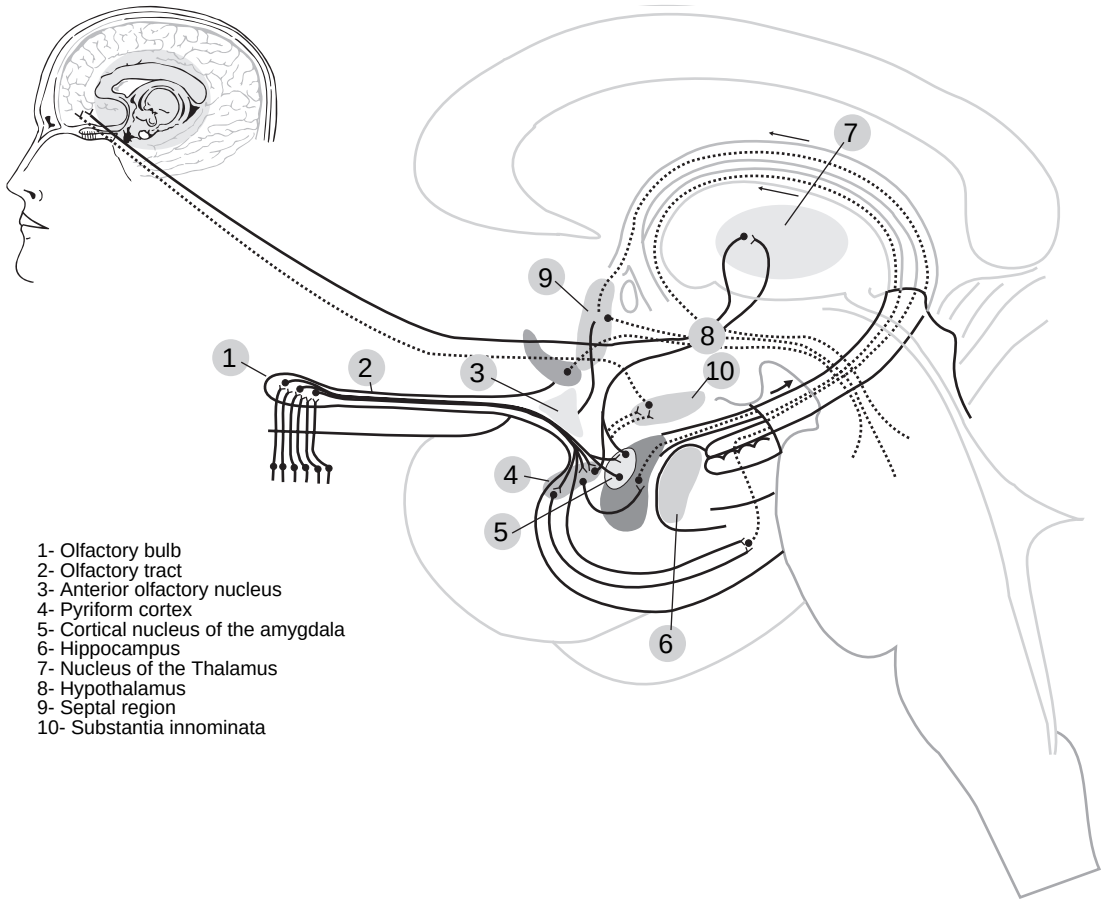

Figure 5.2. The human olfactory cortex and related brain areas.

Linking the seven-transmembrane domains (hydrophobic regions), there are three extracellular loops that alternate with three intracellular loops, as detailed in Figure 5.3 (Lancet and Ben-Arie 1993, Baldwin 1994, Mombaerts 1999, Firestein 2001, Mombaerts 2001, Man et al. 2004).

Among different ORs, the transmembranal receptor proteins show a similar general structure, with common patterns of amino acid sequence. In fact, this is the reason these transmembrane proteins are referred to as a family, since current classification defines the amino acid sequences sharing more than 40% identity as a family (Ngai et al. 1993, Lancet and Ben-Arie 1993). Nonetheless, there is some diversity among transmembrane receptor proteins in relation to their amino acid sequence. This diversity might provide different mechanisms of interaction between the receptor proteins and the odorants containing different functional groups, chemical affinity, size, shape, etc., explaining why humans can discriminate thousands of distinct odors and detect more than 10,000 structurally distinct odorous compounds. In fact, minor alterations in the molecular structure of an odorant are capable of significantly changing its odor quality (Buck and Axel 1991).

The family of olfactory proteins can be segmented into as many as 172 different subfamilies (Malnic et al. 2004), in which the divergences in amino acid sequences are restricted to a small number of residues (≅ 60% identity). Based on this,

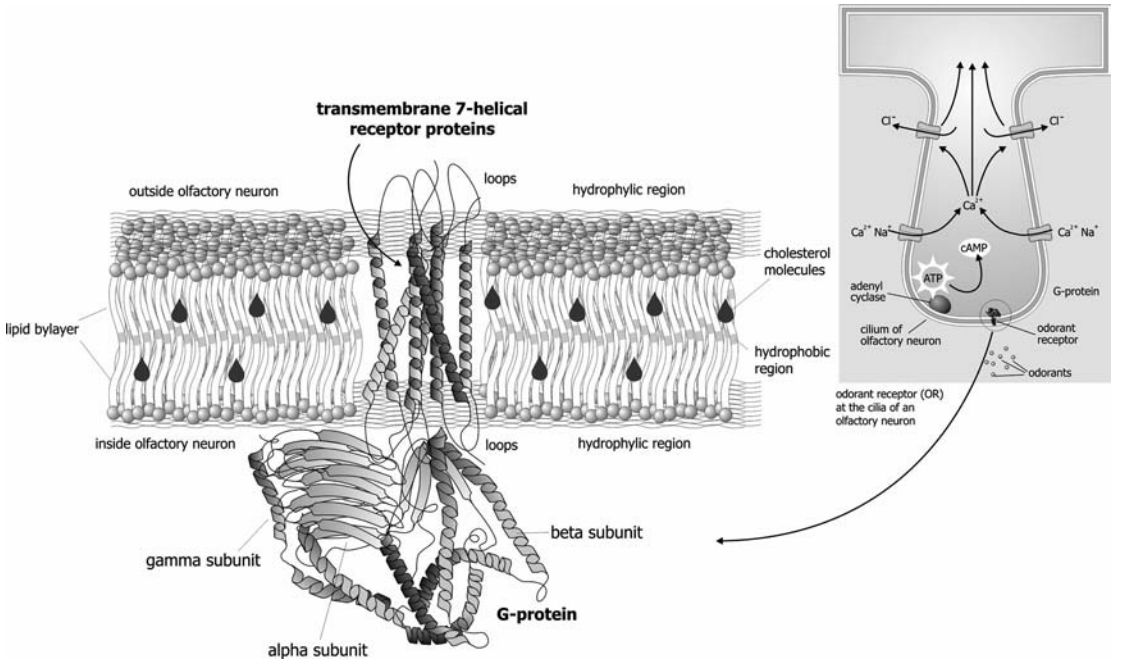

Figure 5.3. Odorant receptor (OR) at the cilia of an olfactory receptor cell with details of the seven hydrophobic regions of an OR inside the membrane of an olfactory receptor cell.

Buck and Axel (1991) and Malnic and others (2004) hypothesized that while members of different families must associate with odorants showing large differences in their molecular structures, the members of a subfamily can recognize odorants with related structures and identify more subtle variations among them such as differences in the substitute group in a benzene derivative molecule, i.e., benzene, toluene, xylene, or phenol.

The divergence in amino acid sequence among the olfactory transmembranes, as proposed by Buck and Axel (1991) and sustained by several authors, appears to concentrate in the third, fourth, and fifth transmembrane domains (Figure 5.3), suggesting that these segments are potential regions for direct contact and recognition of odorants. In three-dimensional models, these three alpha-helical barrels seem to face each other and form a pocket, which several scientists believe is the probable binding site for ligands (Baldwin 1994, Afshar et al. 1998, Floriano et al. 2000, Singer 2000, Firestein 2001, Vaidehi et al. 2002, Man et al. 2004). This subject will be discussed later in this chapter.

At its base (Figure 5.3), the transmembranal 7-helical receptor protein is coupled to a heterotrimetric GTP-binding protein, usually referred to as G-protein. This structure is located inside the receptor neuron cell and is called G-protein because it binds guanosine triphosphate (GTP). G-proteins are comprised of three subunits: an alpha, considered the active portion, and beta and gamma subunits. At rest, the alpha unit binds GDP (guanosine diphosphate).

It is believed that when an odorant molecule binds to an odorant receptor protein as shown in Figure 5.3, it possibly causes the transmembrane receptor protein to change shape and trigger the G-protein. The GTP then replaces the GDP in the alpha subunit, causing its dissociation from the beta and gamma subunits, and to associate with the enzyme adenylyl cyclase, activating it. Once activated, the enzyme breaks adenosine triphosphate (ATP) down into cyclic adenosine monophosphate (cAMP), which causes channels in the membrane of the olfactory neuron to open, allowing the flow of extracellular inorganic ions (Ca^{++}) to enter the cell. The influx of Ca^{++} activates the chloride channels causing them to

open and the Cl^- ions leave the cell through these channels. One activated receptor activates in turn tens of G-proteins, each of which will activate a cyclase molecule capable of producing thousands of cAMP per second. Three cAMP molecules are required to open a channel, but hundreds of thousands of ions cross the membrane through just one open channel. These occurrences cause the membrane charge to change from approximately 65 millivolts (mV) to approximately −35 mV, generating an action potential (electrical signal) that represents the transduction (Pace et al. 1985, Sklar et al. 1986, Firestein 2001). These signals travel down to the receptor axon, and from there, to the brain, as described earlier.

As intracellular calcium increases during the odor response, it decreases the sensitivity of the ion channels to cAMP, leading to odor adaptation and requirements for stronger odor stimulus to produce enough cAMP to open the ion channels (Liu et al. 1994, Firestein 2001). Additional mechanisms to explain the olfactory adaptation to odorants appear to involve both the existence of a protein (regulator of G-protein signaling) that decreases the adenylyl cyclase activity (Sinnarajah et al. 2001), and a kinase, that phosphorylates activated receptors desensitizing them (Dawson et al. 1993).

Recently it has been reported that signal transduction occurs in certain mammals involving IP3 (inositol 1,4,5-trisphosphate), cyclic guanosine monophosphate (GMP) and carbon monoxide, but the mechanisms associated with these events are only beginning to be explored.

PREDICTIONS OF ODORANTS' BINDING SITES AND THREE-DIMENSIONAL STRUCTURES

Since Buck and Axel (1991) sequenced OR proteins and reported that the transmembrane helices 3 to 6 (TM3 to TM6) (Figure 5.3) showed the highest variability and, as a consequence, they were good candidates to participate in odorant binding, several studies have been conducted with the aim of predicting odorants' binding sites and their three-dimensional (3-D) structures.

The most recent work of Man and others (2004) reported 22 amino acids positioned on TMs 2 to 7 and on the second extra-cellular loop (EL2) (Figure 5.3), which may play a major role as odorant binding sites of the OR protein super family. Of these 22 positions, one was at TM2, seven at TM3, one at TM4, four at TM5, two at TM6, three at TM 7, and two at EL2. According to the authors, the TM positions were all clustered around a pocket-shaped region and were all located in the extra-cellular two-thirds of TM helices 2 to 7, where ligands are known to be bound in other G-protein-coupled receptors, similar to previous reports of Baldwin (1994) and Pilpel and Lancet (1999). All the 22 amino acid residues predicted by Man and others (2004) as the binding sites of ORs, have been previously proposed by Afshar and others (1998), Floriano and others (2000), Singer (2000), and Vaidehi and others (2002).

Besides finding odorant binding sites, several studies have focused on predicting the 3-D structure of ORs. These studies usually employ a sequence of software programs that estimate not only the OR 3-D structure, but also, the location and affinities of the binding sites. Using such an approach, Floriano and others (2004) predicted the binding sites, affinities, and 3-D structures of six mouse olfactory receptors involved in the detection of 6 aliphatic alcohols (C4–C9), 12 acids (from butyric to azelaic acid), and 6 bromo acids. The binding sites of odorants on all six ORs were located between TM3 and TM6, positioned 10Å below the extra-cellular loops of the helical barrel. Two ORs (S18, S19) recognized both acids and alcohols while one OR (S46) recognized only acids. The differences among them were found at position TM3–9. In S19 and S18, it was occupied by phenylalanine and in S46 by threonine. Several amino acids patterns associated with the recognition of short aliphatic alcohols and monoacids were proposed in this research. Vaidehi and others (2002) and Hall and others (2004) developed similar studies, in addition to the authors already cited in this section.

THE HUMAN OLFACTORY CODE FOR ODOR DISCRIMINATION

In 1999, Linda Buck's group—2004 Nobel Prize winners—conducted an enlightening study to analyze the OR ligand specificities for a series of aliphatic odorants with related structures but varied odors (Malnic et al. 1999). Twenty-four odorants were studied: six aliphatic alcohols—butanol, pentanol, hexanol, heptanol, octanol, and nonanol—and the corresponding carboxylic acids, bromocarboxylic acids, and dicarboxylic acids. The authors

verified that a single OR could recognize multiple odorants. On average, each OR recognized four test odorants. The length of the chain appeared to be important for OR recognition; for example, five specific ORs only recognized odorants with seven, eight, or nine carbon atoms, while one OR only recognized C5–C6 odorants. In addition aliphatic alcohols of increasing carbon chain length were recognized by increasing numbers of ORs. This was an interesting finding, since, as pointed out by Malnic and others (1999), the detection threshold for aliphatic alcohols decreases with increasing carbon length (Cain, 1988). Thus they pointed out that the larger the size or complexity of an odor code, the higher the cumulative intensity of signals transmitted to the olfactory cortex, and the easier the odorant's olfactory detection.

Malnic and others (1999) also reported that the functional groups seemed to influence OR recognition. Some ORs recognized only one of the four classes of odorants; others recognized odorants from two or three classes, but no OR recognized odorants from all four classes. Comparisons of ORs that recognized acids and alcohols with the same carbon chain showed that though some ORs recognized both classes, invariably different classes were recognized by different combinations of OR. The dicarboxylic acids were recognized by fewer ORs than the remaining classes of odorant.

Overall, one of the most interesting findings of this study was perhaps the fact that different odorants were recognized by different combinations of OR. A number of odorants were recognized by overlapping but never by identical sets of OR.

In addition, the results obtained by Malnic and others (1999) suggested that changes in odorant concentrations could also result in a change in the ORs code. This supports the fact that for some odorants, changes in concentration imply differences in odor quality.

Based on all the above findings, Linda Buck's group proposed that: (i) each OR recognizes multiple odorants and, in turn, different odorants are recognized by several ORs, but each odorant is recognized by a unique combination of Ors, and (ii) the human olfactory system possibly uses a combinatorial coding scheme to discriminate odorants, by "reading" the combinational responses of the ORs triggered by the odorant molecules in the olfactory epithelium. Considering the hundreds of OR genes present in the human genome, this combinatory receptor coding allows for the discrimination of a very large set of distinct odorants by the human olfactory system (Malnic et al. 1999).

Recent work from Linda Buck's group (Malnic et al. 2004) continues to support the idea that ORs that are $\geq$60% identical, as occurs in ORs subfamilies, can recognize odorants with related structures, allowing for the fine discrimination of odorants with highly related structures. The findings of Kajiya and others (2001) also support a model in which each OR subfamily recognizes a particular class of odorant structures or structural features.

MOLECULAR THEORIES OF OLFACTION

The relationship between a volatile molecular feature and its odor quality has always interested flavor chemists, physiologists, and several other groups of scientists. In the early 1950s, Amoore (1952, 1963a, 1963b) proposed the existence of seven primary odors: camphor, musk, floral, peppermint, ether, pungent, and putrid. According to Amoore's stereochemical theory, the volatile molecules showed seven general shapes, which could be recognized by seven correspondingly shaped olfactory receptors, generating each of the seven cited odor qualities. This theory was very appealing and stimulated several debates, however, solid evidence to support it never appeared and it was soon dropped.

Currently, the molecular basis of odor remains unclear. Nonetheless, several studies relating odorants' structural properties with OR activation have been developed. Using 70 different odorants to study the specificity of mouse OR912–93, Gaillard and others (2002) verified that this receptor was only activated by ketones and aldehydes with carbon chain length $\geq$ 4, with preferential activation for ketones with the carbonyl group located in position C2 or C3.

Bieri and others (2004) studied the activation profile of rat ORs to sandalwood oil, to three synthetic sandalwood odorants showing an electron-rich structural feature similar to that of sandalwood oil and to one sandalwood odorant with a slightly different structure. Two control odorants were comparatively tested: octanal and 5-alpha-androst-16-3n-ol (urine-like odor). Sixteen types of OR were activated by the structurally similar sandalwood odorants, natural

and synthetic. Each sandalwood odorant activated a particular set of ORs generating a specific "fingerprint" of that odorant. None of the 16 above-mentioned Ors responded to the controls or to the structurally distinct sandalwood odorant. This was the first study showing that an important class of perfume compounds slightly different in their molecular structures, activated specific ORs. It supports the existence of a relation between a molecule's structure and its odor quality. Previous studies conducted by Katoh and others (1993), Mori and Yoshihara (1995), and Mori and Shepherd (1994) also supported the assumption that both the stereochemical structure and the type and position of the attached functional(s) group(s) of the odorant might explain the molecule odor.

Recently, Turin (1996), based on the earlier postulations of Dyson (1938) and Wright (1977), proposed that the molecular feature related to odor quality was the molecule's vibrational spectra rather than its structure or shape. For Turin (1996), each OR operates as a spectrometer, which detects a single well-defined energy E that he expresses in terms of wave number (1eV = 23.06 kcal/mole = 8,066 cm^{-1}) and is liberated when a molecule with a particular vibrational energy E interacts with it. Thus each OR responds to one or a few vibrational bands ranging from 0 to 4,000 cm^{-1}, explaining why each OR responds to one or a few odorants. Evidence supporting the vibrational spectra theory comes from the results of an experiment conducted by Firestein and others (1993) where salamander olfactory neurons were exposed to three odorants: isoamyl acetate, cineole, and acetophenone. Some cells responded only to cineole, others to both acetophenone and isoamyl acetate, and others to all three odorants. After calculating each odorant's vibrational spectra in the range from 0 to 4,000 cm^{-1}, Turin (1996) proposed that the ORs responding to cineole were tuned to the band of 1,200 cm^{-1}, which he found to be absent in the remaining odorants. The ORs responding to all three molecules, were tuned to the band 1,000 cm^{-1} present in all three compounds, and finally, the ORs responding to acetophenone and isoamyl were tuned to the band of 1,800 cm^{-1}, associated to the C=O group present only in these two molecules.

One very interesting approach made by Turin (1996) to support the vibrational spectra theory was his explanation of how a mixture of guaiacol (phenolic-like) and ethylbenzaldehyde (bitter almond-like) produced a vanilla-like odor. Although each compound possesses different predicted convolved spectra, the sum of the spectra of guaiacol and benzaldehyde is very similar to the spectra of vanillin.

According to Turin's analysis, the vibrational spectra theory accounts for several occurrences relating to molecules and their odors: (1) it explains why several structurally distinct odorants such as cedramber, karanal, Jeger's ketal, and timberol, possess very similar ambergris-like odors—the predicted convolved spectra of the compounds are very similar; (2) it also explains why very structurally similar molecules such as 2-undecanone and 6-undecanone possess different odors—their predicted convolved spectra show large differences at bands ≅ 550 cm^{-1}, 650 cm^{-1}, 1,000 cm^{-1}, and 1,200 cm^{-1}; and (3) it accounts for odor differences between enantiomers such as S-carvone (mint-like) and R-carvone (caraway-like)—when the molecule binds to the OR, the carbonyl group of S-carvone is unfavorably arranged, so the group is detected less intensely or it is not detected at all. Several other intriguing occurrences among molecules and their odors were explained by Turin (1996) using his vibrational spectra theory.

To provide convincing evidence for the vibrational spectra theory of olfaction, Haffenden and others (2001) generated three isotopes with identical molecular structures but different vibrational spectra from benzaldehyde, and tested the sensory and spectral differences between benzaldehyde and each of the three isotopes: $^{13}C_6$(ring)-benzaldehyde, ^{13}CHO-benzaldehyde, and benzaldehyde-d_6. Molecular modeling studies confirmed that the shape of the molecule was retained in each isotope and its volume increased less than 1%. Sensory analyses indicated that benzaldehyde-d_6, the only isotope to significantly differ ($p=0.002$) from benzaldehyde, was the molecule that experienced the most drastic shift in absorption frequencies of the aromatic and aldehydic C-H stretching bands. The smallest shifts occurred for the ^{13}CHO-benzaldehyde, which failed to show significant odor differences ($p = 0.05$) in relation to benzaldehyde. These results strengthen the support for the vibrational spectra theory.

Contradicting Turin's predictions, sensory studies conducted by Keller and Vosshal (2004) failed to corroborate that the smell of a mixture of guaiacol and benzaldehyde produces a vanilla-like odor not found in its individual components. Additionally,

Keller and Vosshal (2004) did not confirm that isotopes with identical molecular structures but different vibrational spectra, such as acetophenone and completely deuterated acetophenone, possess different odors, as found by Haffenden and others (2001) for deuterated and regular benzaldehyde. Keller and Vosshal (2004) demonstrated skepticism regarding Turin's vibrational theory and concluded that alone, it cannot explain the relationship between a molecule feature and its odor quality.

Despite all the advances relating to molecular features and OR activation, one must remember that what ultimately counts from a coding perspective, is how the human brain decodes OR responses. In this sense, studies involving odorants and brain imaging are of fundamental importance and will certainly dominate the olfaction research field in the next decade.

NEUROIMAGING AND NEUROPHYSIOLOGICAL STUDIES OF OLFACTORY EVENTS

Knowledge of how the human brain processes the signals generated by the olfactory receptors (OR) improved greatly after the advances made in neuroimaging and neurophysiological techniques in the last decade. Nowadays two classes of noninvasive technique are used in brain research involving olfaction: (i) those that measure electromagnetic events, such as electroencephalography (EEG) and magnetoencephalography (MEG), and (ii) those that measure changes in the regional cerebral blood flow (RCBF) in order to estimate neural activity in the brain, such as functional magnetic resonance imaging (fMRI) (Royet and Plailly 2004).

Measurement of brain activity evoked by olfactory events using EEG can be made with extra-cellular electrodes that sense action potentials in brain neurons. Extra-cellular recording detects the synchronized activity of large numbers of neurons; such signals are called field potentials. EEG represents a set of field potentials as recorded by multiple electrodes on the surface of an individual scalp. Thus the electrical activity of EEG is an attenuated measurement of the extra-cellular current flow from the summated activity of many neurons close to the EEG electrode (Kandel et al. 2000).

The set of locations for electrodes placed on the scalp is called a montage. It may be a referential montage (also called monopolar montage) in which each electrode records the electrical activity at a site (active electrode) relative to a distant site ("indifferent" or referential electrode), or a bipolar montage, where pairs of electrodes are interconnected in line and thus measure the potential difference between them (Kandel et al. 2000). It must be understood that EEG does not measure the "absolute" fluctuation of the electrical activity of the brain. It always measures the difference in voltage between two points (two electrodes), and it is therefore always a relative measure. For example, if, in a given situation, two electrodes (A and B) have the same potential, there will be no activity in the channel recording the EEG between A and B, even though electrical activity is present in both electrodes.

Scalp EEG recordings have excellent temporal resolution (a few milliseconds) but poor spatial resolution. The electrodes reflect the activity of a large number of neurons close to the skull, making this technique unsuitable to monitor the activity of small olfactory areas located deep in the brain such as the pyriform cortex, enthorinal cortex, and hippocampus, which are related to odor discrimination and memory (Kandel et al. 2000, Royet and Plailly 2004).

A normal human EEG shows activity in the range of 1–30 Hz, which for brain observational purposes, is divided into the following groups: alpha (8–13 hertz [Hz]), beta (13–30 Hz), delta (0.5–4 Hz), and theta (4–7 Hz). Moncrieff (1962) was one of the first researchers to report a decrease in alpha activity when subjects were presented with several odorants. Measurements of EEG changes in the frontal and temporal regions of the brain associated with hedonic responses to olfactory stimuli (Kobal et al. 1992, Brauchli et al. 1995, Roscher et al. 1998, Kline et al. 2000, Owen and Patterson 2002) and to the subjects' age, gender, and health conditions (Aufferman et al. 1993, Murphy et al. 1994, Evans et al. 1995, Pause et al. 1996) appear to be the main focus of EEG olfactory research.

Even though EEG shows reasonable test-retest reliability for measuring chemosensory events, with correlations ranging between 0.40 and 0.75 (Welge-Lussen et al. 2003), the use of EEG in olfactory research is limited. It mostly informs us if the brain

processes odors differently or not, but it cannot satisfactorily identify which region of the brain is associated with which odor-event (Lorig 2000). This is one of the main reasons why neuroimaging techniques such as fMRI are rapidly gaining enormous popularity among brain researchers.

In fMRI, the assessment of the functional status of different regions in the brain occurs by measuring changes in the blood oxygen level dependent signal. fMRI is noninvasive and does not involve radiation, thus subjects can be scanned several times, improving the data analyses. Additionally, it is less expensive, simpler, quicker to perform, and usually more available than other neuroimaging techniques, such as positron emission tomography (PET) (Zald and Pardo 2000). Measurements generated by fMRI have also proved to be very reliable for quantitative analyses.

Studies by Levy and others (1997) involving fMRI and olfaction showed quantitative brain activation due to subjects' olfactory stimulation. This study was one of the earliest in the area and involved 17 normal subjects from 22 to 41 years old. The stimuli consisted of amyl acetate (banana-like), L-menthone (peppermint-like) and pyridine (very pungent). The authors reported uniform activation notably in the orbitofrontal and entorhinal cortex and cingulate gyrus. Activation was also present, but less intense, in the hippocampal region, amygdaloid complex and pyriform cortex (Figure 5.2). Gender differences were quantitatively detected with women showing lower responsiveness than men to all three stimuli in all afore-mentioned brain sections. Nonetheless, no differences in response localization were observed between men and women. Kobal and Kettenman (2000) confirmed some of these findings by relating human brain activation to odorous stimulation using fMRI. They concluded that fMRI allows the identification not only of the primary areas involved in olfactory processing, but also the secondary and tertiary areas.

The chemosensory systems are distinct from the other systems in the sense that the intensity of the stimulus can be dissociated from the stimulus valence (pleasantness). Anderson and others (2003) took advantage of this and analyzed the fMRI responses of 16 subjects to the stimulation by four stimuli: citral (pleasant lemon-like odor) at low and high concentrations and valeric acid (unpleasant sweaty/rancid odor-like) at low and high concentrations. The authors observed that amygdala activation (Figure 5.2) was associated with odor intensity but not valence. The amygdala pattern of activation to low concentrations of valeric acid (still reported as unpleasant) was lower than that generated by high concentrations of citral (still a pleasant odor). By contrast, the highest amygdala activation was observed for both high-intensity stimuli: the unpleasant (valeric acid) and the pleasant (citral) ones. Assessing the orbitofrontal cortex, the authors verified that activation of the medial orbifrontal gyrus was greater for pleasant than for unpleasant odors at their highest and lowest concentrations. Comparisons of regions of interest (ROI) and activation profiles with clean air confirmed all cited results.

Neuroimaging of human responses to odorants may provide useful information not only for rapid advances regarding OR signal processing in the brain, but also to assess areas in the brain related to pleasantness of aroma and flavor (Araujo et al. 2003, Rolls et al. 2003), odor-memory (Herz et al. 2004), hunger and satisfaction (Tataranni and DelParigi 2003), and between subject differences due to age, gender, and health conditions (Kareken et al. 2003, Ferdon and Murphy 2003). However, brain researchers must be aware that the patterns of activation of an individual's cortical areas to different odorants are somewhat variable. Thus, if the experimental design fails to identify and control all of the significant effects, the effect of the main variable might erroneously be confounded with unknown factors, leading to incorrect conclusions. For example:

1. Odorants can stimulate three different systems, depending upon the nature of the molecule: the olfactory, the trigeminal, and the vomeronasal systems. For example, vanillin, a unimodal odorant, was found to activate the amygdala and pyriform cortex on both sides of the brain (Figure 5.2), whereas acetone, a bimodal odorant, only activated a minor portion of these regions, showing an accentuated stimulation of the areas also activated by painful stimuli (Yousem et al. 1997, Savic et al. 2000, Savic 2002).
2. Odorants have the unique ability to elicit associative responses related to memory and to hedonic and emotional feelings, as well as judgments of intensity and familiarity, among others.

Different odorants elicit different associative responses. For instance, unpleasant odors activate the left orbitofrontal cortex and the left amygdala significantly more than pleasant odors (Zald and Pardo 1997, Royet et al. 1999). Depending on the task performed by the subject, such as reporting odor intensity, quality, or recognition, different regions of the brain are activated (Savic et al. 2000). So, brain-imaging studies associated with odors must be rigorously accompanied by knowledge of the odorant and control measures: the nature of the odorant, the associative responses it provokes in the tested subjects, and all the tasks the individuals perform.

On the other hand, in studies involving odorants, it is well known that the human perception, memory, behavior, acuity, and discrimination toward the same odor stimuli, and possibly brain activated patterns, show great variability among subjects, depending on age (Murphy 1983, Doty et al. 1984, Stevens and Cain 1985, Evans et al. 1995, Kaneda et al. 2000, Choudhury et al. 2003) and gender (Doty et al. 1984, Choudhury et al. 2003). Variation between subjects is especially present in the evaluation of a large set of odorants using a continuous-flow olfactometer (Da Silva et al. 1994, Garruti et al. 2003). As a consequence, studies associating an individual's brain activation with olfactory stimulation must concomitantly conduct a series of sensory tests in order to assess each subject's capabilities toward odor detection, identification, discrimination, recognition, and memory (Doty et al. 1995, Martzke et al. 1997, Kobal et al. 2000), to guarantee independence among experimental variables.

Finally, airflow rates (as occur during sniffing) may well obscure the subject fMRI responses to the odorants (Sobel et al. 1998). Pulsatile and gross movement artifacts can also make it difficult to image the brain, especially in olfactory studies, due to their link with respiration. Thus, while the application of fMRI to olfactory studies may well generate new exciting information, a research group applying this technique is first challenged to overcome the cited difficulties by the careful development of suitable imaging methodology (Cerf-Ducastel and Murphy 2004).

Keywords: human olfaction, olfaction physiology, olfactory receptor, odorant receptor, olfactory code, theories of olfaction, odor discrimination.

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6
Sensory Methodology of Muscle Foods

Patti C. Coggins

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INTRODUCTION TO SENSORY EVALUATION

Sensory testing has been defined as predominantly evaluating the attributes of any consumed product (Meilgaard et al. 1991). Measuring the sensory properties of products and being able to determine the importance of them is a major accomplishment for sensory evaluation. Food companies exploiting this knowledge and applying it to their in-house quality maintenance and/or marketing program are utilizing a valuable research tool that will ultimately enhance overall perception of the products produced. The information that applied sensory evaluation provides is invaluable to a company. Without complete product acceptance from the end consumer, a company could encounter financial difficulties that could otherwise be avoided.

There has long been a need for a more detailed approach to sensory evaluation practices within the corporate environment of muscle foods companies. Muscle foods often fall fate to a detrimental perception by the American consumer who demands fresh, high quality products for consumption. It can be said that the muscle foods sector of the food industry has a greatly reduced quality acceptance range than other natural foods. The typical American consumer will not accept a muscle food that is not "perfect" in their opinion. This aspect makes determination of quality and acceptance sometimes difficult to decipher due to biases of the consumer as well as inherent differences within the muscle food product.

SENSORY ATTRIBUTES

According to Civille, we are apt to perceive sensory characteristics of a food item in the following order: appearance, odor/aroma/fragrance, consistency and texture, and flavor. However, most of these overlap, and the subject will have a simultaneous sensory impression. Without proper training and experience in sensory methodology aspects, the subject will find it more difficult to distinguish between each attribute (Meilgaard et al. 1991). Sensory evaluation, as well as the consumer, has matured in the last decade. With this in mind, it is important for the muscle foods industry to realize that consumers are much more sophisticated in their tastes, and ability to detect differences overall, as well as what they are willing to accept as a quality muscle food product. Sensory attributes are defined as appearance, odor/aroma/fragrance, flavor, texture, and specific feeling/chemical factors.

Appearance

Appearance is the visual properties of a product including size, shape, color, texture, gloss, transparency, cloudiness, and so on (Lawless and Heymann 1998). It is often the only attribute on which one can base a decision to purchase or consume a product. One becomes comfortable and trained in doing this; panelists will do the same in a test booth. General appearance characteristics include:

- Color involves both physical and psychological components and is commonly expressed in terms of hue, value, and chroma of the Munsell color system. The evenness of color as opposed to uneven or blotchy appearance is important. Deterioration of food is often accompanied by a color change.
- Size and shape include length, thickness, width, particle size, geometric shape, distribution of pieces; and size and shape as indication of defects.
- Surface texture includes the dullness or shininess of a surface, and the roughness versus evenness. Does the surface appear wet or dry, soft or hard, crisp or tough?
- Clarity includes the haze or opacity of transparent liquids or solids, and the presence or absence of particles of visible size (Seibert et al. 1981, McDougall 1988).
- Carbonation, for carbonated beverages, involves the degree of effervescence observed upon pouring (Meilgaard et al. 1991).

The effects of appearance on flavor perception are sometimes overruling. Human beings are a visually driven species. In many societies with mature culinary arts, the visual presentation of a food is as important as its flavor and texture characteristics (Lawless and Heymann 1998).

Visual appearance is a key factor that influences consumers when they are assessing the quality and palatability of meat and meat products. Certain colors influence food acceptance, although the color of the meat itself may be influenced by its moisture and fat content and also by the content of hemoprotein, particularly myoglobin and its relationship with the surrounding environment. The development of the characteristic color of fermented products is the result of the action of nitrite with myoglobin, producing the red color (Hui et al. 2001).

Odor/Aroma/Fragrance

Odor is the characteristic smell of a substance (Lawless and Heymann 1998). As defined by Civille (Meilgaard et al. 1991), the odor of a product is detected when its volatiles enter the nasal passage and are perceived by the olfactory system. Odor is sniffing of volatiles through the nose. Aroma is the odor of a food product most often associated with applied heat or thermal property, and fragrance is the odor of a perfume, a cosmetic, or natural plant extractives. The amount of volatiles that escape from a product is affected by the temperature and by the nature of the compounds. Volatility is also influenced by the condition of a surface (Meilgaard et al. 1991).

Many volatile compounds have been identified in fermented products belonging to the following classes: alkanes, alkenes, aldehydes, ketones, alcohols, aromatic hydrocarbons, carboxylic acids, esters, terpenes, sulfur compounds, furans, pyrazines, amines, and chloride compounds. Different pathways are responsible for the formation of these volatile compounds. However, the impact of an odor component on the total aroma depends on a number of factors, such as odor threshold, concentration, solubility in water or fat, and temperature as reported for dry-cured ham flavor (Hui et al. 2001).

Consistency and Texture

Consistency, texture, and viscosity are attributes that are perceived by sensors in the mouth other than taste and chemical feelings. Consistency can also be measured by sensory evaluation; in practice, some standardization is possible by the aid of consistometers. Texture is defined by Civille as the sensory manifestation of the structure or inner makeup of products in terms of their reaction to stress and tactile feel properties. Viscosity refers to the rate of flow of liquids under some force, such as gravity (Meilgaard et al. 1991).

The International Organization for Standardization (ISO) defines the texture of a food product as all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors (ISO, 1981). The texture of a product is perceived by the senses of sight (visual texture), touch (tactile texture), and sound (auditory texture) (Bourne 1982). In many products, one of these texture definitions may be used to describe a product's texture, whereas in others, all definitions could be applied. Ball and coworkers (1957) were pioneers in distinguishing between sight/visual and feel/tactile definitions of texture. Their studies on muscle foods greatly influenced how quality of meat is scientifically perceived today. One important discovery is how visual texture is a determination of product quality and freshness. Also, visual texture creates expectations of mouthfeel, which to the consumer is also an indicator of quality and/or freshness. Many surface characteristics of a food product not only affect the perceived appearance of the product but also the perception of the texture (Lawless and Heymann 1998). For example, the surface roughness of a biscuit can be assessed visually as well as orally; the viscosity of gravy can be assessed visually by pouring as well as orally. Tactile texture can be divided into oral tactile texture, mouthfeel characteristics, phase changes in the oral cavity, and the tactile texture perceived when manipulating by hand (Lawless and Heymann 1998). Oral tactile texture is often a measurement of size and shape, mouthfeel, phase change, as well as tensile strength. Oral tactile texture is an important measurement of the texture of a muscle food. For instance, if a piece of meat is mushy or soft, a negative perception could possibly form. Handheld tactile is most often used in evaluating certain consumer and/or personal care products. However, it is still of importance in the texture measurement of muscle foods, especially luncheon meats and meat sticks.

Flavor

Flavor, as an attribute of foods, has been defined as the perceptions resulting from stimulation of the sense ends that are grouped together at the entrance of the alimentary and respiratory tracts (Meilgaard et al. 1991). It is a complex group of sensations comprising of olfactory, taste, and other chemical sensations, such as irritation or chemical heat (Lawless and Heymann 1998). Flavor includes:

- The aromatics caused by volatile substances released in the mouth via the posterior nares
- The tastes caused by soluble substances in the mouth
- The chemical feeling factors, which stimulate nerve ends in the soft membranes of the buccal and nasal cavities (Meilgaard et al. 1991)

Meat flavor is derived from many different sources. Fresh uncooked meat has a metallic and blood-like flavor. Cooked meat, dependent upon the length of cooking cycle, and process, can vary in flavor as well. Fermented meats have yet another interesting flavor. The characteristic flavor of fermented sausages mainly originates from the breakdown of carbohydrates, lipids, and proteins through the action of microbial and endogenous meat enzymes. But other substances added to the sausage, such as salt and spices, should be taken into account because of their important contribution to flavor. Additionally, there are other pathways, such as autooxidation, that form flavor compounds without direct enzymatic participation (Hui et al. 2001).

The carbohydrate fermentation is responsible for the typical tangy or sour taste. The interactions between carbohydrate and protein metabolism during meat fermentation determine the rate of pH decline and flavor development. During carbohydrate fermentation, significant amounts of acetic acid, besides lactic acid, are generated. On the other hand, pH is partially neutralized during drying as the result of further ammonia and free amino acids generation. All of these compounds have an impact on flavor (Hui et al. 2001).

There are internal and external parameters that influence flavor. The internal parameters are chemical (added sugars or spices) or microbiological (starter cultures); external parameters are physical, such as the temperature and humidity during the process (Hui et al. 2001).

SENSORY EVALUATION PERFORMANCE

A sensory evaluation study involves several steps. Sensory studies are difficult and time consuming to perform. Following is a guide for sensory evaluation studies.

Project Objective

As with all research and any project, an objective must first be defined. Often many questions must be answered prior to the start of a project. If the project objective is not clearly defined, the appropriate test is unlikely to be used and the data gathered could possibly not answer the questions at hand about the product.

Test Objective

The test objective is defined once the project has been determined and an objective established. The type of sensory study is then defined at this point.

For example, the project objective may be to identify a less costly flavor-masking agent for the soy additive in a meat binder formulation that a meat company purchases from a seasoning supplier. Knowing this information and how it will be ultimately used is beneficial because it not only identifies the specific test objective but also alerts sensory evaluation to the potential of additional testing and to the fact that current production is the target (Stone and Sidel 1993).

Sample Screening

While determining the project and test objectives, all sensory properties of the samples to be tested should be examined. Biases inherent or otherwise can be determined at this point.

This issue precedes a decision that the test is warranted and most often arises during the initial stages of sensory evaluation. At this time, any samples with significant differences may be weeded out, because it would be redundant to test such samples.

Pretest screening also will enable sensory evaluation to clarify any special requirements related to preparation and serving and to identify any additional background material required as it relates to the product or the test (Stone and Sidel 1993).

Test Design

Designing the sensory methodology that will be utilized and the correct number of panelists, trained or untrained, must then be decided. The use of a properly designed score sheet is of extreme importance as to the outcome of the test results and data obtained.

Selection of the specific test method can be challenging. Industry personnel often are familiar with one particular sensory method and utilize that method for all of the work with which they are involved. This can be a "death sentence" to an internal sensory program or any other area of the company. In cases such as this, outsourcing is often necessary for objective opinions.

Sensory evaluation must first consider product and objective before reaching a decision about the most appropriate method for that problem. All discrimination methods have the same basic goal and all are essentially different forms of the two-sample choice situation. Consumer studies likewise have an end goal; define what the consumer likes or wants, for example.

Test method selection is the result of a series of probing questions and answers by the sensory professional. The focus is on test objective, type and availability of product, preparation procedures, product sensory characteristics, test method capabilities, availability of qualified subjects, and the use of the results. Such an approach leads to a much higher quality database, and also provides more reliable and valid product information (Stone and Sidel 1993).

Test Conducting

The evaluation should be performed in a controlled setting and a professional environment maintained at all times. Biases should always be kept to a minimum and control implemented at all times. Professionalism is a must in sensory evaluation studies.

Data Analysis

The necessary statistical programs should be used when the study is complete. The data should be analyzed according to the procedure decided upon at the test design stage. The data should be examined for the main treatment effect as well as other test variables.

Interpretation of Results

The original objectives of the project and test should enable the sensory analyst to review the analyzed results of the data and come to a conclusion about a range of variables. The analyst can then express the results in terms of the stated objectives and make recommendations for the product (Meilgaard et al. 1991).

STUDY CONTROL

When performing a sensory test, many variables must be controlled. These variables include test controls, product controls, and panel controls, to mention a few (Meilgaard et al. 1991).

Test Controls

The Booth or Divided Table Setting

Sensory testing booths are desirable, but not all companies have an internal sensory program or testing booths. If formal booths are not available, divided areas of a table or other secluded areas have been used with few problems. Booths may be arranged side by side, in an L shape, or with two sets of three to four booths facing each other across the serving area. Dividers between the booths should extend 18 inches above the countertops to provide privacy between panelists. A small stainless steel sink and water faucet can be included for rinsing. However, many scientists opt to exclude the sink and provide water in cups for drinking and an additional set of cups for rinsing and expectoration if necessary. Sinks can harbor odors if not properly maintained so thought must be given as to whether or not the utilization of sinks is of that importance. A signal system is frequently used to let the technicians know when the panelist is ready or has a question. This is usually accomplished using a form of a switch in each booth that will trigger a signal light (Meilgaard et al. 1991). However, there are other ways of administering contact between panelists and technician if necessary or if there are questions.

The booths and the surrounding area should be odor-free and easy to clean. The ventilation for this room and especially for the booths is critical. Meat companies tend to have a great deal of different odors and aromas filtering through the research and development area. Meat samples are cooked for various studies and the smoking of meats can infiltrate a facility. For these reasons, the entire booth, or testing area should have a slight positive pressure relative to other areas.

Facing each panelist seated at a booth will be a small door, referred to as a "sample pass-through door" allowing samples to be passed to the subject. The most commonly used door type is the "bread-box" design. The bread box is so constructed that when one side is open, the other is closed. This minimizes the likelihood of the subject having any view of the preparation area. Major disadvantages of the bread box include the increased amount of space required on the subject's side when the door is down, the height of the box relative to serving containers, and the inability to easily communicate with subjects (Stone and Sidel 1993).

Descriptive Evaluation and Training Area

A large conference style room is recommended for expert panels and descriptive evaluation. Several tables work well because they can be arranged as necessary by the size and objective of the group. Audiovisual equipment may be useful. Separate preparation facilities may also be used for reference samples. When working with muscle foods or meats in general, use of a descriptive panel is of most importance.

Preparation Area

The preparation area should permit preparation of all test samples of any kind that is anticipated. The laboratory should be equipped with the necessary equipment that will meet the needs of the evaluations performed.

General Design Factors

Color and lighting should be provided to give adequate viewing of the sample, but should also minimize

distractions. Walls should be an off-white color; the absence of hues of any color will prevent unwanted difference in appearance. Many panel booths have colored lighting (red, green, and/or blue) at low intensity obtained through the use of colored bulbs or special filters. These lights are used to hide individual differences between samples in difference tests to determine by taste which samples are identical (Meilgaard et al. 1991).

When performing studies with meats, the red lights are very useful at hiding small color differences that could be a result of smoking or fermentation procedure(s).

Product Controls

Equipment that is used to handle samples should be controlled in order to reduce biases being introduced to the product. An example is the limitation on the use of plastics as food storage containers and in some cases, food-serving containers. Controlled preparation of products requires careful regulation and monitoring of procedures used. All aspects of the test must be kept "constant" to ensure that the responses reflect only the variable being evaluated. Always keep in mind that the subjects are "looking" for something to be different with the test products.

TYPES OF TESTS

The tests discussed are for use with any food. However, the focus in this chapter is muscle foods and/or meat. It is important for the muscle foods expert to realize when a particular type of test should be utilized. The following sections provide a brief overview of the different types of tests available for use in sensory evaluation.

Discrimination Testing

There are many different methods for consumer testing. The different tests available are divided into two categories: discrimination testing and affective testing. Discrimination testing represents one of the most useful tools available for sensory analysis (Stone and Sidel 1993). This class of sensory testing compares two products or stimuli ultimately looking for differentiation on a sensory basis (Lawless and Heymann 1998). Methods include triangle tests, paired comparison, duo-trio, and other lesser-known tests. However, all the methods are intended to answer a seemingly simple question, "Are these products perceived as different?" (Stone and Sidel 1993).

Triangle Test

The triangle test is a discrimination test in which three samples are presented, two being the same and a third that is a different version of the variable under investigation. The judge's task is to choose the item that is most different from the other two (Lawless and Heymann 1998). Larmond (1982) recommended a simple but accurate method utilizing a statistical chart in which the task is to count the number of correct replies and refer to a chart for interpretation. This is probably the most widely used application for determination of statistically significant differences when using a difference test method.

This method should be used when the test objective is to determine whether a sensory difference exists between two products. It is especially useful in situations where treatment effects may have produced product changes, which cannot be characterized simply by one or two attributes. The triangle test is statistically more efficient than the paired comparison and duo-trio methods. However, it has limited use with products that involve sensory fatigue, carryover, or adaptation, and with subjects who find that testing three samples is too confusing. This method is effective when determining whether product differences result from a change in ingredients, processing, packaging, or storage and whether an overall difference exists, where no specific attribute can be identified as having been affected. It is also useful when selecting and monitoring panelists for the ability to discriminate given differences (Meilgaard et al. 1991).

Paired Comparison Tests

There are two sensory tests of this type. One is the directional paired comparison method (the two-alternative forced choice method) and the other is the difference paired comparison (simple difference test). With the former, the panelist is presented with two samples and asked to determine whether they differ in a specific attribute, such as sweetness, crispness, etc. With the latter method, the panelist is presented with two samples and asked simply

whether or not the samples differ (Lawless and Heymann 1998). If the sensory analyst knows that the two samples differ in a specific sensory characteristic, then the two-alternative forced choice method is used. It is always more efficient and powerful to use a directional paired comparison test specifying the sensory attribute in which the samples differ than to ask the panelists to indicate the different sample. However, if the sensory analyst does not know if or how the sensory characteristics of the product differ, then the simple difference test may be more useful (Lawless and Heymann 1998).

Duo-Trio Test

In this test, the panelist receives three samples simultaneously. Two of these are the same and the other is different. On the duplicate samples is marked "reference." The panelist is then asked to identify which sample corresponds or matches the reference sample. These tests allow the sensory analyst to determine if two samples are perceptibly different, but the analyst will not know in which attributes the samples differed.

A-Not-A Test

This test is essentially a sequential paired difference test or simple difference test. The panelist evaluates the first sample then it is removed. The panelist does the same with the second sample. The panelist is asked to determine whether the two samples were perceived to be the same or different. The results of this test, again, will tell the analyst whether the panelists could significantly discriminate between the samples when they are not presented simultaneously. This test is useful when the experimenter cannot make the two formulations have exactly the same color, shape, or size, yet these characteristics are not relevant to the objective of the study.

Affective Testing

Affective tests assess the acceptability of products or the relative preference among a set of products (Lawless and Heymann 1998). These tests usually follow discrimination and descriptive tests, which have reduced the number of product alternatives to some limited subset, and precedes larger scale testing done outside of research and development by others, such as marketing research (Stone and Sidel 1993). Affective testing includes preference tests, acceptance tests, and assessing individual attributes.

Preference Tests

If the project is specifically designed to compare one product directly against another in situations such as product improvement or parity with competition, then a preference test is indicated. The preference test forces a choice of one item over another or others. However, it does not indicate whether any of the products are liked or disliked. Preference tests can include paired preference test with two samples (one sample is chosen over another, A-B); ranked preference test with three or more samples (samples are ranked in a relative order, A-B-C-D); multiple paired preference test with three or more samples (a series of paired samples with all samples paired with all others, A-B, A-C, A-D, B-C, B-D, C-D); and multiple paired preference test with three or more samples (a series of paired samples with one or two select samples [e.g., control] paired with two or more [not paired with each other], A-C, A-D, A-E, B-C, B-D, B-E) (Meilgaard et al. 1991).

Acceptance Tests

When a product researcher needs to determine how well a product is liked by consumers, an acceptance test is the correct choice. The product is compared to a well-liked company product or that of a competitor, and a hedonic scale is used to indicate degrees of unacceptable to acceptable, or dislike to like (Meilgaard et al. 1991).

Attribute Assessment

As part of a consumer test, researchers may want to determine the reasons for any preference or rejection by asking additional questions about the sensory attributes (Meilgaard et al. 1991). Questions may be asked regarding how intense an attribute is or how well they like a specific characteristic.

Descriptive Analysis Techniques

Descriptive analyses are the most complex methods in sensory evaluation. These tests are useful when a

detailed description of the innate and added attributes of any given product is needed.

Descriptive analyses utilize a developed language for determination of a product attributes' description. There are three types of language: commonly used or "everyday," lexical, and scientific. Commonly used or "everyday" language is applied in daily conversations and varies in cultural aspects such as regions or countries. Lexical language is found in the dictionary and may also be used in common or "everyday" language conversations (Lawless and Heymann 1998). Scientific language is for technical work and is very specific and is formally applied (Civille and Lyon 1996).

Descriptive analyses require a training phase for the development of a descriptive language by the panelists. A unique scientific language is thereby created for the product of study. This scientific language can often be formed into a lexical language if the situation allows. The goal of descriptive analyses is to have all trained panelists use the same concepts developed to describe a given attribute of a product.

Descriptors developed for a product should be singular rather than combinations of several terms. Combination or holistic terms such as "thick," "slimey," "hard," and "dirty," are complex and should be avoided. Terms such as these are impossible to describe and mean different things to different panelists. Terms such as these should be assimilated into their primary, analytical/technical, or elemental components. Panelists can use suitable descriptors with reliability and repeatability.

There are several descriptive analyses techniques. These include the Flavor Profile® method trademarked to Arthur D. Little company; Quantitative Descriptive Analysis®, developed during the 1970s by Stone and Sidel; Texture Profile® method, created by scientists employed by General Foods during the 1960s; Sensory Spectrum® method, created by Civille; Generic Descriptive Analysis, in which techniques have been adapted to meet the needs of a particular situation; and Free Choice Profiling, created in the 1980s by British scientists.

Regardless of the method of descriptive analysis chosen for sensory evaluation studies of a given product, it is of greatest importance to ensure the development of a descriptive language to be as complete as possible. This ensures an excellent instrument available for description of the product in question and builds quality and reliability into the trained panel.

CONCLUSION

Sensory evaluation has proven to be invaluable to the muscle foods industry. All of the mentioned applications, procedures, and methods are exceptionally useful in the muscle foods area and should be utilized on a broader scale. Consumer demand drives this need, and the industry as a whole thrives on consumer perception. With this in mind, any company that does not utilize sensory evaluation techniques to their fullest extent in the evaluation of the food product(s) that it produces should expect failure of their product(s) in the marketplace sooner than later. Sensory evaluation is a tool that if used properly can solve problems before they arise. There is a depth of information that can be obtained from the proper utilization of sensory methodology.

The meat industry and muscle foods in general often have it somewhat more difficult than other industries and products in maintaining certain key attributes in the food (muscle). Sensory evaluation should not be overlooked because it is an invaluable tool in assisting in the control and monitoring of many of these key attributes.

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7
Objective Methods of Sensory Analysis

Jayden L. Montgomery

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INTRODUCTION

This chapter will review a number of objective measurements, or instrumental techniques, that can be used either in combination with other traditional sensory measurements to further explain differences or used to simulate traditional sensory techniques. Consumer preferences for meat quality from a sensory standpoint are influenced by appearance, texture (tenderness), flavor, and juiciness (Resurreccion 2003). The primary meat quality factors that can be measured currently are appearance, predominantly color and discoloration, aroma or odor, flavor, juiciness, and meat texture, which is primarily meat tenderness in nonprocessed meats. For each of the individual objective sensory measurements discussed, a brief overview of the technique will be discussed and each technique's relevance. The instrumental techniques discussed in this chapter are briefly summarized in tabular form with a reference in Table 7.1.

For most analytical measurement techniques of meat products, the primary factor that affects measurement accuracy is in obtaining a representative sample. Care should always be taken in selection of samples regardless of the means to be used for evaluating the sample. Sample selection and preparation requires standardized procedures that are repeatable and reproducible. Samples should always be handled in exactly the same manner to reduce variability and inaccuracy. The instrument being used for measurements should be calibrated by the manufacturer once a year when possible. When possible, instruments should be standardized and calibration/proper working order should be verified. It is important that when measurements are made that measurements be accurate and reproducible; for example, certified weights should be used to verify that scales are working properly. In general, good scientific methods should be employed at all times.

Table 7.1. Major instrumental techniques used for and in combination with sensory evaluation of meat and meat products.

Method/Measurement	Typical Test Product	Number of Samples	Citation
	Color Methods		
Hunter & CIE L, a, and b values	Fresh & Processed	2 or more	AMSA 1991
Saturation index (Chroma) and hue angle	Fresh & Processed	2 or more	AMSA 1991
Reflectance data to determine pigment %	Fresh & Processed	2 or more	Krzywicki 1979
Pigment extraction	Fresh & Processed	at least 1	AMSA 1991
TBARS (oxidation and peroxidation)	Fresh & Processed	at least 1	Tarladgis et al. 1960
	Odor and Aroma Methods		
GC	Fresh & Processed	at least 1	Sheldon et al. 1997
GC/MS	Fresh & Processed	at least 1	Vercellotti et al. 1987
HRGC/MS	Fresh & Processed	at least 1	Guth and Grosch 1994
GC/Sniffer port analysis	Fresh & Processed	at least 1	Bett and Grimm 1994
Electric Nose	Fresh & Processed	at least 1	Arnold and Senter 1998
TBARS (oxidation and peroxidation)	Fresh & Processed	at least 1	Tarladgis et al. 1960
	Flavor Methods		
GC	Fresh & Processed	at least 1	Sheldon et al. 1997
GC/MS	Fresh & Processed	at least 1	Vercellotti et al. 1987
HRGC/MS	Fresh & Processed	at least 1	Guth and Grosch 1994
GC/Sniffer port analysis	Fresh & Processed	at least 1	Bett and Grimm 1994
Electric Nose	Fresh & Processed	at least 1	Arnold and Senter 1998
TBARS (oxidation and peroxidation)	Fresh & Processed	at least 1	Tarladgis et al. 1960
HPLC	Fresh & Processed	at least 1	Bett and Grimm 1994
LC/MS	Fresh & Processed	at least 1	NA
	Juiciness Methods		
Drip loss	Fresh & Processed	at least 2	NA
Purge	Fresh & Processed	1	NA
Cook loss	Fresh & Processed	1	NA
WHC—Electron microscopy & X-ray diffraction	Fresh & Processed	at least 1	Diesbourg et al. 1988
WHC—filter paper press method	Fresh & Processed	at least 2	Wierbicki and Deatherage 1958
Muscle pH	Fresh & Processed	at least 1	NA

Table 7.1. Continued

Method/Measurement	Typical Test Product	Number of Samples	Citation
	Texture (Tenderness) Methods		
Warner-Bratzler Shear Force	Fresh & Processed	6–10	AMSA 1995
Slice Shear Force	Fresh	1	Shackelford et al. 1999
Star Probe (Puncture Probe)	Fresh & Processed	at least 2	Cain et al. 2003
Hardness, springiness, cohesiveness, gumminess, & chewiness	Processed	10	Bourne 1978
Sarcomere length	Fresh & Processed	25	Cross et al. 1981
Collagen content	Fresh & Processed	at least 1	Goll et al. 1963
SDS-Page	Whole Muscle & Processed	1	Laemmli 1970
SDS-Page & Western Blot	Whole Muscle & Processed	1	Huff-Lonergan et al. 1996
MFI (Myofibrillar Index)	Fresh	2	Culler et al. 1978
MFL (Myofibrillar Length)	Fresh	10	Heinze and Bruggemann 1994
Calpain & calpastatin enzyme activity	Fresh	1	Koohmaraie 1990, Geesink and Koohmaraie 1999

NA = Not Applicable

MEAT APPEARANCE (COLOR)

The appearance of fresh meat color strongly influences consumer quality assessment of the meat product (Chan and Decker 1994). Visual appraisal of meat products by consumers and sensory panels can lead to highly variable and sometimes nonreproducible results, which is why instrumental objective measurements can lead to much more accurate and reproducible results. However, areas within a given muscle can vary in color, discoloration, marbling amount, or connective tissue. When this occurs, it is important to determine the proportion of the surface each section represents and to measure each separately. When measuring meat appearance, it is important that samples are thick enough to be opaque and any backing used should be white rather than black (AMSA 1991). Additionally, the type of film or packaging that over wraps the sample can influence readings, thus each instrument should be standardized for each packaging used. Suggested sample preparation techniques for instrumental methodology techniques to appraise meat appearance are outlined in the American Meat Science Association (AMSA) Guidelines for Meat Color Evaluation (1991).

Colorimeter Data

Color of meat is due to a balance between oxymyoglobin oxidation and metmyoglobin reduction (Faustman and Cassens 1990, Chan and others 1998). The development of discoloration (typically a brown color or a darkening in color will significantly reduce consumer acceptability of meat products and especially fresh meat products) is due to the oxidation of deoxymyoglobin to metmyoglobin (Faustman and Cassens 1990). The two types of instrumental methodologies used to measure meat color are color reflectance or pigment extraction. The two reflectance scales typically used for meat products are the Hunter Lab values or Commission Internationale de l'Eclairage (CIE) values (known as L* a* b*). In addition, Munsell, tristimulus values, and reflectance at specific wavelengths all have been used to express color data. L and L* values are indicative of lean whiteness, a and a* values are indicative of lean redness (positive values), while b and b* values are indicative of lean yellowness (positive values). Currently, most researchers use the L*, a*, and b* values to document treatment effects on color while L, a, and b values were used in older

color data. Additional reflectance data collected include hue angle, a measurement where a vector radiates into the red-yellow quadrant, and Chroma (color saturation index).

Hue angle = arctangent (b*/a*) × [360°/(2 × 3.14)]
Chroma (Color Saturation Index) = $(a^{*2} + b^{*2})^{0.5}$
(Minolta 1993)

Reflectance values have been used to indicate color changes and to quantify myoglobin forms. The two major types of machines used to collect reflectance data are the Minolta chromameter (Osaka, Japan) or the HunterLab Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA; see Figure 7.1). Reflectance data measurements tend to be very precise and can indicate treatment differences in color prior to sensory panel differences or consumer panel measurements. For measurements of meat color factors, L-, a-, b-values, saturation index, and hue angle, the instrumental measurement guidelines explained by AMSA (1991), should be followed. Typically, illuminant C or D_{65} lighting conditions are used. Colorimeters should be calibrated prior to use each time by reading calibration plates wrapped in the same packaging materials as the meat samples (typically a white, black, and green calibration plate are used although a variety of other calibration plates such as red, brown, orange, or light purple may be used). Typical measurement aperture openings are approximately 0.5 cm^2 to several square centimeters for colorimeters with spectrophotometric capabilities (Swatland 1995). The CIE Lab and Hunter scales are essentially parallel with differences between the scales tending to occur due to variation in aperture diameter within and among instruments. Meat browning is seen in the CIE Lab system in the effect of change of C* (chroma; saturation index) and hue angle, h*.

Pigment Extraction

Extraction techniques give sharp peaks and better separation than reflectance measurements in indicating the quantity of myoglobin, but extraction procedures overestimate oxymyoglobin and metmyoglobin and underestimate deoxymyoglobin because of changes that occur during extraction and measurement (AMSA 1991). Obtaining a representative sample for extraction techniques is difficult because myoglobin forms vary at the meat surface compared to internal regions, thus, grinding an entire sample will lead to inaccurate evaluation values. Because extraction techniques do not prevent the conversion of one myoglobin form to another and provide no reliable information on pigment form stability (AMSA 1991, Krzywicki 1982), extraction procedures are mainly helpful in the determination of myoglobin pigment. There are a number of different extraction techniques that can be used to quantify meat pigments. Each use a thin (1- to 2-mm) slice of the sample at the surface. Methods include a cyanometmyoglobin derivative method in which samples are homogenized and filtered and activity determined by

Figure 7.1. Handheld colorimeters, Hunter and Minolta.

measuring absorbance (Hagler et al. 1979, Warriss 1979). A less toxic reagent method (Karlsson and Lundström 1990), and high performance liquid chromatography (HPLC) methods (Oellingrath et al. 1990) have also been described. The Hornsey method (Hornsey 1956) can be used in determining pigment quantities in cured meat products.

Pigment Estimation with Reflectance Data

Estimation of deoxymyoglobin, oxymyoglobin, and metmyoglobin pigment quantities are possible through reflectance data because two or more of the pigments have the same reflectance at 474, 525, 572, and 610 nanometer (nm) (see Figure 7.2). The oxidation of oxymyoglobin to metmyoglobin is accompanied by a loss of absorbance at 578 nm relative to absorbance at 542 nm (Krzywicki 1979). The reflectance data at specific wavelengths are converted to K/S values (K = absorbance coefficient; S = scattering coefficient) that then are put into equations requiring standard values to calculate the pigment quantities (1 to 100%) being calculated as explained by Judd and Wyszecki (1963) and modified by Krzywicki (1979).

When cured meat products are displayed in lighted cases, a tan to brownish tinge may develop. Color fading can be measured as decreases in a-values and increases in b-values. Because a- and b-values tend to be most affected in cured meats, hue angle and saturation index are typically more indicative of color changes than separate individual measurements. In cooked meats, degree of doneness can significantly affect reflectance measurements. Reflectance ratios and differences correlated well to degree of doneness scores (Flores et al. 1985). As degree of doneness increases, a-values and meat redness should decrease. However, Howe and others (1982) reported that hue angle was the most useful reflectance measurement in monitoring color changes in cooked pork samples. Because brown color is difficult to measure directly compared to the loss of redness in cooked samples, the percentage of

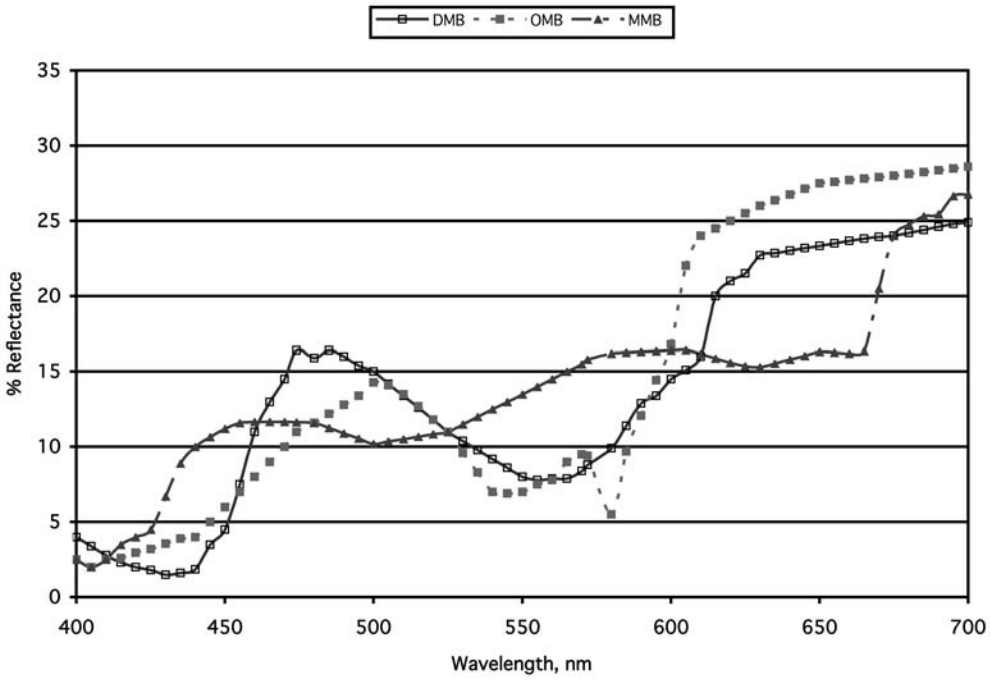

Figure 7.2. Reflectance spectra of deoxymyoglobin (DMB), oxymyoglobin (OMB), and metmyoglobin (MMB) modified from Snyder 1965 and AMSA 1991.

denatured pigment may be useful in color evaluations of cooked meat (AMSA 1991).

Lipid oxidation and pigment oxidation in skeletal muscle occurs initially at the membrane level (Whang et al. 1986). The thiobarbituric acid reactive substance (TBARS) method can be used as a measurement of meat oxidation (although predominantly lipid oxidation and peroxide formation). Because oxidation of deoxymyoglobin or oxymyoglobin to metmyoglobin lead to a brown color, TBARS measurement can be indicative of the total oxidation process. Additional instrumental color techniques that have been reported include an image processing system (Sony) that measures surface metmyoglobin (Demos et al. 1996) and other online color measurement systems that measure carcass color and marbling (Swatland 1995) and near-infrared reflectance (Leroy et al. 2003).

MEAT ODOR AND AROMA

Historically, qualitative assessment of meat odors was conducted by human panelists who were presented serial dilutions of air samples. However, use of sensory panels is labor intensive (requiring a number of panelists), time consuming, expensive, prone to errors, and generally problematic. Qualitative characterization was next instrumentally measured for specific organic compounds by analytical procedures to characterize aromas or odors. The major instrumental techniques used to measure meat odor include gas chromatography (GC), mass spectrometry (MS), the electric nose, and TBARS. For each of these methodologies, each procedure will vary by laboratory due to differences in equipment, techniques, columns, and the like. For each specific procedure, it is suggested that individual researchers reference the exact procedure being utilized in each of their own laboratories. Because odor greatly affects the senses of flavor, many of the processes and sample preparation for flavor analysis and odor/aroma analysis are similar if not the same. Each instrumental procedure listed typically involves the use of blanks and a range of reference standards either to produce a standard curve for reference and/or to verify calibration of equipment and proper working of the equipment. When chromatography techniques are implemented in which samples are run through the same machine and column for analysis, it is important that a clean up step occur between samples so that there is no carry-over affect between samples.

Gas Chromatography and Sniffer Port

The compounds responsible for odor development and perception must first be released from the muscle matrix and then transported to the olfactory receptors in the nose. The compounds generated in meat that contribute to odor and flavor are typically referred to as volatile compounds, and will contribute to aroma depending on their concentrations, odor thresholds, and interactions with other food components (Gianelli et al. 2003). Odor volatiles of meat from different species or from animals with different nutritional treatments typically are similar in quality but may vary quantitatively. A detailed GC analysis may result in hundreds of peaks (Gasser and Grosch 1988) and can be grouped into categories. One way is grouping by compound type while another approach is the use of statistical data-grouping techniques. Cluster analysis, discriminate analysis, principal component analysis, and factor analysis are all statistical methods that may be used (Bett and Grimm 1994) depending on study objectives.

The first step in chromatographic analysis of meat samples is the sample preparation for the collection of volatile compounds. Typically, heat, water, organic solvent, or gases are used to denature and remove odor volatiles. A shortcoming of all these processes is the potential for new odor/flavor compounds to be formed during sample preparation. In steam distillation, the volatiles are steam distilled from an aqueous meat sample, and a solvent may be used to capture the volatiles. The steam distillate is brought into contact with a nonpolar solvent and the odor/flavor components partition between the two phases; this leads to a rather indiscriminate and relatively large quantity of undesirable compounds being carried over (Bett and Grimm 1994). Solvent extraction is another sample preparation method in which volatiles are extracted using common solvents (hexane, methylene chloride, ethanol, methanol, diethylether, pentane, water, and mixtures of each).

Gas chromatography techniques are the most often used instrumental technique to identify the volatiles contributing to meat odor/aroma as well as flavor. Headspace gas analysis involves use of a gaseous phase above a sample in a sealed container (Sheldon et al. 1997). Dynamic headspace analysis continually removes the headspace gases by running a carrier gas through the sample and collecting on a trap or cryofocusing at the head of a capillary col-

umn (Bett and Grimm 1994). This is a very typical method for meat analysis, where a purge-trap apparatus is used in combination with a GC, because it is ideal for analysis of lighter more volatile compounds. Additionally, a "supercritical fluid extraction" method may be used in which carbon dioxide is used as the solvent and remains in neither a gaseous nor liquid phase. Determination of the sample preparation to be used should be based on the equipment available and the compounds of interest. The two types of columns used for GC analysis include a packed column or capillary column. Another method of determination that can be used with a GC is the use of a sniffer port. In sniffer port techniques, a portion of the sample on the GC column is routed through a port to be sniffed by a human subject for quantitative evaluation (Bett and Grimm 1994). The human nose at the sniffer port can be more sensitive than instrumental detectors.

Mass Spectrometry

Mass spectrometry is commonly used in combination with a GC to identify organic volatile compounds. The GC-MS combination with a purge and trap apparatus is widely used in odor analysis of meat samples to identify and quantify volatile compounds (Vercellotti et al. 1987). Also, high-resolution gas chromatography (HRGC) in combination with an MS has been used to identify small quantities of volatiles such as thiols (Guth and Grosch 1994, Specht and Baltes 1994, Kerscher and Grosch 1998).

Electric Nose

Digital aroma technology, or the electric nose, uses a semiconductor multisensorial system to detect odors. The electric nose system is designed to mimic biological olfactory functions of sensory panels by absorbing and desorbing the volatile compounds at the surface of sensors, which causes changes in electrical resistance (Arnold and Senter 1998). The electric nose functions to classify odors based on previous readings and information. The sensitivity and discriminating ability of the instrument improves as more data points are collected and processed (Arnold and Senter 1998). The electric nose has been used in the analysis of ham aroma (Otero et al. 2003) and for meat "warmed-over flavor" (O'Sullivan et al. 2003). To compare GC analysis and the electric nose, the GC separates headspace volatiles into volatile peaks while the electric nose integrates measurements of the total headspace compounds as they cross the array of sensors (Hodgins and Simmonds 1995, Arnold and Senter 1998).

Lipid Oxidation (TBARS)

The TBARS method in which samples are homogenized, centrifuged, and distilled and read at approximately 531–538 nm on a spectrophotometer is used in a wide variety of experiments as an indication of lipid oxidation and peroxidation formation through analysis for malondialdehyde (mg/kg of samples; Tarladgis et al. 1960).

Because numerous data can be collected on a number of volatile compounds, data interpretation is very important. Concerning which volatiles are of importance Ulrich and Grosch (1987) and Grosch (1990) presented a screening procedure for important volatile odor compounds called aroma extract dilution analysis. Specht and Baltes (1994) identified pleasant and unpleasant volatiles with high aroma values (Table 7.2) in cooked beef samples. The majority of compounds with high aroma values are responsible for fatty, sweet, or roasted aroma/flavor qualities that contribute to typical roasted meat (Specht and Baltes 1994). Odor thresholds for major meat aromas have also been reported which allow researchers to determine if the quantity of a specific volatile compound is contributing to the odor/aroma being evaluated (Sutherland and Ames 1996). Processing and cooking parameters will affect aroma such that different cooked odor/flavor compounds are based on different cooking methods. There are a number of different volatiles that typically occur in meat as either leading to a normal aroma or to off odors (see Table 7.2).

MEAT FLAVOR

Flavor as a sensory attribute of meat has been defined as the impressions perceived via the chemical senses from meat in the mouth (Meilgaard et al. 1991). The chemical senses include (1) the detection of the four basic mouth sensations on the tongue (salty, sweet, sour, and bitter); (2) the olfactory perceptions caused by aromatic volatile compounds released from the meat in the mouth; and (3) the chemical feeling factors in the mouth for example,

Table 7.2. Aroma and flavor compounds with high aroma/flavor profiles and sensory descriptions.

Compound	Sensory Description
1,8-cineole	Eucalyptus, mint
2-Acetyl-1-pyrroline	Roasted, sweet
2-Acetylthiazole	Roasted
2-Acetylthiophene	Sulphurous, sweet
2-Methyl-3-(methylthio)furan	Sulphurous
2-Methylfuran-3-thiol	Meaty, sweet, sulphurous
3-Acetyl-2-5-dimethylthiophene	Sulphurous
3-methyl butanoic acid	Rotten peas, excrement, cheese
5-Methylthiophene-2-carboxyaldehyde	Moldy, sulphurous
A deca-2, 4-dienal (not E,E)	Fatty
Allyl isothiocyanate	Sulfurous, sewer, cheese
Benzothiaxole	Pyridine, metallic
Benzylthiol	Sulphurous
benonol	Violets
bis(2-methyl-3-furyl)disulphide	Meaty
Butanoic acid	Fruity
Cinnamaldehyde	Cinnamon
Deca-2(E), 4(E)-dienal	Fatty, fried potato
Decan-2-one	Musty, fruity
Dimethyl sulfide	Onion, garlic
Dimethyl disulfide	Fermented, plastic, onion, garlic
Dimethyl trisulphide	Cabbage, sulphurous
Dodecan-2-one	Musty, fruity
Eugenol	Spicy, cloves
Hept-2(E)-enal	Fatty, tallowy
Heptan-2-one	Fruity, musty
Heptanal	Green, fatty, oily (Unpleasant)
Hex-2(E)-enal	Green
Hexanal	Green
L-carvone	Mint, chewing gum
Linalool	Orange, flower, licorice
Menthol	Mint, mint sweets
Methional	Cooked potatoes, grassy (Pleasant)
Non-2(E)-enal	Tallowy, fatty
Nona-2(E), 6(Z)-dienal	Cucumber
Nona-2(E), 4(E)-dienal	Fatty
Nonan-2-one	Fruity, musty
Nonanal	Tallowy, green (Unpleasant)
Oct-1-en-3-ol	Mushroom
Oct-1-en-3-one	Mushroom
Oct-2(E)enal	Fruity, fatty, tallowy
Octa-1,5(Z)-dien-3-one	Geranium, metallic
Octan-2-one	Fruity, musty
Phenylacetaldehyde	Honey, sweet (Pleasant)
Thymol	Thyme, eucalyptus
Tridecan-2-one	Rancid, fruity, tallowy
Undecan-2-one	Tallowy, fruity

Adapted from Golovjna and Rothe 1980, Gasser and Grosch 1988, Specht and Baltes 1994, Guillard and others 1997.

astringency, spice heat, cooling, bite, and metallic flavor (Meilgaard et al. 1991). The total sensation of flavor is a combination of taste and smell stimuli that stimulate flow of saliva and gastric secretions. Thus, meat aroma and flavor are intertwined with aroma significantly affecting what is perceived as flavor. Total intensity of flavor is a measure of the overall flavor impact that includes the aroma, tastes, and feelings factors contributing to flavor.

Flavor and Odor Methods (HPLC)

As analytical methodologies and instrumentation have improved, the identification of new flavor-active compounds contributing at threshold levels has become possible. The breakdown of muscle by applying heat during cooking results in the release of flavorous juices and volatile aroma compounds that are responsible for the characteristic flavor of meat (Cross et al. 1986). Processing of meat such as curing, cooking, and smoking brings about characteristic flavors in each meat product. Thus, meaty flavor compounds are typically water-soluble components of muscle tissue. Because aroma and flavor are very related and often result from the same volatiles the same instrumental techniques used for aroma/odor determination are used for flavor determination and include GC, GC/MS, HRGC/MS, GC/sniffer port, and the electric nose (for further information on these instrumental techniques please see the section above). As with aroma, TBARS can be used to measure meat rancidity flavor that involves lipid peroxidation sometimes due to transitional metal ions such as iron and copper.

While volatile flavors are readily analyzed by GC, other compounds such as sugars, amino acids, fatty acids, and other fat soluble compounds must first be derivatized prior to analysis. Liquid chromatography (LC), which is performed at ambient temperatures, permits the analysis of compounds but with somewhat less separation efficiency than gas chromatography (Bett and Grimm 1994). This has led to the use of high performance liquid chromatography (HPLC) for analysis of flavor contributing compounds (see Figure 7.3). Another difference between the GC and LC methods is that sample fractions of compounds can be collected for further investigation (for example on a GC/MS system). HPLC machines have a wide variety of detectors available that will be somewhat specific for the type of compounds being investigated and lead to a typical detection limit of compounds in parts per thousands to parts per million. However, new methods using an LC/MS (see Figure 7.4 for an example) combination are gaining popularity in analyzing flavor compounds with accuracy as GC/MS instrumental techniques.

MEAT JUICINESS

Prediction of meat juiciness can be somewhat difficult. There is not a significant or consistent relationship between juiciness or muscle moisture or fat content. Juiciness scores tend to be more related to water release on cooking (cook loss), drip loss, or water-

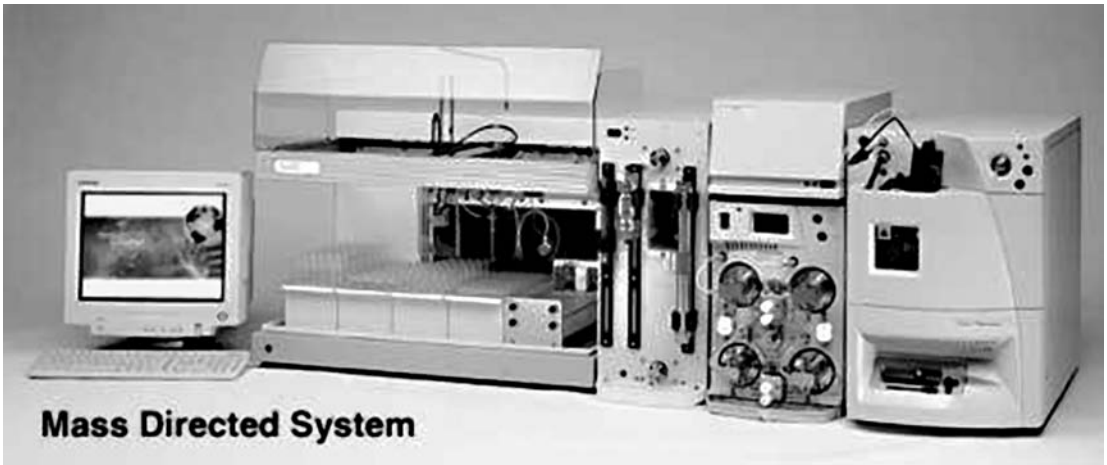

Figure 7.3. High performance liquid chromatography (HPLC) with collection equipment.

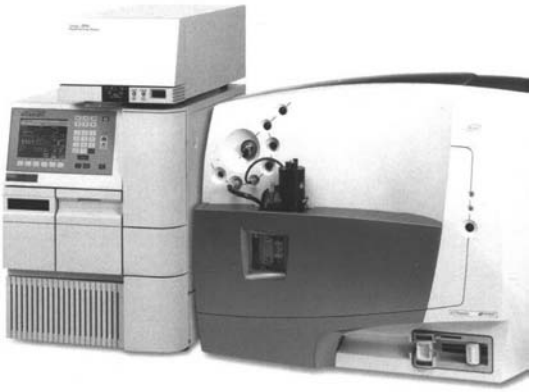

Figure 7.4. Liquid chromatography/mass spectrometry (LC/MS) equipment.

holding capacity. Water within meat exists in the bound, immobilized, and free forms. Because water molecules are charged molecules, they associate with electrically charged reactive groups of muscle proteins. About 5% are located to charged muscle proteins, and are referred to as bound water. This moisture remains tightly bound after the application of severe mechanical force. Other molecules are attracted to the bound molecules in layers that become successively weaker as the distance from the reactive group on the protein becomes greater (Judge et al. 1989). Such water is termed immobilized water, and water held on by weak surface forces is known as free water.

Drip Loss, Purge, and Cooking Loss

Drip loss can be measured on wholesale cuts or parts of cuts (small meat samples or steaks). For repeatable data, it is advisable to use samples with a defined weight and shape because drip loss is affected by surface area. For wholesale cuts, purge can be measured by weighing cuts in packaging (e.g., vacuum) prior to removal and after removal and expressing the water loss as a percentage. For drip loss measurements, the fiber direction can impact measurements. If small samples are used (not steak samples), a cube sample of 30 to 100 g size should be used. Samples should be suspended by means of a net, thread, or hook inside a plastic pouch or plastic container and sealed under atmospheric pressure. Samples should be held at 0 to 4°C for at least 24 hours with a 48-hour duration and longer decreasing variability and improving correlations to juiciness scores especially in aged meat. Pouches or containers should be utilized in a manner in which meat exudates and is not in contact with meat samples. Drip loss can be expressed as milligrams per gram (mg/g) of sample or as a percentage. Because pH can greatly affect moisture binding in meat, it is always suggested that the pH of samples be collected. An additional technique that is associated with drip loss and is related to juiciness scores is cooking loss, which is measured by weighing samples at consistent temperatures before and after cooking.

Water-holding Capacity Methods

Water-holding capacity (WHC) is the ability of meat to hold its water during application of forces. Fresh meat typically contains 70 to 75% water (moisture). The typical method or measurement of WHC is via a filter paper press method originally described by Wierbicki and Deatherage (1958). The method is a simple and rapid method in which fresh, ground, water-added, and cooked meat samples can be analyzed. However, the method is not accurate with high fat products or meat products with added salt. In the method by Wierbicki and Deatherage (1958), a 0.5-g sample of muscle tissue is placed on filter paper and pressed between two Plexiglass plates to a thin film. The meat free water is squeezed out and absorbed by the filter paper. The area of the ring of fluid, which is obtained by subtracting the area of meat film from the total area, is proportional to the percent free water. Percent bound water is calculated as 100 minus the percent free water, and the percent immobilized water is the percent bound water minus the percent free water.

Electron microscopy and X-ray diffraction have been used to determine WHC of meat (Diesbourg et al. 1988, Irving et al. 1989). This is an exacting technique that requires carefully dissected slips of muscles and exposure times of several hours, but it does provide a superior method of establishing the WHC of the filament matrix (Swatland 1995).

MEAT TEXTURE (TENDERNESS)

Meat Cookery

For meat texture measurements, meat cookery can have profound effects if samples are raw samples. Degree of doneness greatly affects texture scores by

sensory panels as well as instrumental readings (Machlik and Draudt 1963, Schmidt and Parrish 1971). Because of this, it is important that all samples within an experiment be cooked uniformly with the same cooking equipment starting at the same temperature (typically 0–4°C) and ending at the same final temperature (typically 71°C). If samples are cooked with a cooking method where one side of the sample is cooked at a time, then samples are typically flipped one time at approximately 40°C. Temperatures should be measured using a thermocouple when possible to minimize disrupting the muscle structure. Once samples are cooked, they are typically cooled down to a consistent temperature, room temperature (approximately 25°C) or refrigerated temperature (0–4°C overnight) prior to textural measurements. For a full outline of cooking procedures, please review the cookery guidelines in AMSA (1995).

Warner-Bratzler Shear Force

There are a variety of different instrumental techniques that can be used to measure meat tenderness. The method most often used is Warner-Bratzler shear force (WBSF). For WBSF 2.5-cm steaks are cooked, and at least six 1.27-cm round cores obtained parallel to the muscle fiber. Cores should be removed by hand or by using a machine drill core and should be uniform in diameter and free of significant connective tissue. Shearing is conducted perpendicular to the longitudinal orientation of the muscle fibers (AMSA 1995). Two types of machines are used to shear the cores—the Warner-Bratzler shear machine (Figure 7.5) or an electric universal testing machine (e.g., Instron) with an attached WBSF head. With universal testing machines, a WBSF attachment is used with a recommended crosshead speed between 200 and 250 mm/min. Procedures for WBSF testing are outlined in AMSA (1995). The shear force of the six or more cores per sample are typically colleted in kg of peak shear force, although pounds and newtons are used. The six shear force measurements per sample are then averaged to produce an average peak shear force mean. Wheeler and others (1996) reported that the repeatability of mean WBSF values did not significantly increase with the use of more than five cores in longissimus samples. However, more than six

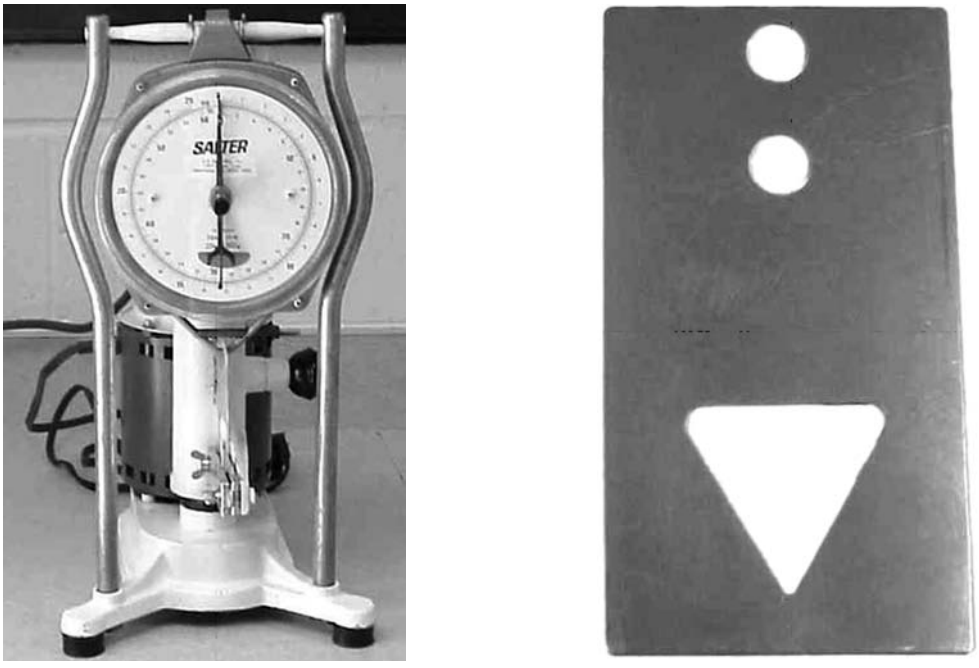

Figure 7.5. Warner-Bratzler shear force machine and shear force blade.

cores may be necessary if steak samples are from larger or more variable muscles. For ground beef patties or processed samples (e.g., ham), at least 10 strip samples 2.5-cm wide should be taken per formulation. Each sample should be sheared three times with a straight-edged WBSF attachment on a universal testing machine. For these measurements, a crosshead speed of 250 millimeters per minute (mm/min) is typically used. Sensory panel scores for tenderness and WBSF correlations vary from experiment to experiment and by product type but a correlation of $r = -0.60$ or more (Caine et al. 2003, Platter et al. 2003, Rhee et al. 2004) should be expected (WBSF values increase indicating more resistance or toughness). Some researchers may indicate that WBSF is a variable indicator of meat tenderness. However, Lorenzen and others (2003) reported that sensory panel tenderness scores had a moderate correlation ($r = 0.34$) to consumer panel tenderness scores in longissimus steaks and lower correlations in other muscles.

Slice Shear Force

As a means to reduce variability in WBSF measurements and to produce a method that could be used online, Shackelford and others (1999a) created a slice shear force. Procedures were later defined further in Shackelford and others (1999b). Slice shear force assessment involves cooking a 2.5-cm thick steak and then removing a 1-cm-wide by 5-cm-long slice parallel to the muscle fibers. The slices are then sheared once with a flat, blunt end blade using a universal testing machine at a crosshead speed of 500 mm/min (Figure 7.6). Slice shear force values correlated well with sensory panel tenderness values ($r = -0.76$) and with WBSF values ($r = 0.80$; Shackelford et al. 1999b).

Figure 7.6. Slice shear blade attached to a universal testing machine.

Star Probe Measurements

Caine and others (2003) and Huff-Lonergan and others (2002) reported using a star-shaped probe or cherry-pitter probe (Instron) to measure shear resistance. The probe is attached to an electric universal testing machine, such as the Instron. Samples are aligned so that the star probe punctures the samples perpendicular to the muscle fiber orientation, and each sample is compressed twice at 80% with a crosshead speed of 200 mm/min. This method is also useful for small muscle samples where collection of 2.5-cm steaks or at least six cores is impossible. In the study by Caine and others (2003), the star probe correlated to beef tenderness sensory panel scores very well ($r = -0.64$) and proved to be a better indicator of panel scores than WBSF values, whereas Huff-Lonergan and others (2002) reported the star probe had a correlation of $r = -0.54$ to pork tenderness scores.

Compression Measurements

Hardness, springiness (originally referred to as elasticity), cohesiveness, gumminess, and chewiness can be determined for ground beef and processed meat products according to the procedures of Bourne (1978). One core, 2.54 cm in diameter, should be removed from the center of at least 10 samples from the same formulation and compressed twice at 70% of the original sample height. Hardness, which is an indication of first bite, is the peak force during this compression. Cohesiveness is a ratio of peak force area during the second and first compression cycles (the first area is the denominator). Springiness is the amount of height that the sample recovers between the two compression cycles. Gumminess is the product of hardness and cohesiveness measurement, while chewiness is the product of gumminess and springiness. A cross head speed of 100 mm/min is used for these measurements. Hardness and chewiness tend to correlate well with initial panel tenderness scores while the other measurements are important in ex-

plaining other texture profiles of meat products (Cain et al. 2003).

Sarcomere Length

Sarcomere length has been reported to be related to tenderness, with shorter sarcomere lengths being related to tougher meat (Wheeler and Koohmaraie 1994, Koohmaraie et al. 1996). Rhee and others (2004) reported that sarcomere length across 11 different beef muscles had a correlation to panel tenderness scores of r=0.68, while WBSF correlated to panel tenderness scores at r=0.73. Cross and others (1981) compared a number of methods for measuring sarcomere length including a laser diffraction method, filar micrometer method, and Shearicon size analyzer method. While all three methods were found to be equal, the laser diffraction method is the one typically used. In the laser diffraction method, samples are fixed in a glutaeraldehyde solution and sarcomere length is measured using a helium laser with a microscope slide placed on a stage underneath and a measuring screen underneath (see Figure 7.7). This method is fairly rapid, although sample preparation should occur at least 1 day prior to sarcomere length measurement. After fixation of the samples, 25 individual fibers are teased from the muscle bundle and placed on a microscope slide with a drop of solution. Slides are placed horizontally in the path of the vertically oriented laser beam to give an array of diffraction bands on the beam, which are perpendicular to the axis of the fibers (Cross et al. 1981).

Collagen Protein Determination (Hydroxyproline Analysis)

Collagen is a protein typically found in muscle tissues with a quantity that varies by muscle, species, and age of animals. Collagen in meat is of concern because it leads to meat toughness in fresh meat and is of concern in processed samples because of shrinkage and conversion to gelatin upon heating. To determine collagen content samples are hydrolyzed in acid, filtered and brought to volume. A portion of the diluted sample is reacted with a solution, for development of a yellow to red color. Samples are then read on a spectrophotometer and compared to standards (Goll et al. 1963). Sample preparation may also include separation by HPLC (Wheeler et al. 2000).

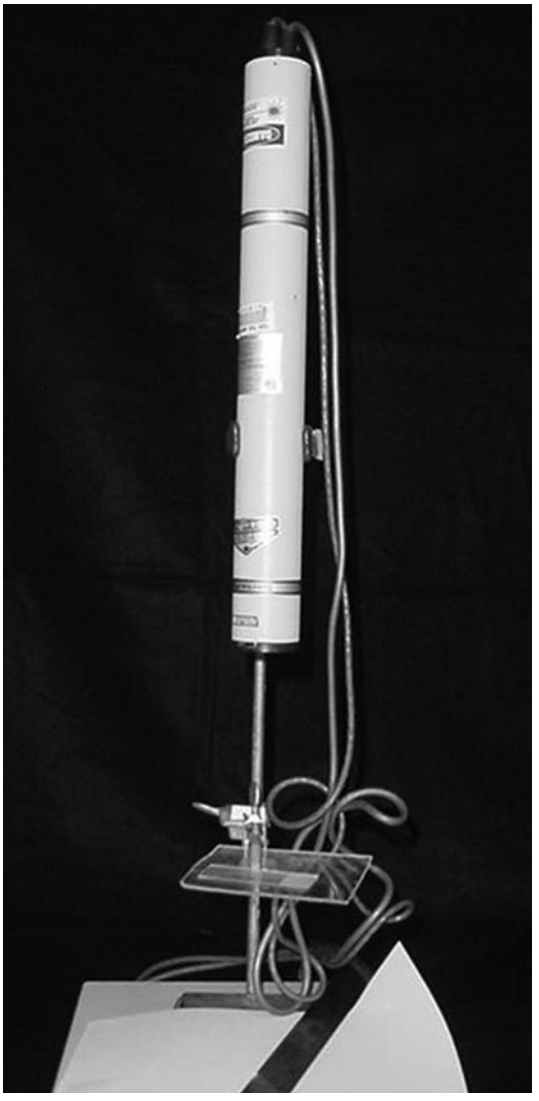

Figure 7.7. Helium laser for measuring sarcomere length.

SDS-Page and Western Blots

One of the most effective ways of improving fresh meat tenderness is through postmortem aging. Myofibrillar proteolysis, as a result of the intracellular calcium-binding proteases, μ-calpain and m-calpain, has been shown to enhance meat tenderness (Koohmaraie et al. 1987). The calpains degrade

myofibrillar proteins such as nebulin, titin, vinculin, desmin, dystrophin, and troponin-T, at or near the Z-line of muscle sarcomeres, leading to postmortem tenderization of beef (Koohmaraie 1992, Taylor et al. 1995, Koohmaraie et al. 1996). Wheeler and Koohmaraie (1994) reported the relationship between WBSF and postmortem aging, in which WBSF decreased primarily due to myofibrillar proteolysis during the aging period after rigor mortis (see Figure 7.8). Because myofibrillar proteolysis can have a profound effect on meat tenderness, a number of techniques explained below are typically used in conjunction with sensory panel ratings and/or instrumental techniques.

One of the most often used methods to look at degradation of myofibrillar proteins is protein electrophoresis with polyacrylamide gel (PAGE). Procedures will vary from laboratory to laboratory and vary based on the myofibrillar proteins of interest. Basically, meat samples are homogenized in a sodium dodecyl sulphate (SDS) solution and then samples are separated by molecular weight on an SDS-PAGE gel over an electric field (see Figure 7.9). The system was first described by Laemmli (1970) and a number of changes for meat samples were proposed by Thomas (1978). While these techniques are useful in describing postmortem changes to myofibrillar proteins, the methods are considered more qualitative in nature. Some researchers will use an imaging computer package to determine differences in pixels at specific locations to add more of a quantitative nature. This is possible when an internal control is used on each gel with a protein at the molecular weight of interest.

Although SDS-PAGE gels are a useful tool, it can be practical to use Western blotting procedures to look at specific myofibrillar proteins of interest. In this procedure, SDS-PAGE gels are transferred to a polyvinylidene chloride (PVDC) membrane via an electric current. The membrane is next blocked with a protein (milk proteins, for example) to minimize nonspecific binding, then with a primary antibody and a second antibody that will react with some sort of detection protocol (e.g., radioactive label or chemiluminescence). The detector is then transferred to film in a darkroom. These procedures allow for excellent detection of intact and degraded proteins. The major problem with the procedure is that only one specific myofibrillar protein can be detected at a time from each SDS-PAGE gel. Thus, if a researcher is interested in three different myofibrillar proteins per meat sample, then three SDS-PAGE gels will need to be run as well as three different Western-blots. For an excellent example of Western-blotting techniques, see the procedures described by Huff-Lonergan and others (1996).

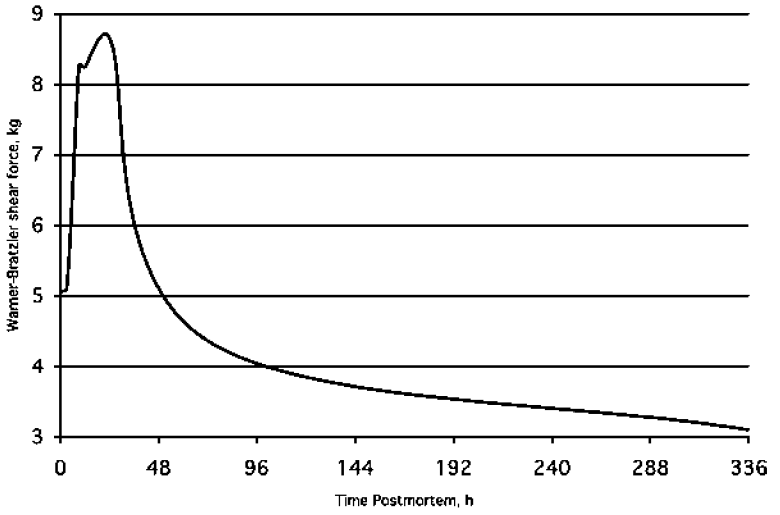

Figure 7.8. Changes in Warner-Bratzler shear force of lamb longissimus during postmortem aging, modified from Wheeler and Koohmaraie (1994).

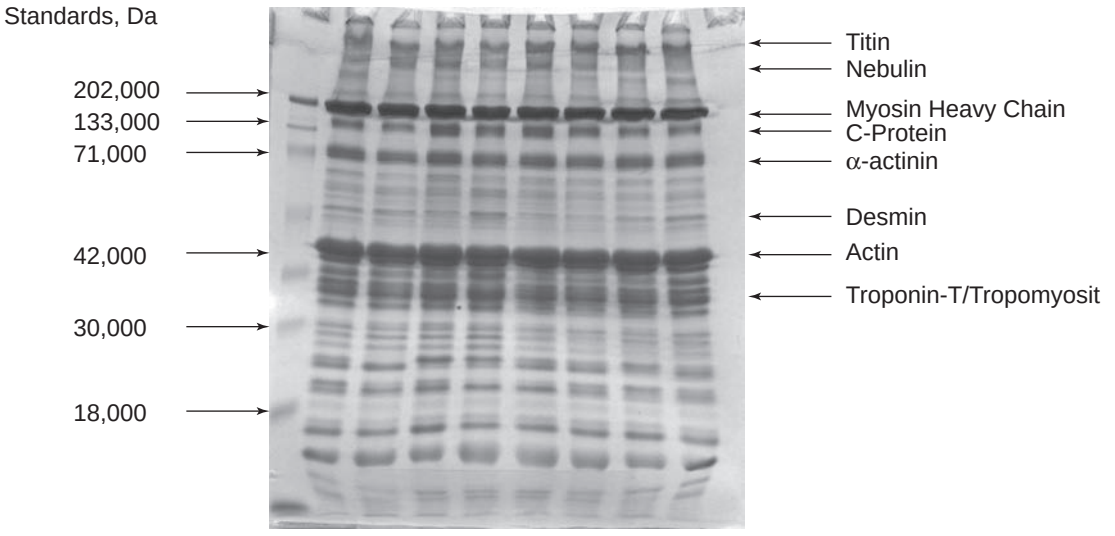

Figure 7.9. 15% SDS-PAGE gel of aged beef longissimus muscle showing the migration positions of the major myofibrillar proteins.

Myofibrillar Fragmentation Index

Myofibril fragmentation that occurs during postmortem aging can account for a high percentage of the variation in tenderness of steaks. The myofibril fragmentation index (MFI) is a measurement of fragmentation of the myofibril that offers the ability to objectively measure tenderness. The MFI procedure predominantly measures the degradation of the myofibrillar protein troponin T into an approximate 28,000- to 30,000-dalton component. The correlation between MFI and WBSF has been shown to be as high as $r=-0.72$ and the correlation between MFI and sensory panel tenderness was $r=0.75$ (Culler et al. 1978). The procedures used typically are the procedures of Olson and others (1976) as modified by Culler and others (1978). The procedures involve homogenization of 4 g of raw muscle tissue, brief purification steps, determination of the extracted protein concentration, and then reaction with biuret reagent and reading on a Spec 20 at 540 nm. Proteins are quantified by the biuret reaction (Layne 1957). Things to note are that samples must be shaken just prior to reading. Some laboratories will use spectrophotometers other than the Spec 20. Myofibril length (MFL) has also been measured as an indication of fragmentation due to proteolysis (Heinze and Bruggemann 1994). In the MFL procedure, the same initial sample preparation as in Culler and others (1978) is used, and samples are read using an automated computer image analysis system for myofibril lengths.

Calpain and Calpastatin

Koohmaraie and others (1987) were one of the first researchers to identify that the calcium-activated calpain system played a major role in the effects on meat tenderness during postmortem aging. The calpain system represents μ-calpain (micro-molar calcium requiring), m-calpain (milli-molar calcium requiring), and their inhibitor calpastatin. Koohmaraie (1990) developed a procedure, involving hydrophobic and ion-exchange chromatography, that isolates μ-calpain, m-calpain, and calpastatin. Shackelford and others (1994) developed a shorter procedure for calpastatin measurement, referred to as heated calpastatin, while Doumit and others (1996) developed the first enzyme-linked immunosorbent assay (ELISA) to quantify bovine skeletal muscle calpastatin. In addition, Geesink and Koohmaraie (1999) developed a two-step method gradient procedure where calpastatin activity is subtracted prior to heat inactivity of μ-calpain. The stepwise gradient method does not require use of a fraction collector or pump as in the continuous gradient method. Calpastatin activity has

been shown to correlate to WBSF (r=0.41) while calpain activity accounted for 41% of the variation in beef WBSF values (Johnson et al. 1990, Shackelford et al. 1991). Thus, calpain and calpastatin measurement techniques can help to explain some of the variation in meat tenderness.

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8
Attributes of Muscle Foods: Color, Texture, Flavor

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INTRODUCTION

The sensory attributes of muscle foods are related to the senses of taste, smell, sight, feel, and sound. Of all the foods consumed, muscle foods have the lowest tolerance for complete sensorial acceptability. A muscle food is either acceptable or unacceptable with little in between. Predominately, the consumer assesses visually the color and surface texture of the muscle. The preparation technique of consumer choice is utilized, thereby altering the sensory attributes, usually completely. The consumer cooks or prepares the muscle food as they prefer, changing the surface color, appearance, and texture. The internal altering of texture and flavor is a result of the preparation or cooking process as well. This of course varies depending on the many methods applied. For instance, the muscle may be grilled, baked, broiled, or otherwise prepared, all with different fluctuating end results. Consumption of muscle foods is one of the most pleasurable eating experiences. The satiety value applied by the consumption of a muscle food is great when comparing food(s) satisfying effect in general. This is why the sensorial properties of muscle foods can be viewed as often more important than that of other foods.

There are many different flavors associated with the sensory evaluation of muscle foods in general. Each, of course, is dependent on the type of muscle food evaluated. To evaluate the basic flavor scenario, we must first begin with a discussion of the innate taste parameters in most all of us.

The basic tastes of salty, sweet, bitter, sour, and umami can be identified in all muscle foods. These basic tastes can vary depending on the cut of a particular muscle, the muscle in general, or the source of the muscle food. For instance, the basic tastes are slightly different when comparing fresh beef to that of fresh mutton.

Smell is a very important sensory property. It is typically the second sensory attribute that is depicted. Fresh uncooked meat is evaluated by smell threshold, whereas cooked meat is evaluated by aroma threshold. Meat that is old, unfresh, or borderline, whether cooked or uncooked may be evaluated by odor threshold. The detection of aromatics and/or odors by

the olfactory nerve comprises what is acceptable or unacceptable in all types of muscle food aroma.

Texture of food influences perceptions and acceptability. Tenderness of muscle foods affects consumer acceptability. Tenderness is the third sensory attribute that must be acceptable to the consumer. This attribute is of course dependent on the type of meat and the cut of that particular meat. For instance, a rib eye steak is anticipated and expected to be much more tender than a flank steak, even though taken from the same meat source. Of course, preparation and processing and/or treatment technique have a great deal to do with the end result and acceptability of a muscle food. A particular cut or source of meat has a perception acceptance value. Another influence on perception and acceptability of a muscle food is mouth coating, which can be residual, fat, or substance in the mouth and throat after the consumption of high fat meat products. If one eats a rib eye steak, there will be little mouth coating or fat residual in the mouth and throat. However, if lamb is considered, there is a great deal of mouth and throat coating (Miller 1994).

Quality characteristics of muscle foods are influenced by muscle appearance, color, surface texture, internal texture, tenderness, juiciness, mouthfeel characteristics, fat content, connective tissue, muscle fiber characteristics, and storage temperature to mention a few. These innate quality characteristics are of course dependent on many predetermining factors that affected the animal prior to its function as a muscle food.

The sections following will depict a brief summary of the "generic" quality characteristics of muscle foods in general. Muscle foods will be discussed as a category and not singled out by breed, unless otherwise noted.

APPEARANCE

The general appearance of a muscle food, regardless of originating species, is the first sensory attribute viewed by a consumer. Appearance is therefore perhaps the most important attribute. Entailed in appearance is muscle color. The consumer is looking for that "bright cherry red" color that industry has deemed and trained the consumer to appreciate and demand. Any skewing from what is typically expected can result in a nonacceptance of the muscle food in question.

Appearance also intakes inherent parameters such as marbling, connective tissue visibility, fat pockets, in some cases "stringiness," moistness of surface, and a perception of moisture level within the muscle. There are many other factors that can affect perception and the general view of the appearance of the muscle food. The attributes mentioned, with the exception of the "bright cherry red" color, would pertain to all muscle foods.

Color

The color of a muscle food is dependent on several factors. The species of the muscle food, the inherent characteristics of various treatments (i.e., vitamin A) applied to the animal from which the muscle food was derived, the food source of the animal, the postslaughter processing and/or treatment methods, the length of time the muscle is kept either frozen or refrigerated prior to packaging and sale, the type of packaging the food is placed in for sale, the environment the package endures (i.e., light source), as well as the length of time on the meat market shelf. There are of course inherent variables that influence color as well. These will be discussed later in this section.

Color reflects or emits a specific amount of energy at wavelengths able to stimulate the retina in the eye. The characteristic red color of meat is due to the heme pigments contained in the muscle. Colors of muscle foods vary depending on species, muscle function within the animal, age of the animal, and storage conditions to name a few variables that influence color. The range of color for muscle foods is influenced predominately by the content of myoglobin. Myoglobin is one of the major proteins in the sacroplasm, and is the main pigment in meat. It accounts for 90–95% of the heme proteins. At increased levels of myoglobin, the color intensity of the muscle ranges from white to pink to very dark red.

The heme pigments take up oxygen to produce "bloom" of fresh meats, but other characteristics are important because of the more widely varied conditions to which muscle tissue is exposed. The protein and heme structures are affected by oxidation, heat, acidity, and chemicals thereby affecting color. Not only do these pigments serve as indicators of freshness, since they turn brown with age, but they also indicate physical and chemical contaminations (Fox 1987). Therefore, this adds importance to the weight

that color and appearance provides as a sensory attribute (Table 8.1).

As has been previously mentioned, beef color is expected to be "bright cherry red" as compared to other muscle foods' ranges of color. Higher myoglobin content in beef muscle is the major factor that differentiates the bright cherry red color of beef when compared to the lighter color of pork or poultry meat. As the animals within the species increase in age, myoglobin content increases within the muscle tissue, and thus muscle from mature animals is darker than muscle from younger animals (Tarladgis 1962a, 1962b).

Meat color, although strongly influenced by myoglobin concentration, is also affected by handling and storage. The anaerobic environment created during vacuum packaged storage versus the use of modified atmosphere packaging where oxygen, nitrogen, and carbon dioxide can be used as the storage environment affects the meat color. The lack of oxygen in vacuum packaged meat systems causes beef exposed to the atmosphere to change from a bright cherry red color to a purplish color in the vacuum package (Castro 1971).

As previously mentioned, the sensory attributes of muscle foods change drastically during the cooking or heating process. This process is one that the end consumer will indeed perform and the end result color greatly affects overall perceptions and acceptability.

The brown pigments produced from proteins and sugars at cooking temperatures also alter color in muscle foods. These dark brown pigments are formed through interactions of deposits from smoke on the meat surface as well as endogenous compounds. Other brown hues are observed in fat, which contains a number of endogenous compounds with color hues ranging from yellow to bronze red (Fox et al. 1967).

Color of Cured Meat

Sensory acceptance of cured meat is somewhat different than that of fresh meat. The overall sensory profile of a cured meat product is changed from that of a profile for fresh meat. The curing process alters the appearance, color, texture, flavor, basic taste(s), and possibly acceptance. For instance, a cured meat product can pass for acceptable, regardless of age, cooking, packaging, or other pertinent variables. But, a fresh meat product might be found unacceptable due to one or more of these variables.

The color of cured meats is dependent on three factors: (1) the concentration of pigment in the tissues, (2) the degree of conversion into nitrosyl pigment, and (3) the proteins in the meat. In fermented beef products, the color is a deep dark red, partly because the tissue proteins are largely denatured (George and Stratmann 1952).

The color of the surface of cured meats is the result of browning reactions or possibly smoking. Smoked meats develop surface colors due to interaction of phenols, aldehydes, and acids (Zimmerman and Snyder 1969).

Color of Frozen Meats

The color of fresh frozen meats is similar to that of chilled meats. However, over time, the muscle will change to more of a brown color. This is accelerated

Table 8.1. The colors of meat.

Pigment	Color	Source	Comments
Hemes	Purple	Reducing conditions	Interior of fresh meats,
Myoglobin			exclusion of air
Hemoglobin			
Oxymyoglobin	Red	Oxygenation	Surface of fresh
			Meat, exposed to
			air, "bloom"
Metmyoglobin	Brown	Oxidation	Old meat, low
			Oxygen pressures
Cytochromes	Red		Very low
			Concentrations

(Zimmerman and Snyder 1969).

if the type of packaging used is not suitable for muscle food frozen storage.

In general, the quality of frozen muscle foods decreases with storage and age in the freezer. Appearance, predominately color, is the first thing the consumer views upon making the decision on whether to purchase a muscle food. However, the consumer's acceptance of frozen meat products is not as dependent upon color as that for fresh muscle food.

AROMA (VOLATILES)

Aroma is the sensory attribute that develops the desire for one to want to consume the cooked or prepared muscle food.

Nearly 1,000 compounds have so far been identified in the volatile constituents of meat from beef, chicken, pork, and sheep. Shahidi (1998) provides a list of volatiles responsible for flavor and believes that sulfurous and carbonyl-containing volatiles are the predominant contribution to aroma.

The chemical nature of many flavor volatiles of meat from different species is similar qualitatively; however, there are quantitative differences. While most of the sulfurous volatiles of meat exhibit a pleasant meaty aroma at concentrations present in meat, at high levels, their odor is objectionable. Therefore, both qualitative and quantitative aspects of volatiles have to be considered when assessing the flavor quality of muscle foods (Shahidi 1998).

Nonvolatile precursors of meat flavor include amino acids, peptides, reducing sugars, vitamins, and nucleotides.

In the evaluation of flavor quality of meat, the contribution by amino acids, peptides, and nucleotides are important. These compounds not only interact with other compounds to produce flavor volatiles but also contribute to sweet, salty, bitter, sour, and umami sensations of muscle foods.

Reactions Leading to Meat Aroma

Many different temperature conditions exist during meat cookery and preparation. Based on these different temperature conditions, a wide range of different flavor sensations is perceived in cooked meats.

The primary reactions occurring on heating that can lead to meat flavor include pyrolysis of amino acids and peptides, caramelization of carbohydrates, degradation of ribonucleotides, thiamine degradation, interaction of sugars with amino acids or peptides, and thermal degradation of lipid. The thermal decomposition of amino acids and peptides and the caramelization of sugars normally require temperatures over 150°C before aroma compounds are formed. Such temperatures are higher than those, which are normally encountered during the cooking of the meat (Mottram 1998).

FLAVOR

Flavor is one of the most important components of the eating quality of muscle foods. A tremendous amount of research has been applied to the understanding of the analysis and chemistry of meat/muscle food flavor. In general, meat flavor depends on the stimulation of the senses of taste and smell. While most of the sensory research and chemistry applied in this area has focused on the volatile compounds that contribute to aroma, there is ongoing research on the contribution of nonvolatile compounds and their relationship with taste sensations.

There are many different compounds that contribute to and provide meat flavor. However, the desirable quality attribute that is perceived as meat flavor cannot be attributed to a single compound or group of compounds. (See Table 8.2.)

The flavor of uncooked meat is "bloodlike" and metallic. The flavor that cooking imparts is unique and dependent upon the length and procedure used. It can vary, and each individual has a preferred way of consuming prepared cooked meat.

Flavor is the result of compounds stimulating the olfactory and taste receptors in the oral and nasal cavity. The muscle system can be divided into the lean portion and the lipid or fat portion, with each component contributing to meat flavor. According to Miller (1994a and 1994b),

> Meat flavor is composed of: 1) Meat like flavor derived from water-soluble reducing sugars and amino acids, 2) Species-specific flavors which are due to differences in fatty acid composition and aromatic, water-soluble compounds that are stored in lipid depots of the animal, 3) Off-flavor development as the result of oxidation of lipid double bonds, defined as lipid oxidation or auto oxidation, and other degradation processes.

Table 8.2. Summary of commonly used product quality attributes used in the evaluation of muscle foods.

Quality Attribute	Description of the Quality Attribute
APPEARANCE / VISUAL	
Color	The perception by the eye of light waves that include blue (400–500nm), green, and yellow (500–600nm) can be defined by hue, value, and chroma. Color can be defined by the overall color or an estimation of positive and negative color attributes.
Texture	A visual evaluation of the texture attributes of a product such as surface smoothness, consistency of texture.
Fat content	An estimation of the amount of internal or external fat content of a muscle food, such as marbling, subcutaneous fat trim level, or amount of steam fat.
Aroma	The volatile compounds detected within the nasal passage by the olfactory system; perceived by smelling (drawing air into the nasal and oral cavities).
Taste	The basic tastes of salty, sweet, sour and bitter that are perceived by gustation through stimulation of taste buds mainly located on the surface of the tongue by water-soluble compounds in the mouth.
MOUTHFEEL/FEELING FACTORS	The perception of senses in the mucosa of the eyes, nose, and mouth through the stimulation of the trigemenal nerves such as perception of heat, burn, cold, metallic, and astringency.

(Miller 1994a and 1994b)

Natural flavor compounds are either fat-soluble or water-soluble. Fat can also serve as a solvent for aroma compounds, or a carrier for flavor compounds. Fat can however mask certain flavors or volatiles (Hornstein and Wasserman 1987, Mottram 1998). To evaluate meat in a semisensory manner, the meat from the older animals is more intense in flavor than the meat from younger animals. Cowy/grainy and serumy/bloody flavor aromatics and metallic descriptors have been associated with increased levels of myoglobin in meat from older animals. The traditional mutton flavor associated with meat from mature sheep is a classic example of how some flavor attributes concentrate in meat from older animals. Diet can also contribute to flavor differences in meat from young versus older animals (Miller 1994a and 1994b).

The terms lamb and mutton flavor are used interchangeably and refer to the characteristic flavor of all sheep meat, regardless of age. Terms like "lamb" and "wooly" have been used for cooked lamb. Fat-rich mutton broths were "lamb-like," "sweet," "oily," and "barny" (Young and Braggins 1998).

Lipid (Fat) Contribution To Meat Flavor

The sensory profile of meat depends largely upon the contribution of fat to the overall flavor complexity.

Lipids can contribute both desirable and undesirable flavors to meats. During cooking, the lipids undergo thermally induced oxidation to give a range of volatiles that contribute to meat aroma. Lipids may also react with the components from the lean tissue to give other flavor compounds. In addition, they may act as a solvent for aroma compounds accumulated during production, processing, and cooking of meat.

Lipids not only impart flavor volatiles by oxidation, but their oxidation products can enter the Maillard reaction, resulting in additional volatiles that are not formed by lean meat precursors. For the most part, the phospholipids of meat rather than triglycerols contribute the fatty acids that interact with Maillard reactions.

According to Miller (1994a and 1994b) most of the volatiles derived from Maillard interactions have a higher boiling point than most of the volatiles

identified in the meat flavor. The possibility exists that some of these compounds might have a meaty odor that is species specific.

Maillard Reaction

According to research done by Bailey (1994), the Maillard reaction (nonenzymatic browning) involves the reaction of aldehydes with amines and through numerous reactions; food flavor compounds and dark pigments (melanoidins) are formed. Factors affecting the progress of the Maillard reaction include temperature, time, moisture content, pH, concentration, and nature of the reactants. The Maillard reaction between reducing sugars and amino compounds does not require the very high temperatures associated with sugar caramelization and protein pyrolysis and readily produces aroma compounds at the temperatures associated with the cooking of food (Mottram 1994).

Sulfur Compounds

According to Mottram (1994), meat would have an entirely different flavor in the absence of sulfur compounds. Large quantities of hydrogen sulfide are produced during the heating of the meat. Under these conditions, concentration of hydrogen sulfide is very prominent. Sulfur compounds are the most important volatiles formed during meat cookery, and sulfur precursors are used in essentially all meat flavor mixtures.

Even though sulfur is not considered one of the pleasant flavor compounds, its very existence defines perceived quality and acceptance of many foods (i.e., sharp cheddar cheese) including muscle foods.

Umami Flavor of Meat

Meat is known to possess a sensory property that many say is one of the innate basic tastes of humans. There has been some argument to this proposed theory, but it is indeed a unique taste sensation, which some can detect while others cannot.

The term "umami" was first proposed by Ikeda (1909). Umami means "deliciousness" in Japanese. Umami can be defined as the taste properties resulting from the natural occurrence or intentional addition of compounds such as monosodium glutamate (MSG) and certain 5′-nucleotides such as 5′-inosine monophosphate (IMP) and 5′-guanosine monophosphate (GMP). These nucleotides have also been referred to respectively as inosinic and guanylic acids, 5′-inosine and 5′-guanylic acids or disodium 5′-guanylate (Maga 1998).

Umami compounds have an ability to act synergistically when used in combination with each other. According to Maga (1983), if MSG is assigned a umami intensity of 1.0, the addition of an equal amount of GMP increases the flavor intensity 30 times. Even addition of as little as 1% GMP to MSG significantly increases flavor intensity.

Intentional addition of MSG and nucleotides significantly modifies meat flavor perception. For example, the addition of MSG to a variety of processed meats resulted in products that were preferred over the same products to which MSG was not added (Girardot and Peryam 1954).

The addition of either MSG or the nucleotides, thus providing the "umami" taste sensation, greatly enhances the acceptance as well as the preference of select meat items, whether utilized in an emulsion system, or directly applied to the muscle surface during cooking.

TENDERNESS

Tenderness is one of the most important sensory attributes when discussing the acceptance of a muscle food. Tenderness entails many parameters to the consumer. The more tender a muscle food is, the better perception of freshness and quality resides in the mind. However, muscle foods' experts know that this is not always the case.

Tenderness of meat may be simply defined as the ease of teeth to cut meat fibers during mastication. For intact or noncomminuted meat, tenderness to toughness is determined by two groups of meat components: the connective tissues and the muscle fibers (Koohmaraie et al. 1988). Tenderness of muscle foods strongly influences and drives consumer's perceptions of acceptability and quality of muscle foods. Muscle tenderness can be segmented into the influence of connective tissue and muscle fiber characteristics. Total amount of connective tissue from within different muscles of the same animal affects tenderness between muscles. For example, the muscles from the round that are used in movement

are higher in connective tissue content than muscles from the loin that are mainly used for structural support. Muscles have been generally classified into tender, intermediate, and tough categories. Between animals, the age associated increase in collagen solubility significantly affects muscle tenderness. As animals increase in age, their meat is tougher. Identification or measurement of animal age, either through ossification of the verbal column of an animal, identification of dentition, or evaluation of animal chronological age has been used as a benchmark for the effect of connective tissue on meat quality between animals (Cross 1987).

Connective Tissue

Connective tissue is a fibrous structure composed of primarily collagen fibrils. There are three types of connective tissue in meat: epimysium, perimysium, and endomysium. The epimysium is a thick sheath of connective tissue surrounding the entire muscle; the perimysium is a thin layer enveloping muscle bundles or muscle fibers (myofibers). The perimysium and endomysium connective tissues present a realistic toughness problem to meat and meat products. The specific influence of both connective tissues depends upon their thickness (i.e., the amount of collagen present, as well as the density and type of cross linkages between collagen fibrils). Muscles of movement contain larger quantities of connective tissue than muscles that support the structure (Koohmaraie et al. 1988).

Muscles in mature animals have higher contents of collagen cross-links than the same muscles from young animals. The type of collagen cross-links is also different; as the animal's age increases, many of the collagen cross-links are transformed from a soluble type to an insoluble type, resulting in increased toughness of meat (Koohmaraie et al. 1988).

Myofibers

Myofibers regulate meat tenderness in several aspects. Collagen in the connective tissue experiences minimal chemical and biochemical changes and exhibits little structural changes other than slight improvement in solubility (Koohmaraie et al. 1988).

JUICINESS

Juiciness is a sensory term, which refers to mouthfeel of the moisture released from food during mastication. Thus, juiciness is indicative of the moisture content in meat, which is critically affected by the water-holding capacity as well as the hydration ability of the meat. Water in meat is confined via capillarity in the spaces between myofilaments, between the myofibrils, and outside the fibers (cells). Myofibril proteins are believed to be largely responsible for water immobilization in meat. The hydration is attributed to the expansion of the interfilamental spaces within the myofibrils and is facilitated by mechanical actions such as massaging and tumbling (Cross 1987).

TEXTURAL PROPERTIES

According to Kauffman and Marsh (1987), texture is an important characteristic of muscle foods especially for processed meats that require some degree of comminution. For intact meat, texture of muscle refers to the definition and fineness of muscle fibers and the amount and distribution of fat in the muscle. In intact meat, the texture is determined by the age of the animal, the type of muscle, gender, and the growth condition. In comminuted meats, however, textural properties are characterized by smoothness and homogeneity of the product, which can be visualized, as well as by the rheological properties (hardness, deformability, elasticity, etc.) and cohesiveness of the bind formed between meat particles. These texture-related attributes are closely related to the functionality of muscle proteins particularly their gel-forming and emulsification properties.

CONCLUSION

In general, the sensory attributes of muscle food products provide great satisfaction in eating and satiety to the end consumer. Acceptance is the predominant factor applied to all of the sensory attributes of muscle foods. The attributes of appearance, aroma, flavor, juiciness, tenderness, and texture, to mention a few, must all be found acceptable when a muscle food is consumed. Muscle foods, unlike other foods, are completely scrutinized by the end consumer and

there is very little, if any, tolerance for any deviation of complete perception of quality for all the pertinent sensory attributes. Sensory evaluation of muscle foods can be one of the most difficult analyses to perform due to the inherent differences of the muscle(s). However, it is one of the most important functions the industry utilizes to determine estimates of perception(s) ultimately to be derived by the end consumer.

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Part III
Flavors

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9
Sensory Characterization

Karen L. Bett-Garber

Descriptive Flavor Analysis
- The Flavor Profile
  - Quantitative Descriptive Analysis
  - The Spectrum Descriptive Analysis Method
  - Free-Choice Profiling
- Chemical Nature of Flavor Characteristics
  - Isolation and Extraction of Compounds
  - Sensory Issues with GC-O
  - Intensity Rating Methods for GC-O
  - Relating the Compounds of GC-O to Food Aroma

References

Flavor, according to Amerine et al. (1965), is an attribute of foods, beverages, and seasonings resulting from stimulation of the sensory receptors that are grouped together at the entrance of the alimentary and respiratory tracts, and includes odor and taste. Caul (1957) restricted flavor to the impressions perceived via the chemical senses from a product in the mouth. This would include aromatic compounds perceived retronasally or volatile substances released from product in the mouth via the posterior nares, gustatory perceptions (tastes) caused by soluble substances in the mouth and chemical feeling factors, which stimulate nerve ends in the soft membranes of the buccal and nasal cavities (Meilgaard and others 1999). For the purpose of this chapter, flavor will include aroma/odor inhaled through the nose in addition to flavor-by-mouth and taste. The underlying principle is that flavor must be perceived by a human to be flavor. This chapter will discuss methods of measuring flavor characters using human beings, as well as methods of defining flavor characteristics.

Flavor results when chemical compounds are perceived by the human senses. Gas chromatographs (GC) and mass spectrometers (MS) can monitor volatile compounds that may or may not invoke a human response. Therefore, they can be used to measure quantities of compounds that contribute to flavor. But, these instruments cannot confirm if compounds are perceivable by humans. Gas chromatography-olfactometry (GC-O) is a method of configuring a gas chromatograph that splits the flow of compounds into two portions. One portion goes to the instrumental detector such as a flame ionization detector or mass spectrometer and the other portion goes to a port where a human smells the effluent laden with molecules. GC-O methods for characterizing flavor will be discussed later in this chapter.

Early in the study of human olfaction, the idea was that odors could be classified or grouped. The search for the grouping system that universally worked was not fully successful. Humans learn from an early age to classify colors, but aroma classifications are not discussed at a young age in the fashion that color is (Ishii and others 1997). Early efforts to classify odors served to indicate the complexity of odors (Doty 1991). In 1956, Wenger and others concluded that there was not an adequate classification of odors. Ishii and others (1997) found that judges categorizing odors over several sessions did not reach consistency over 8 to 10 sessions. Use of the perfume industry's categories did not come naturally, but needed considerable training. But, as the cues were increased, judges began to sort closer and closer to the perfume industry's category system (Ishii and others 1997). Within an industry, such as perfume, wine, beer, and drinking water, classification systems have simplified the description of odors, but classification has not helped with elucidating basic mechanisms of the olfactory system

(Doty 1991). There is a classic review on aroma classification (Harper and others 1968), and this subject will not be discussed further in this chapter.

Sensory evaluation is the use of humans to evaluate a product using one or more of the five senses (smell, taste, feel, sight, sound). Descriptive flavor evaluation is a sensory evaluation method that describes individual flavor characters and gives an intensity rating to each descriptor. This chapter will contrast the various methods of descriptive flavor analysis and contrast the methods used to determine descriptions of flavors. Panelists are selected on normal abilities to taste and smell and are trained in the methods before data collection takes place. The selection and training process will not be reviewed in the scope of this chapter, but is available in the references cited for each method.

DESCRIPTIVE FLAVOR ANALYSIS

The birth of descriptive flavor analysis resulted from the development of a laboratory method of analyzing flavor comparable to the expert taster in industrial quality control, namely in the wine, tea, and coffee industries (Caul 1957). The laboratory panels needed to consist of multiple persons (5 to 20) and needed to be versatile for work on multiple products that may be dissimilar. Methods for selecting and training new panelists needed to be documented so that panels could continue operation by incorporating new panelists after attrition of original members. The Flavor Profile was developed in the 1940s at Arthur D. Little, Inc. (Cambridge, Massachusetts) to meet the growing analytical needs of sensory evaluation in the food industry (and in some cases, nonfood industries) (Cairncross and Sjöström 1950, Caul 1957). This method was distinct from expert tasters in quality control, because it made no direct judgment about consumer acceptance (Stone and Sidel 2004). Subsequently, new methods have emerged from these ideas and their applications have grown substantially (Stone and others 1974, Meilgaard and others 1999). These methods differ in their ideologies about characterizing flavor and will be discussed in this chapter.

The Flavor Profile

The method originated from the concept that flavor consists of identifiable attributes of taste, aroma, flavor, and chemical feeling factors. The attributes were called "character notes" and were labeled with descriptive or associative terms. For example, eggy, rubbery, cabbage-like, or skunky terms were used to describe sulfury notes (Cairncross and Sjöström 1950, Caul 1957). The terms were objective terms like "vanilla" and not subjective like "good" (Keane 1992). When a specific chemical compound was identified to be associated with the character note, it was used as the reference. For example, phenylacetic acid has represented the "horsy" note in beer (Caul 1957). When necessary, a character note could be further characterized, such as chemical-sweet and sugar-sweet (Caul 1957) or localized, such as dry mouth, cheek puckering, tongue coating, or tooth roughing for variations of astringency (Cairncross and Sjöström 1950). During character note development, panelists start with a blank sheet and record all aromas, flavors, tastes, and aftertastes they perceive in the product. Then they discuss and develop an agreed upon list and duplication of a note under different descriptions is eliminated. It may take two to four sessions to finalize the composite list that completely describes the product and that all panelists agree with (Neilson and others 1988). Reference standards are obtained to represent the notes. It is essential that all panelists understand the scope and connotation of each note in the study. The panel members, who are selected according to their abilities to discriminate odor and flavor differences and communicate their perceptions, develop the character notes (Caul 1957).

Within a sample, the character notes are perceived in a specific order. Adding seasoning to the product can affect the order. For example, monosodium glutamate alters the order of appearance of character notes. In a flavor profile, the character notes are listed in the order they appear and any changes in order are noted (Caul 1957). When using standardized evaluation techniques for smelling and tasting, the order of appearance for character notes is influenced by location of taste buds on the tongue, volatility of aromatics, and texture of the product (Keane 1992).

Intensity is noted on a 5-point scale [0 = not present, just recognizable, or threshold, 1 = slight, 2 = moderate, and 3 = strong]. The panelists evaluate the intensity of the observable character notes individually. Upon completion of all samples, they discuss their scores and arrive at a consensus for the intensity of each note in the sample. The panel session

is not complete until all panelists in attendance agree individually and as a unit that the consensus represents the sample (Cairncross and Sjöström 1950, Caul 1957). The Flavor Profile was not designed as a numerical system for statistical analysis of data but as a consensus or composite of profile terms and intensities in the final product (Keane 1992). Some variations have emerged that make the Flavor Profile method statistically friendly (Miller 1978), but these are not as widely accepted as some of the newer descriptive methods.

Quantitative Descriptive Analysis

As the food industry expanded, and moved further away from the expert taster concept, a need arose for data that could be analyzed statistically. Scientists at Stanford Research Institute developed the Quantitative Descriptive Analysis (QDA) method (Stone and others 1974, Stone and Sidel 2004). This method screened panelists with discrimination tests, used descriptors developed by the panel, used repeated evaluations in sensory booths to avoid group judgments, and used analysis of variance and graphing of means to interpret data (Zook and Pearce 1988).

QDA starts with panelists that are selected based on their product usage and familiarity, discrimination ability within the product or product category, and task comprehension. During the training process, panelists develop descriptive language, group the attributes (terms) by modality (appearance, aroma, and so on), list them by occurrence, develop definitions for each attribute, identify helpful references, and familiarize themselves with the scoring procedure. The panelists work individually and as a group to ensure that the attributes are fully understood and that all of the product characteristics are fully accounted for. Within the QDA philosophy, the panels develop their own language, without being influenced by the information given them by a panel leader. The group of panelists must decide to include or exclude an attribute. They are encouraged to use their own words, provided they are common everyday language. The meaning of each word-sensation experience is defined so the entire group will be familiar with the word and the meaning. Numerous attributes/terms are encouraged, because having too few may limit panelists' ability to differentiate product differences. Statistical analysis is used to determine which terms/attributes differentiate the samples. Distinct concepts assigned to each attribute are not considered critical, because a magically correct set of independent descriptors may not fully describe the sensory properties. Panelists also become familiar with the order of occurrence of each sensation. References are not necessary, but useful for training and retraining; and can help panelists relate to a particular sensation that is not easily detected or described. The references should not introduce additional fatigue or significantly increase training time and should only be used during language development and not in the initial sessions (Stone 1992, Stone and Sidel 2004).

Relative intensity is measured with a 6-inch or 15-cm open line scale to provide an infinite number of possible options and to avoid number biases. Word anchors are placed one-half inch or 1.5 cm from each end. Low intensity is on the left end, and high intensity is on the right end. To be more confident about reliable results, multiple replicates are evaluated. For most products, four replicates result in the desired sensitivity. Results are analyzed by analysis of variance. Replication allows for characterizing reliability of response patterns and does not generalize the results to a population (Stone 1992, Stone and Sidel 2004).

The Spectrum Descriptive Analysis Method

The Spectrum method was developed by G.V. Civille in collaboration with a number of companies that were looking for a method to obtain reproducible and repeatable sensory analysis of products (Meilgaard and others 1999). It is flexible enough to evaluate one to all sensory modalities and to evaluate an array of products, including foods, beverages, personal care, home care, paper, and other products. A Spectrum panel describes accurately and in detail the characteristics/parameters of appearance, flavor, and texture and allows statistical treatment of data (Muñoz and Civille 1992, Meilgaard and others 1999). The Spectrum method utilizes a broad selection of references for describing the sensory attributes and for evaluating intensity (Rutledge and Hudson 1990).

To develop descriptors (terms), trained panelists (after being exposed to underlying principles of appearance, flavor, and texture) start by evaluating a broad array of products (commercial brands, pilot

plant runs, etc.) that define the product category (Muñoz and Civille 1992, Meilgaard and others 1999). A few sessions are devoted to this process. Panelists individually develop lists of terms that adequately describe the attributes in the product category. The group discusses their lists and the panel leader, in conjunction with the panelists, records and organizes the terms provided by the individuals. The organization is based on the underlying structure of flavor categories. After a few sessions of this type of activity, the panel is led to establish the list of terms that describe the sensory attributes of the product category that will be evaluated. This process includes using references to help all panelists understand each term in the same way. The list of descriptors should be comprehensive, but not overlapping. Several sessions may be required to complete a ballot (Muñoz and Civille 1992, Meilgaard and others 1999). Panel leaders can glean descriptor terms and definitions from the literature to suggest references to complete the ballot. The panel is frequently reminded that the truth is always in the sample(s). A good source of descriptors is ASTM International's book and diskette titled *Aroma and Flavor Lexicon for Sensory Evaluation: Terms, Definitions, References and Examples* (Civille and Lyon 1996).

Panelists generally use a 0- to 15-point scale, numbered in tenths, for rating intensity of most flavor descriptors. However, panelists may indicate higher intensities (greater than 15) for very strong stimuli. Panelists may also use a line scale with anchors at each end indicating direction (Muñoz and Civille 1992). Extensive use of intensity reference points and specific reference samples are derived from the collective data from several panels at separate locations over several replicates (Meilgaard and others 1999). Training is never over, since panelists keep adding to their knowledge of the product and evaluating their responses. An internal standard or control that is available from a consistent supply of product can be used as a reference for comparison to experimental samples (Rutledge and Hudson 1990). A variety of uni- and multivariate data analysis techniques are used in the many applications of Spectrum descriptive data.

Free-choice Profiling

Free-choice Profiling is a method developed by Williams and Arnold (1984) in the United Kingdom that allows untrained panelists to invent and use as many terms as they need to describe the sensory characteristics of the product. The use of the generalized Procrustes analysis method makes it possible to reduce the variation in terminology (Williams and Langron 1984). The centroid of each assessor's data space is rotated, expanded, and shrunk until each assessor is made to match as closely as possible to the other assessors, but maintaining each assessor's intersample relationships. A consensus configuration is obtained. These configurations (axes) are then related to external factors (Williams and Langron 1984). All of this depends on the individual assessors' ability to precisely and consistently use the terms they generate. Inexperience and isolation from other panelists results in inadequate vocabulary that may be improved by allowing more time for vocabulary development (Piggott and Watson 1992). Rodriguez and others (2000) found that trained panelists using Free-choice Profiling were more accurate than untrained panelists. Guerrero and others (1997) demonstrated that trained panelists were more efficient in generating terms than semitrained panelists. Gilbert and Heymann (1995) observed that Free-choice Profiling allowed for quick generation of descriptive terms without the need for extensive training; however, interpretation of results was not as straightforward as descriptive analysis methods and was not recommended for use with untrained panelists. Heymann (1994) reported that terms generated by sensory-savvy panelists were more likely to discriminate among samples. In the end, the experimenter/sensory analyst decides what the terms for each parameter mean (Meilgaard and others 1999). Therefore, this method has undergone changes that cause it to approximate the other descriptive methods (Stone and Sidel 2003).

All of these methods place emphasis on determining the full range of variation in the product during generation of terms for describing flavor. The Flavor Profile method and profile derivatives (e.g., the Spectrum method) use a language that tends to be more technical in nature (developed by a technically trained group) and provide technical guidance to the product developer. The QDA method takes the perspective that the panel should represent consumers and the language should reflect consumer language yet be descriptive (Muñoz and Bleibaum 2001). All of these methods develop definitions for the generated terms except Free-choice Profiling. Flavor

Profile and Spectrum incorporate extensive use of flavor references for both the terms and intensities. Having definitions for descriptive terms allows others outside the panel to understand what is meant by the terms.

Chemical Nature of Flavor Characteristics

There has been much effort devoted to relating flavor compounds to specific flavors. Basic tastes and chemical feeling factors tend to be less complicated to relate to compounds, but natural aromas can be quite complex. To determine the influence of chemical compounds on flavor, the gas chromatograph has been coupled with an olfactometry (GC-O) device for sniffing the compounds after they are separated from each other. There are three main uses: (1) to determine the odor qualities of single compounds; (2) to detect odor-active compounds in complex flavor extracts; and (3) to quantify the odor contribution of individual compounds in flavor systems (Dattatreya et al. 2002). Kim and others (2003) devised a method for determining if the aroma of the combined extracted volatiles resembled the aroma of the original sample. The trapped volatiles were injected (splitless mode) into a 0.5-m column at 200°C and evaluated at the sniffing port. If the effluent aroma resembled the original product's aroma, then the essence of the total aroma is being evaluated. If it does not resemble the product's aroma, then one can conclude that key volatiles were lost during the extraction process (Kim and others 2003). There are extraction issues and sensory issues to keep in mind pertaining to GC-O methods.

Isolation and Extraction of Compounds

Numerous techniques are employed to extract aromatic compounds from the various sample matrices. The extraction technique will influence the volatile profiles. Common methods are solvent extraction, static headspace sampling, solid-phase microextraction, simultaneous steam distillation/extraction, and supercritical fluid extraction. A review by Peppard (1999) describes the methods. Some of these methods work better for GC-O than others. Solvent extraction uses organic solvents such as methylene chloride, various pentane/diethyl ether mixtures, or other solvents to extract the flavor compounds from the food or beverage sample. Water-soluble compounds may be incompletely extracted. Flavor compounds are typically present in small amounts and require solvent removal for detection. Concentration of the extract prior to injection may result in loss of some volatiles. Extraction of volatiles from solid particles can be time consuming. Static headspace involves equilibrating the headspace above a sample in a sealed vial under controlled conditions and sampling with a syringe. Headspace sampling is effective for highly volatile flavor compounds. Compounds with low volatility pose a problem and quantitation can be complicated. Dynamic headspace sampling involves passing an inert gas such as helium through or over a sample, then trapping on a charcoal or a Tenax® trap. Volatiles are eluted from the trap with a solvent or desorbed with heat. This is an excellent qualitative method of extracting flavor compounds having a wide range of volatility from difficult-to-extract matrices, but reliable quantitation is difficult due to incomplete extraction. Solid-phase microextraction (SPME) uses a short length of fused silica fiber coated with a thin layer of absorptive material. Several types of coated fiber are available with differing selectivities. SPME can be used in static headspace or the fiber can be inserted into a liquid substance. This extraction method and the subsequent gas chromatography can detect flavor compounds at lower concentrations than traditional static headspace sampling. It is simple, rapid, and requires no solvents. Selectivity depends on the fiber coating. Reliable quantification requires appropriate and consistent care. Simultaneous steam distillation/extraction (Likens-Nickerson) is a steam distillation method with simultaneous extraction of refluxing vapors by a refluxing solvent, such as methylene chloride. It is used with samples that are high in fat or other solvent extractable material. This method is time consuming and requires specific glassware. Only steam distillable volatiles are extracted. High and low volatile and polar compounds are poorly trapped. Extracts tend to contain artifacts due to oxidation, thermal breakdown, and hydrolysis. Supercritical fluid extraction is a powerful method of extracting volatiles from dry, finely ground samples. Liquid or wet samples can be used if the sample is immobilized. Carbon dioxide (CO_2) is pressurized sufficiently to be in the supercritical state so that it has the extracting capabilities of an organic solvent. Adjusting the temperature and/or pressure can change the selectivity by controlling the density of the CO_2.

Using adsorbents and CO_2 modifiers, such as methanol, enhance selectivity and aid in capturing flavor compounds (Peppard 1999). After preparation, samples should be analyzed as soon as possible. Cold on-column injection can avoid thermal induced decomposition of some compounds. Certain thiols dimerize in diethyl ether upon refrigerated storage. Therefore, storage of concentrate should be in pentane at −30°C under inert gas (Blank 1997).

Sensory Issues with GC-O

According to Sell (2000), a majority of the published odor descriptions of compounds were done on mixtures rather than on single compounds. These mixtures include trace impurities, unresolved peaks on chromatograms, the presence of isomers, etc. Historical data need to be substantiated with recent technologies in chiral capillary gas chromatography coupled with olfactometry to confirm descriptions of compounds (Sell 2000).

Threshold concentration varies among compounds, sometimes as much as 11 orders of magnitude (Weyerstahl 1994). Within a single compound, subjects' relative sensitivities to enantiomers (e.g., β-ionone) diverge widely. For example, some panelists were more sensitive to (+)− than to (−)-β-ionone and vice versa. The threshold ratios can vary by four orders of magnitude between the two enantiomers (Weyerstahl 1994). These ranges differ from compound to compound.

There are several factors affecting perception other than concentration. Humidity can affect perception and consequently should be controlled. Adaptation (or fatigue) can affect perception. Cross adaptation can affect perception of odor for succeeding compounds (Sell 2000). Time between breaths that may be longer than the compound elution can result in incomplete experiences (Hanaoka and others 2001). Subjects that breathe more rapidly detected odors more often than subjects that breathe slower (Hanaoka and others 2001). The dilution at which the GC-O is carried out affects the sensory detection of compounds, which could be improved via running multiple concentrations for each sample (Ferreira and others 2001). Enantiomeric form can affect flavor. Carvone is a classic example, the (+)−5 carvone has a typical caraway odor and the (−)−5 form has a spearmint odor. Other compounds, such as camphor, have both enantiomeric forms smelling alike (Ohloff 1986).

Intensity Rating Methods for GC-O

In 1957, Patton and Josephson proposed the idea of odor activity value (OAV). This occurred soon after the introduction of the gas chromatograph. The idea was to relate concentration of a compound to its sensory threshold. This early work demonstrated that the most abundant volatiles may have little if any odor significance in a food. These techniques have been invaluable for determining off odors in foods. Unfortunately, the original ideology has resulted in limited success in flavor duplication (Mistry and others 1997). From this early work, four methods have emerged, dilution analysis method Aroma Extract Dilution Analysis (AEDA) (Ullrich and Grosch 1987), Charm Analysis (Acree and others 1984), the detection frequency method (Linssen and others 1993), and Osme (McDaniel and others 1990).

The AEDA techniques utilize step-wise diluted extracts of volatile compounds, which reveal the most intense flavor compounds in the extract. This results in D-values (lowest dilution at which a substance is still smelled) that are proportional to the aroma values (Ullrich and Grosch 1987). In Charm Analysis, the beginning and end of each odor are recorded. It uses computerized data collection and a sensory procedure based on odor-detection thresholds rather than psychological estimations of stimulus intensity. The dimensionless measure of odor intensity is called charm (Acree and others 1984, van Ruth and O'Conner 2001a). The charm response chromatogram is essentially a plot of a function of the dilution factor and number of responses at a given dilution factor versus retention index (Acree and others 1984). The detection frequency method monitors nasal impact frequency. Six to eight assessors sniff the headspace of a single dilution of a representative sample. The time it takes the aroma to come through the port is monitored. Nasal impact frequency of 100% means the odorant was detected by all the assessors. Peak intensities are not related to aroma intensities, but to their detection frequencies (Pollien and others 1997).

Osme is a quantitative bioassay method used to measure the perceived odor intensity of a compound eluting from a GC olfactometer. The subject rates the intensity of a compound's odor by using a time-intensity device, providing an odor peak similar to a chromatogram. Verbal descriptions are given for each peak recorded (Miranda-Lopez and others 1992).

A comparison of three ideologies was done by van Ruth and O'Conner (2001a, 2001b). They found similar results with Osme (intensity) and nasal impact frequency (detection frequency). The charm (dilution) method correlated only slightly with the other two methods. This may have been due to large dilution steps used to cover the range of thresholds for the mixture of compounds. They did find a large variability between assessors' thresholds. Charm also required numerous GC runs. In the intensity method (Osme), large quantities of volatile compounds do not necessarily relate to high odor intensities, as this property is due to a difference in thresholds and differences in intensity/concentration relationships. Detection frequencies are limited to the concentration at which the maximum frequency possible is attained and measured by the number of assessors that perceive the aroma. Dilution methods have a large variability due to differences in thresholds (up to a factor of 200) and response criteria of assessors. Van Ruth and O'Conner (2001a) recommend using a number of assessors and not relying on one or two judges to achieve reliable GC-O analysis independent of the method used. Both assessors' qualities (e.g., alertness) and analytical conditions clearly influenced the GC-O data and, therefore, require optimization (van Ruth and O'Conner 2001b).

There are inherent problems that occur when panelists try to evaluate aromas that result from a single compound whether it be in a food system or from a GC-O run. In some work done on catfish samples spiked with geosmin or 2-methylisoborneol, the author and colleagues (Bett and Johnsen 1996) found that trained descriptive panelists could distinguish intensity differences in concentration of 2-methylisoborneol within a sensory session, but could not determine intensity differences between sessions on different days. Meanwhile, they were able to evaluate flavor intensities made up of multiple compounds, such as the chickeny flavor of catfish. These authors, Bett, and Dionigi (1997) have also reported that panelists had greater standard deviations when evaluating the off flavor from one compound (geosmin or 2-methyliosborneol) than they had when evaluating flavors containing multiple compounds, such as chickeny and nutty descriptors. When the author worked with sensory panelists to rate intensities of compounds coming through the GC-O, the same phenomena was observed (unpublished data). We concluded that measuring intensity of a single compound is difficult. Detection thresholds vary from day to day and among panelists. Adaptation, fatigue, and enhancement or suppression by other factors affect repeatability. We have determined that using GC-O is most suited for two purposes: (1) determining compounds with aroma impact and (2) describing the aromas of individual compounds eluting from the GC-O.

Relating the Compounds of GC-O to Food Aroma

One purpose of GC-O is to distinguish odor-active compounds from those volatiles without odor impact. GC-O allows one to determine the odor-significant compounds so the scientist can focus on these compounds and can work on their chemical origin or olfactory differences. Correlating GC-O responses to descriptive sensory responses has its limitations. Threshold concentration does not necessarily correlate with aroma intensity. Thresholds can vary with experimental conditions, and the intensity function changes due to concentration differences among volatile compounds. Ideally, intensity ratings of GC-O compounds would correlate better with descriptive aroma (Blank 1997). Kamath and others (2001) compared descriptive analysis of essential oils with GC-O evaluations and found that many descriptors developed in descriptive analysis did not overlap with GC-O descriptors. Generally, only three or four descriptors (out of six to seven descriptive analysis descriptors) were used by both the descriptor panel and the GC-O panel. Meanwhile more descriptors were generated by the GC-O panel than the descriptive analysis panel (Kamath and others 2001). Van Ruth and Roozen (1994) found 10 similar descriptors between GC-O and sensory analysis in rehydrated bell peppers. Meanwhile, there were still 10 GC-O descriptions that did not relate to sensory analysis descriptors (van Ruth and Roozen (1994). Le Fur and others (2003) developed nine aroma categories for French Chardonnay wines. Descriptive panelists and GC-O panelists described aromas for six wines, and all the terms were placed in the nine categories. Correspondence Analysis was used to plot the two sets of data (six samples and nine descriptors). Generalized Procrustes Analysis was used for comparison of the two spatial configurations (six wines and nine descriptor classes). They identified major

aroma compounds but could not account for the distinctive sensory profiles of the wines. The deficiency occurred when the descriptive panel observed a spicy character in each wine that was not characterized in the GC-O analysis. Compounds eluted that had an herbaceous character in the GC-O analysis but no herbaceous character was described by the descriptive analysis panel. Therefore, a relationship between herbaceous smelling compounds and the flavor descriptors could not be determined (Le Fur and others 2003).

GC-O has advanced the understanding of compounds that contribute to flavor, but it has not yet made it possible to determine the chemical make-up of common food flavors. Some off-flavors, consisting of few compounds have had their chemical composition identified, but deciphering flavors such as peanutty flavor has not been successful.

In the last half of the twentieth century, many strides were made in understanding flavor characterization. The developments in descriptive sensory methods and advances in the GC-O methods have advanced the understanding of flavor and aroma. Descriptive sensory analysis has made it possible to characterize the many flavor attributes in a product or product line. Flavor intensity rating methods have made it possible to compare flavor differences due to various treatments, ingredients, and processes. GC-O has made it possible to determine aroma active compounds eluting from the column. It has helped with identification of compounds that make up some flavors, but has shown more success in the identification of off-flavor compounds than in determining compounds responsible for desirable flavors.

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10
Chemical Characterization

Neil C. Da Costa and Sanja Eri

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Infrared Spectroscopy and Gas Chromatography-Infrared Spectroscopy
Nuclear Magnetic Resonance Spectroscopy
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INTRODUCTION

Because of the complexity of food matrices and the low concentration of flavor compounds within them, to characterize the flavor of a particular food, one has to first isolate and/or concentrate flavor compounds. Methods for isolation of flavor compounds include extraction with a solvent, distillation, headspace collection, etc. (Da Costa and Eri 2004). When using these methods, it is important to keep in mind that instrumental analysis will provide a true picture about flavor profile of a food only if the flavor of the isolate/concentrate resembles that of the starting material.

After a flavor has been isolated and concentrated, it is ready for chemical characterization. Several techniques can be employed to determine the chemical composition of flavor isolates. They include mass spectrometry (MS), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR). These principles can be used to identify the structure of most pure organic molecules. However, in flavor work, we are invariably dealing with chemical mixtures. For that reason, MS and IR have been coupled with separatory techniques, namely gas chromatography (GC) and liquid chromatography (LC), to form a powerful tool in dissecting and characterizing these mixtures. This chapter will outline the main techniques available to the analyst for characterizing flavor chemicals.

GAS CHROMATOGRAPHY

PRINCIPLE

The flavor of a food can contain from several hundred to over 1,000 chemicals. To obtain structural information about each flavor chemical in a mixture, they need to be separated. Gas chromatography has been successfully used for separation of volatile flavor compounds for decades. In this technique, flavor chemicals are introduced into a gas chromatograph through the injection port and carried by a carrier gas such as hydrogen, helium, or nitrogen through the analytical column to the detector. The GC column contains a polymeric stationary phase that absorbs and subsequently releases flavor compounds. The carrier gas is inert so it is not absorbed by the column, and it does not react with the flavor volatiles. Compounds entering a gas chromatograph get separated due to differences in partitioning behavior between the mobile phase (carrier gas) and

stationary phase. Depending on the concentration and thermal stability of the sample, different types of GC introduction systems can be used (e.g., split/splitless injector, on-column injection system, programmed temperature injector). Injection volumes usually range from 0.1 to 5 microliters (μL), depending on the concentration of the flavor sample. Due to different affinities of flavor compounds for the column's stationary phase, some compounds are retained longer than others. When compounds get released from the column, they are carried by the carrier gas toward the detector. Once they reach the detector, a signal is generated and their presence is recorded in a form of a "peak" whose area is proportional to the amount of the compound in the sample. The data are represented as a chromatogram, which is an x, y plot where the x-axis represents retention time (minutes) and the y-axis represents intensity of the signal (Figure 10.1). The detection of a compound can be based on several different principles; hence, there are several types of detectors.

Detectors

Due to its universality and low limit of detection (minimum detection level 5 picograms [pg] of Carbon/second [C/s]), the most common detector used for flavor analysis is the Flame Ionization Detector (FID). As its name says, this detector responds to molecules that ionize in an air-hydrogen flame. The sensitivity of detection by FID is dependent on the number of carbon atoms—the more carbon atoms in the molecule of the compound, the better its detection. To compensate for the difference in detection, response factors that take into account carbon atoms and molecular weight of the molecule can be used. The disadvantage of FID is that compounds that contain large numbers of sulfur, nitrogen, or oxygen atoms can be undetected. This is especially important in the case of sulfur- and nitrogen-containing compounds that are among the most powerful flavor chemicals and are often present in trace amounts in foods. Fortunately, there are several selective detectors that can be employed for detection of sulfur and nitrogen compounds. Since these detectors can be tuned so that they respond only to sulfur or nitrogen atoms, they can also provide valuable information toward identification of compounds in a mixture (Westmoreland and Rhodes 1989, Mussinan 1993).

Most commonly used for sulfur compound detection (such as thiophenes, mercaptans, thiols, thioesters) are the Flame Photometric Detector (FPD) (detection limit 20 pg of sulfur[S]/second, and 0.9 pg of phospho-

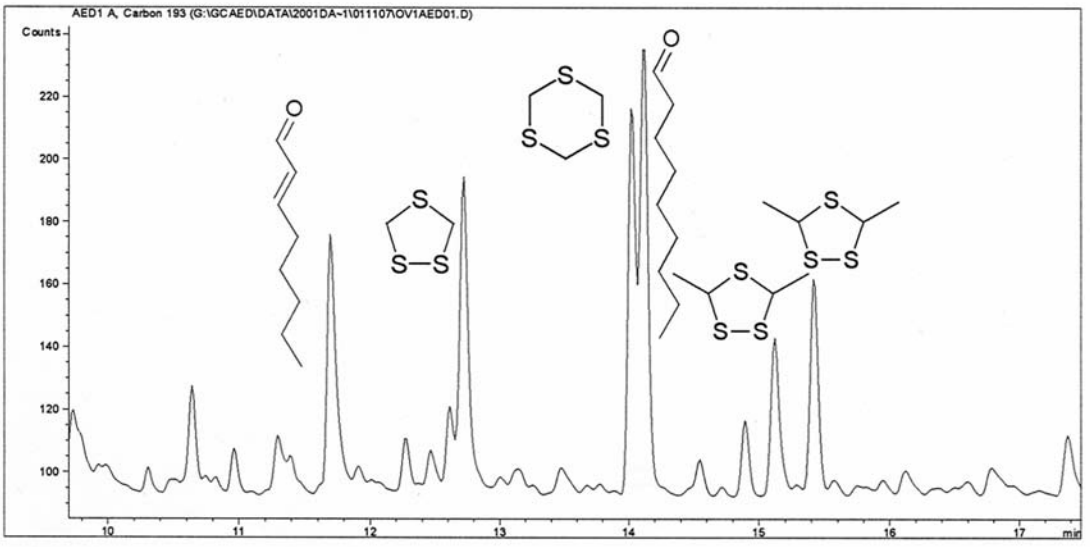

Figure 10.1. GC-FID Chromatogram of Fried Shrimp Steam Distillate (From left to right: trans-2-Octenal, 1,2,4-Trithiane, 1,3,5-Trithiolane, Nonanal, 3,5-Dimethyl-1,2,4-Trithiane I & II).

rus [P]/second), Sulfur Chemiluminescence Detector (SCD), and Atomic Emission Detector (AED) (detection limit 0.1 pg to 1 nanogram [ng]). The most common detectors employed for nitrogen compounds (such as pyrazines, pyridines, pyrroles, amides, amines) are Nitrogen-Phosphorus Detector (NPD) (detection limit 0.4 pg of nitrogen [N]/second, and 0.2 pg of P/second), and AED. Since an AED can be tuned for any element, it can also be used for selectively detecting compounds that contain oxygen (alcohols, aldehydes, etc.) (Mussinan 1993). Figures 10.1 and 10.2 show chromatograms of fried shrimp steam distillate obtained by GC-FID and GC-AED, respectively.

Gas Chromatography-Olfactometry

Soon after the invention of gas chromatography, flavor chemists started "sniffing" the effluents coming out from a GC column. This technique, called gas chromatography-olfactometry (GC-O), is still a very valuable tool in the identification of flavor molecules. In a standard setup, a gas chromatograph is fitted with an FID and an odor port. The end of the GC column is attached to a "splitter" or Y-connector, which splits the effluent between the FID and odor port. The odor port is also connected to a small water reservoir to humidify the effluent and prevent drying out of nasal passages. In the past, the sample was passed through a thermal conductivity detector (TCD), which was nondestructive and allowed 100% of the effluent to pass through to the odor port. However, this detector was not as sensitive as the destructive FID.

An experienced flavorist sits by the GC and sniffs the carrier gas as it flows from the column. The odor of each component is recorded and makes the basis for creating an "aromagram." At the same time, the FID records a regular chromatogram that can be compared with the "aromagram" to identify areas that contain odor-active compounds. If the results of a GC-O analysis are used in conjunction with GC-MS analysis, the analyst can focus further on identifying the chemicals that are odor-active. The GC-O technique has also been used to identify odor thresholds of flavor compounds by analyzing a series of dilutions (Drawert and Christoph 1984, Leland and others 2001, van Ruth 2001).

Retention Times

So far, we have shown that gas chromatography can be used for the separation and detection of volatile

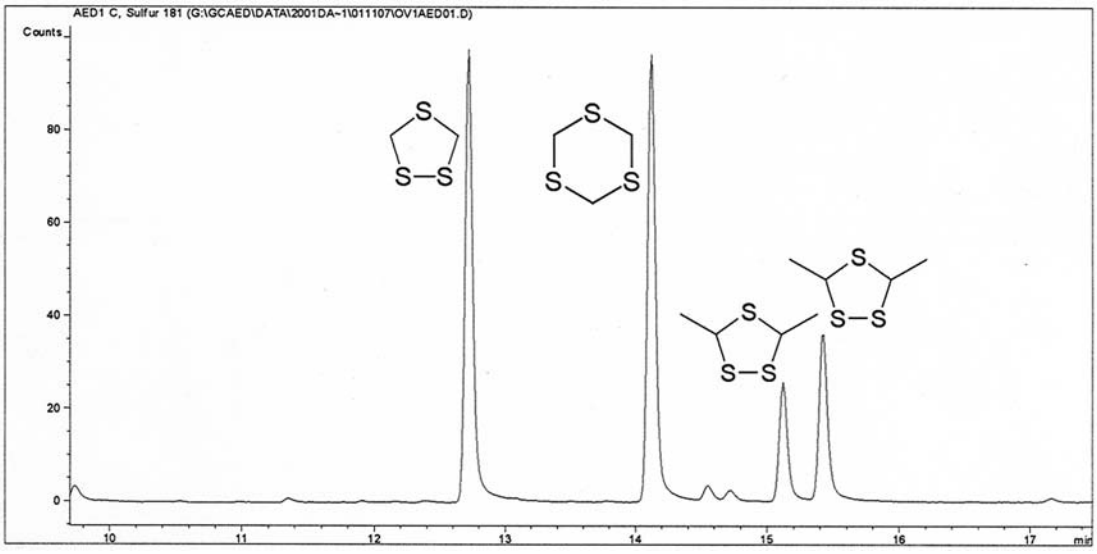

Figure 10.2. GC-AED Chromatogram of Fried Shrimp Steam Distillate (From left to right: 1,2,4-Trithiane, 1,3,5-Trithiolane, 3,5-Dimethyl-1,2,4-Trithiane I & II).

compounds. However, gas chromatography can also provide valuable information toward the identification of flavor compounds. Upon introduction to a GC, volatiles spend a finite period of time on the GC column before being detected. If the conditions under which the GC is operated remain constant, the retention time of a compound will remain constant as well. The retention time can therefore be used toward identification of a compound. If a standard of the suspected compound is available, it can be analyzed under the same conditions and should elute at the same time as the unknown. Due to the differences between individual GC instruments and also the length of a GC column (if a GC column gets moved from instrument to instrument, it may be cut a little bit each time), this retention time can be slightly different even if the GC conditions are the same. To overcome that, relative retention times (retention indices) that relate the retention time of a compound to that of a standard compound or a series of standard compounds have been employed. Most commonly used for that purpose is an n-paraffin standard mixture, which is used for calculation of Kovats indices (Kovats 1958), and McReynolds constants (McReynolds 1966, Jennings and Shibamoto 1980). Another example of the standard mixture is an ethyl esters mixture (Mussinan 1993).

As mentioned before, a GC column retains volatiles based on their affinity for the column's polymeric stationary phase. Stationary phases can have different functionalities. Most commonly used for flavor work are methylsilicone based, nonpolar phases such as OV-1, and polyethylene glycol based, polar phases such as Carbowax. The basis for separation on these two phases is quite different. While a nonpolar phase separates volatiles based primarily on their boiling point (the highest boiling compounds elute last), volatiles are separated on polar columns primarily according to their polarity (the most polar compounds elute last). To make use of these two different separation principles, it is standard practice to analyze flavor samples on both columns. That way, co-elutions on a nonpolar phase can be resolved on a polar phase and vice versa. For example, polar compounds such as acids do not elute well on nonpolar columns; they tend to broaden and cover the surrounding peaks. On polar columns, acids form nice, sharp peaks. Moreover, the peaks that were in the vicinity of the acid in question on a nonpolar column most probably elute in a completely different place on a polar column. The number of detected compounds is often much higher when two analyses are combined as compared to the analysis obtained by using only one phase.

With a few exceptions, the standard retention index of a particular compound is rarely the same for polar and nonpolar columns. For identification purposes, both values are used. To unambiguously identify an unknown compound, in addition to mass spectrometric identification, both retention indices have to match that of a standard. The retention indices can be very helpful in cases where two different compounds have very similar mass spectra or in the case of *cis* and *trans* isomers. The retention index then provides crucial evidence for confirmation of the identity of an unknown.

Another example of using GC for identification purposes would be if we already suspect the identity of the compound in a sample. In this case, we can "spike" the sample with the standard of that compound and reanalyze it. If the peak area of the unknown becomes larger on polar and nonpolar columns, there is a pretty good chance that the compound in question is indeed the one we suspected.

Chiral Separations

Chiral compounds are compounds whose molecules contain one or more chiral carbon atoms (i.e., a carbon atom connected to four different substituents). For each chiral carbon atom present, chiral compounds have two forms (enantiomers), which differ in the direction in which they rotate plane-polarized light and are nonsuperimposable mirror images of each other.

The importance of chiral compounds in flavor work lies in the fact that enantiomeric pairs can have different organoleptic properties. The best example is carvone, where d-carvone smells like caraway and l-carvone smells like spearmint. It is also possible that enantiomers have the same odor description but different strengths (Pickenhagen 1989).

The ability to resolve enantiomers of a particular chemical can be very helpful in proving authenticity. Most natural materials contain mostly one enantiomer of the chiral compound while synthetic chemicals generally contain a racemic mixture comprising of equal amounts of d- and l-isomers.

Two enantiomers of the same compound elute at the same time on a normal GC capillary column. To resolve them, a special chiral column is needed. It is

also possible to make diastereoisomeric derivatives that can be analyzed by achiral columns (Konig 1984, Allenmark 1988). Other nonchromatographic methods that can be used for resolving enantiomers are optical rotation measurements, differential scanning calorimetry, isotope dilution, and NMR spectroscopy (Mussinan 1993).

Multidimensional Gas Chromatography

If the mixture contains many components that are hard to separate, or many trace components, an analyst may use a technique called multidimensional gas chromatography (MDGC) or GC-GC (Schomburg and others 1984, Cronin and Caplan 1987). In this technique, the GC contains two columns, which usually differ in polarity. Selected fractions from the first column are directed onto the second column (a technique called "heartcutting"), which can provide better resolution of the compounds within that fraction. Also, if multiple injections are performed, trace components can be significantly enriched on a second column and thus the possibility for their identification by MS increased.

A new version of the multidimensional gas chromatography, called comprehensive two-dimensional gas chromatography (GC×GC), has recently been developed. With this technique, instead of transferring only specific fractions of the chromatogram from the first to the second column, the entire effluent from the first column is reanalyzed by the second, short GC column (second "dimension" is fast GC). The interface between the two columns is a modulator that increases the amplitude of the signal that goes from the first column into the second one. The modulator traps and releases signal in portions. Each fraction is analyzed on the second column separately. By this approach, peak capacity becomes significantly increased and a better picture of complex mixtures can be obtained (Dimandja 2003, Dimandja and others 2003).

Preparative Gas Chromatography

In addition to MS and IR, it is often the case that NMR analysis is needed to confirm the structure of the compound. For NMR analysis, the compound needs to be as pure as possible. Unfortunately, the amount needed for the analysis is usually many times more than the amount present in an extract.

If an unknown component is found at a significant concentration within an extract, and it is well separated from neighboring compounds, it may be possible to use preparatory gas chromatography to isolate enough of a pure component to perform a proton NMR. The preparatory process involves injecting large amounts of a sample into a GC column and collecting the fraction of interest from multiple runs. In a typical setup, the column effluent is split in such a way that one part goes to the FID and the other part can be collected for further analysis. The analyst can monitor the GC display indicating the peak elutions and attach a glass capillary tube (with some sort of cooling involved) to the column outlet at the point at which the unknown elutes. After the unknown stops eluting, the tube is removed from the outlet. Repeating this exercise several times in the same glass tube would usually yield enough sample to rinse with deuterated solvent into an NMR tube and hence determine the structure. This manual process has been made easier by automated preparatory-GC instruments, which can collect the same fraction from many runs with great reproducibility. For other aspects of gas chromatography not described in this section, the reader is encouraged to choose among many books and papers describing GC methods and applications (Grob 1985, Mosandl 1992).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Principle

In gas chromatography-mass spectrometry (GC-MS), compounds are separated on a GC column and subsequently introduced into a mass spectrometer. A mass spectrometer consists of three parts: an ion source, a mass analyzer, and an ion detector. Upon introduction to the mass spectrometer, molecules enter an ion source. There, due to the input of energy, the molecules are fragmented into ions. Each ion has a mass and a charge and therefore its characteristic mass-to-charge ratio (m/z). Based on their mass-to-charge ratios, generated ionic fragments are separated by the mass analyzer and focused toward the ion detector. Several types of mass analyzers available nowadays (i.e., quadrupole, time of flight, ion trap, double focusing magnetic/electrostatic field analyzers) differ in the way they separate the ions (i.e., by radio frequency, by magnetic field, etc.)

and subsequently in their sensitivity and resolution. Once ions reach the detector, a signal that provides a measure of the abundance of the fragments (ions) is produced. At the end of a mass spectrometry run, a Total Ion Chromatogram (TIC), which looks like a gas chromatogram, is generated. Each peak in the TIC, however, contains not only information about the abundance of the compound eluting at a certain time, but its fragmentation fingerprint (mass spectrum) as well. A mass spectrum is a representation of the ions observed by the mass spectrometer. The spectrum is normalized so that the most abundant fragment has 100% intensity. It could be in the form of a written report or a graphic representation (a bar graph where x-axis is the mass-to-charge ratio and the y-axis is the intensity scale). Figure 10.3 shows a graphic representation of a mass spectrum of the monoterpene, carvone.

Due to the fact that organic compounds have a characteristic fragmentation pattern by which they can be recognized, mass spectrometry can be used for identification purposes. Libraries containing mass spectra of organic compounds are commercially available. Most of the flavor volatiles can be identified by comparing individual spectra with those in a reference spectrum library. If a compound has *cis* and *trans* isomers, a retention index will come in handy. If, however, the mass spectrum of the unknown cannot be found in the library, its structure has to be deduced using knowledge about the rules that govern fragmentation (McLafferty and Turecek 1993, Kitson and others 1996, Lee 1998).

Electron Ionization (EI)

There are two ionization techniques used in mass spectrometry, namely electron ionization and chemical ionization. The most common ionization technique is electron ionization. In this ionization mode, molecules in an ion source are bombarded by a stream of electrons having energy of 70 electron volts (eV). Due to such high-energy input, this technique produces many fragments and therefore, each compound has a very detailed "fingerprint" that is used for identification purposes (commercially available libraries are EI spectra libraries).

However, with the use of EI, some compounds may undergo extensive fragmentation and may lack their molecular ion in the spectrum. This is often the case with long chain aliphatic compounds. When an EI-MS spectrum cannot provide information about molecular weight, a different, softer ionization technique called chemical ionization (CI) can be employed.

Chemical Ionization

In chemical ionization (CI), instead of high-energy electrons, the sample is bombarded with ions of a reagent gas such as ammonia, methane, or isobutene, which are initially ionized by high-energy electrons. During the collision, reagent gas molecules can transfer a proton to the molecule or take a proton from them (depending on the molecule and the reagent gas), hence positive ion and negative ion chemical

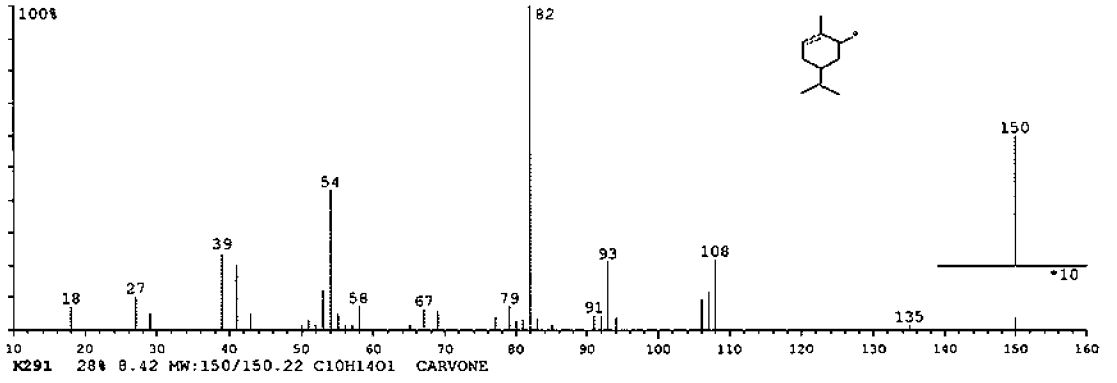

Figure 10.3. EI mass spectrum of carvone.

ionization. In positive ion CI, the molecular fragment produced is a protonized molecular ion having molecular weight increased by 1 (M+1). The fragmentation of the molecules in CI is significantly reduced when compared to that occurring when EI is used. Therefore, CI spectra can provide useful information about the molecular weight of the compound but much less about its structure. Note that methane is a more energetic molecule than isobutane and when used in CI tends to cause more fragmentation than isobutane, but less than in EI.

Figures 10.4, 10.5, and 10.6 show the mass spectra of lavandulyl acetate as obtained by EI and by CI using ammonia and isobutane as reagent gases, respectively. For more information about the use of mass spectrometry in flavor characterization and specific applications, please refer to the many excellent books and papers published on this subject (Cronin and Caplan 1987, Gilbert 1987, Herderich 1999).

Tandem Mass Spectrometry

In tandem mass spectrometry (MS-MS), two or more mass analyzers are coupled together. The first mass analyzer separates the ions produced in the ion source of mass spectrometer and provides a mass spectrum of the mixture. After selecting one ion of interest, the first mass analyzer is set to pass only that ion. The selected ion (parent ion) travels to the collision chamber where it undergoes collisionally activated dissociation (CAD) and produces "daughter ions." Daughter ions get separated by a second mass analyzer and subsequently recorded. The daughter ions spectrum can be used to identify the compounds by comparison to reference spectra. An advantage of this technique is that it can be used for the analysis of mixtures without prior separation of flavor compounds, since fragment ions derived from a parent ion can be recorded without interference from other ions in the primary spectrum. In addition to structural elucidation, this technique is very useful when looking for a specific compound in the mixture (for example in stability studies). Moreover, MS-MS could be helpful for the functional group analysis by looking at the losses of specific neutral molecules from the parent ion (Startin 1987).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

So far, we have described instrumental methods used for characterization of volatile compounds. However, nonvolatile compounds such as sugars,

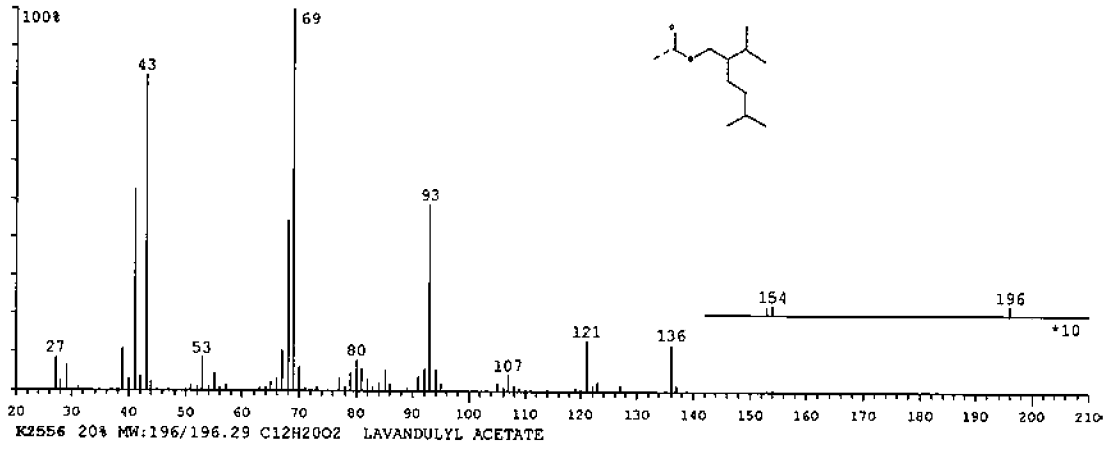

Figure 10.4. EI mass spectrum of lavandulyl acetate (MW 196).

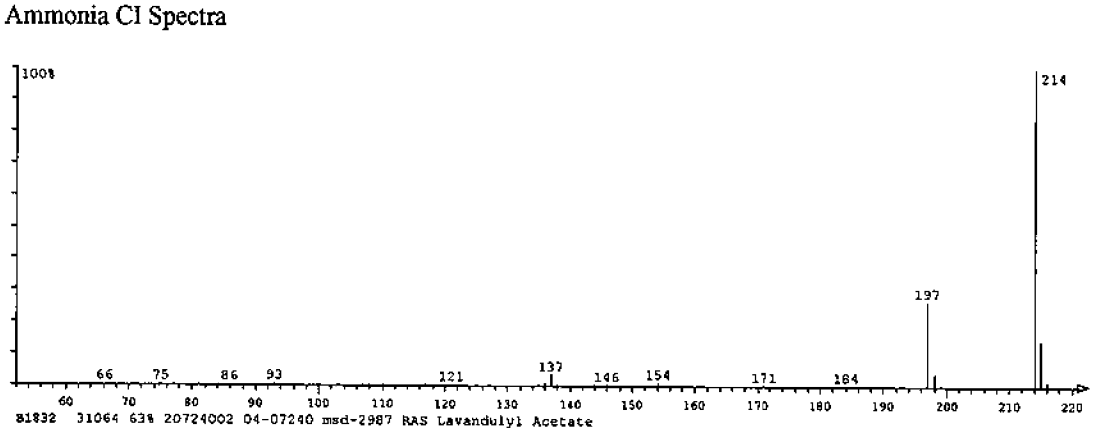

Figure 10.5. Ammonia CI mass spectrum of lavandulyl acetate (Note the ammonia adduct 214 m/z).

proteins, and fats play an important role in the perception of food flavor. The identification techniques used for characterization of nonvolatile compounds include the ones described for volatiles (MS, IR, NMR). The difference is in the technique used to separate nonvolatiles before their detection. For that purpose, the technique of choice is generally high performance liquid chromatography (HPLC). As in gas chromatography, a sample is introduced onto a column and carried toward the detector, except in HPLC it is carried by a liquid phase (solvent or mixture of solvents) rather than a gas phase. The detectors used for HPLC are generally nondestructive (such as ultraviolet-visible [UV-VIS] detector) in which case fractions of the sample can be collected after detection and used for sensory evaluation and spectroscopic identification. By using standard dilutions of known compounds, it is possible to determine the concentration of these compounds in food. If, however, we need the identification of an un-

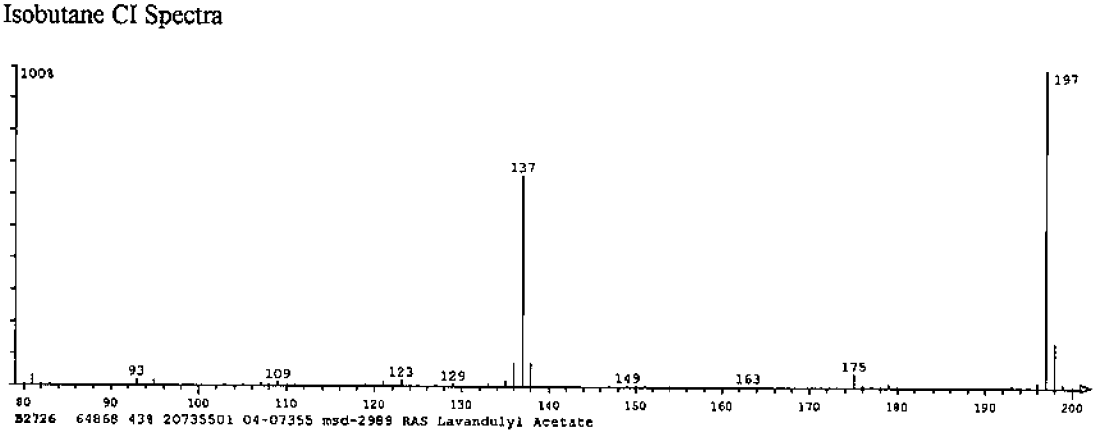

Figure 10.6. Isobutane CI mass spectrum of lavandulyl acetate (Note the proton addition 197 m/z).

known compound, mass spectrometry coupled with HPLC (known as LC-MS) would be the method of choice (Games 1987).

In addition to the determination of nonvolatiles, HPLC and LC-MS are often used for identification and quantitation of very polar or semivolatile compounds (such as vanillin, maltol, furaneol). These compounds do not extract well into a nonpolar solvent during sample preparation for GC analysis. Also, they are usually under detected by a flame ionization detector or in the case of vanillin can sublimate in the column reappearing in subsequent runs.

Due to the high temperatures used for volatilization in GC injector port, thermally labile compounds could break down. For characterization of these compounds, in addition to the on-column injection, HPLC and LC-MS may be the methods of choice (Hartman and others 1989).

INFRARED SPECTROSCOPY AND GAS CHROMATOGRAPHY-INFRARED SPECTROSCOPY

Infrared spectroscopy has been a valuable technique to the analytical chemist for many decades. In this technique, IR radiation is passed through a sample so that when the frequency of the radiation delivered and absorbed corresponds to that of a vibrational mode of a molecule, an IR band in the spectrum is produced. The spectrum usually consists of a plot of wavelength versus transmittance or absorbance. Commercial libraries are available for matching against (Sadtler Standard Spectra). The development of Fourier Transform Infrared (FTIR) has led to faster and better quality spectra. FTIR refers to a fairly recent development in the manner in which the data are collected and converted from an interference pattern to a spectrum.

Infrared spectroscopy gives important information about the functional groups attached to any given molecule. It is able to determine the presence of alcohols, amines, carbonyl compounds, etc., as well as differentiate between aldehydes and ketones and *cis* and *trans* isomers, which is not always possible by mass spectrometry. Figure 10.7 shows an FTIR spectrum of the monoterpene, carvone.

To get a clean interpretable spectrum, a pure substance is usually required. Mixtures are difficult if not impossible to interpret. The sample may be run on a spectrophotometer in several ways. Traditionally, potassium bromide (KBr) plates were used for running liquid samples. This material had few if any interfering bands in the infrared region. A drop applied between two plates produced a perfect spectrum. Solids were similarly handled by forming a thin paste or "mull" using nujol, organic oil. However, this gave interfering bands in the IR spectrum. A more convenient technique for solids is a KBr disc. A small amount of sample is ground into a fine powder with crystalline KBr and poured into a small die within a press. Physical pressure is applied and reduced gas pressure removes any low boiling vapors and moisture. Practice is required to produce a fine translucent disk, which gives a clean spectrum.

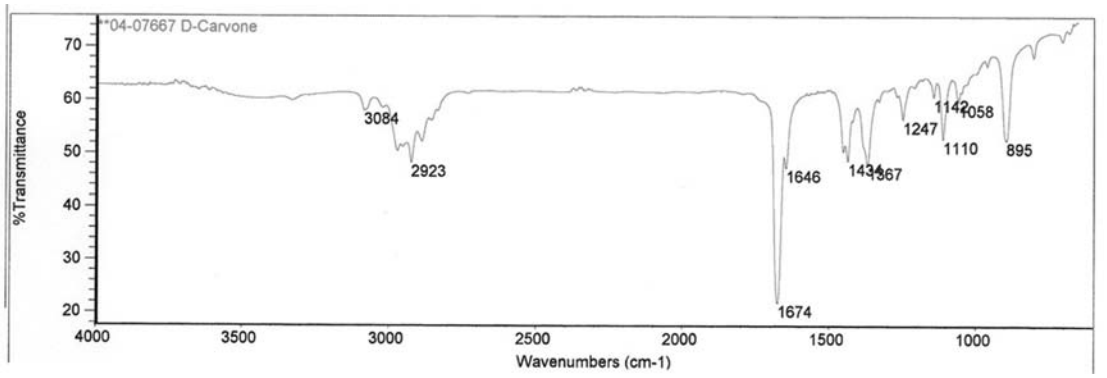

Figure 10.7. FTIR spectrum of 1-carvone.

More recently, IR has been combined with GC and even MS to give a more powerful technique for analysis of mixtures. In these techniques, all spectra obtained are vapor-phase spectra. They still give important molecular data, but tend to be less detailed than liquid or solid spectra. When compared to mass spectroscopy, a much higher concentration of sample is required to give detailed spectra. However, the technique is nondestructive thus enabling recovery of the sample for further use (Idstein and Schreier 1985).

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

NMR is a technique that makes use of the fact that nuclei of a material placed in a strong magnetic field absorb radio waves supplied by a transmitter at par-

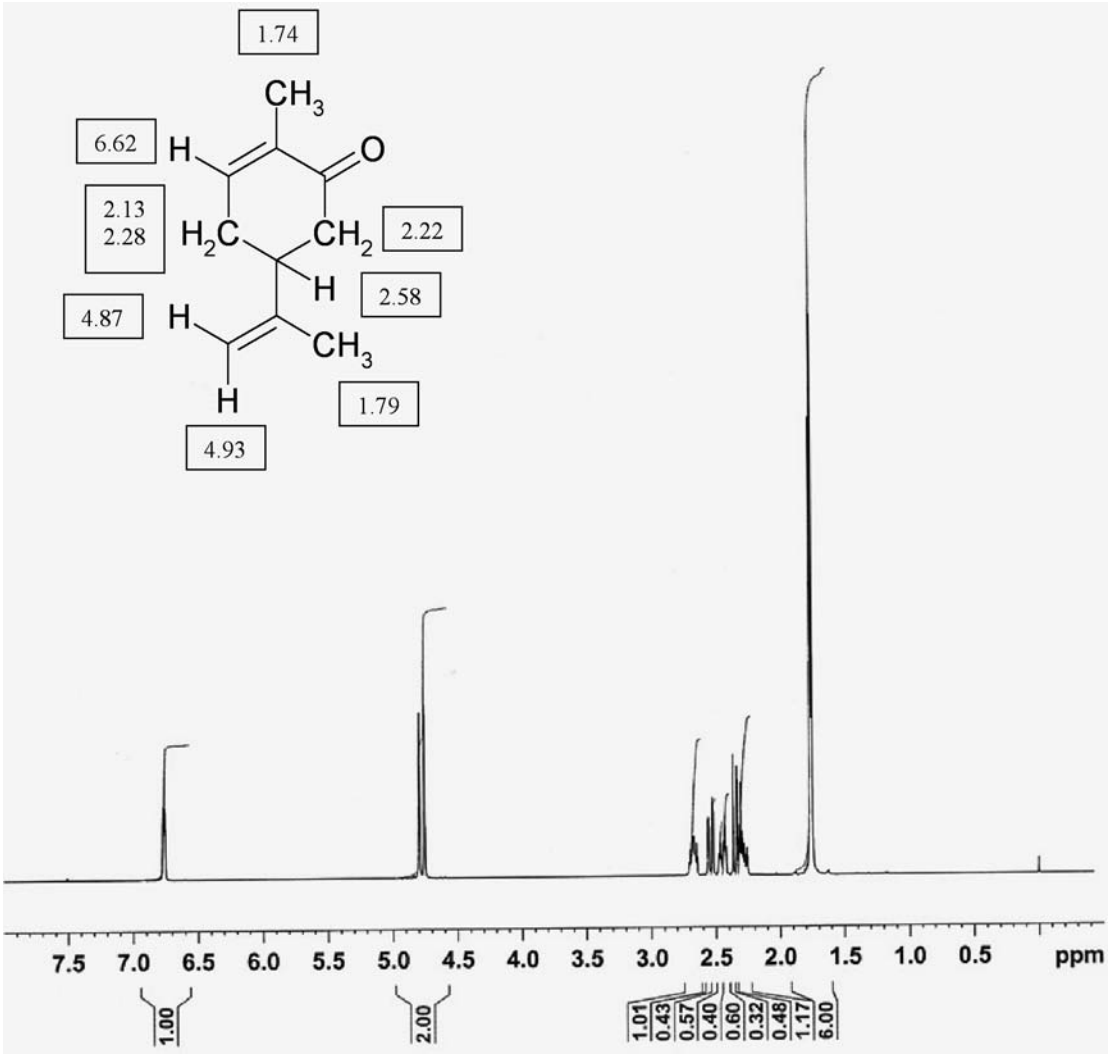

Figure 10.8. Proton ^{1}H spectrum of carvone.

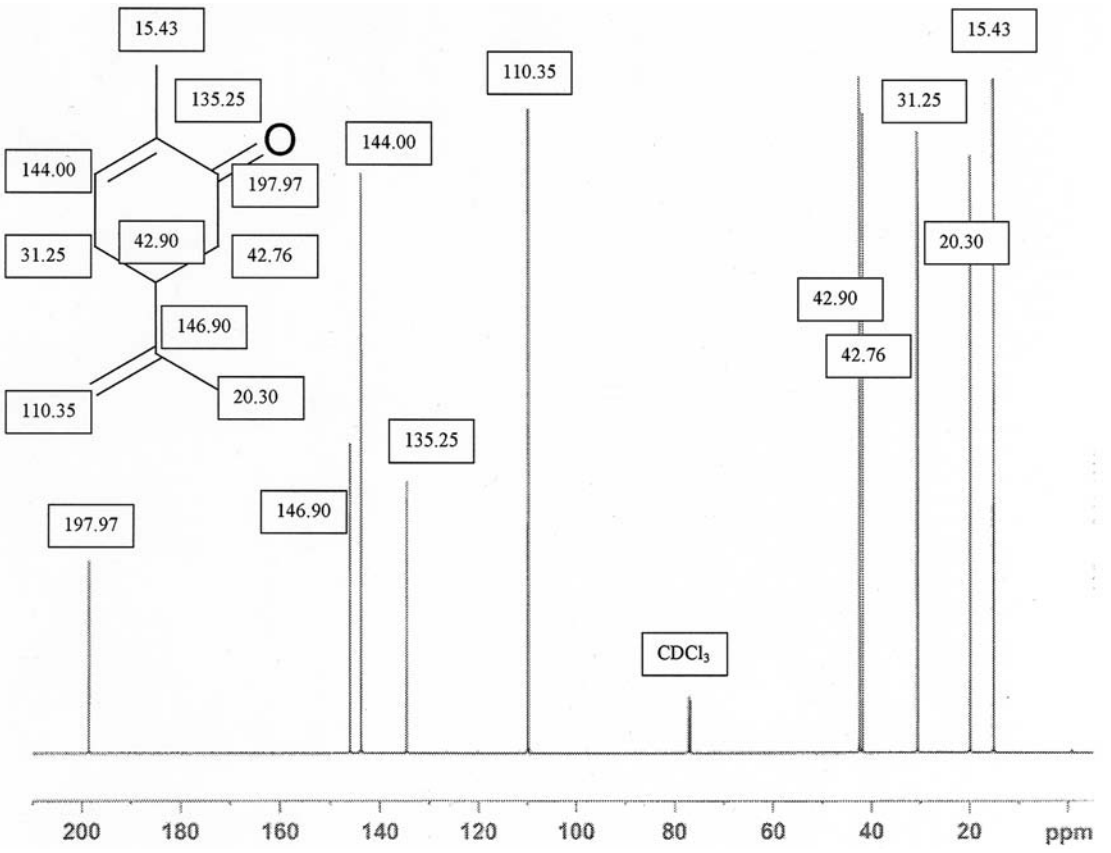

Figure 10.9. Carbon ^{13}C spectrum of carvone.

ticular frequencies. The energy of the radio-frequency photons is used to promote the nucleus from a low-energy state, in which the nuclear spin is aligned parallel to the strong magnetic field, to a higher-energy state in which the spin is opposed to the field. When the source of the radio waves is turned off, many nuclei will revert to the lower energy state by emitting photons at characteristic resonance frequencies, thus providing information about the sample structure.

NMR, like X-ray crystallography, can determine the absolute structure of an unknown molecule. In its main mode of operation, it yields information about the position of the hydrogen ^{1}H and carbon ^{13}C atoms in a molecule (Kubeczka and Formacek 1984). NMR is generally used to identify pure unknowns since, unlike IR and MS, there are no current commercial instruments available that link NMR with GC. The time required to obtain sensitive and detailed NMR spectra is too long for normal GC techniques. However, NMR has been successfully coupled with liquid chromatography. Fourier transform application to data acquisition has greatly enhanced this technique.

Like IR, NMR is also a nondestructive technique enabling the sample to be used for further tests. Solvent is required for each sample and that can cause interfering bands and peaks. Solvents most often used are deuterated chloroform, methylene chloride and dimethyl sulfoxide. In practice about 20 μg of sample is required for proton NMR and 300 μg for carbon NMR. Figures 10.8 and 10.9 show proton and carbon spectra of carvone in deuterated chloroform ($CDCl_3$), respectively. Figure 10.10 shows the 135° Distortionless Enhancement by Polarization Transfer (DEPT) spectrum of carvone. DEPT is a

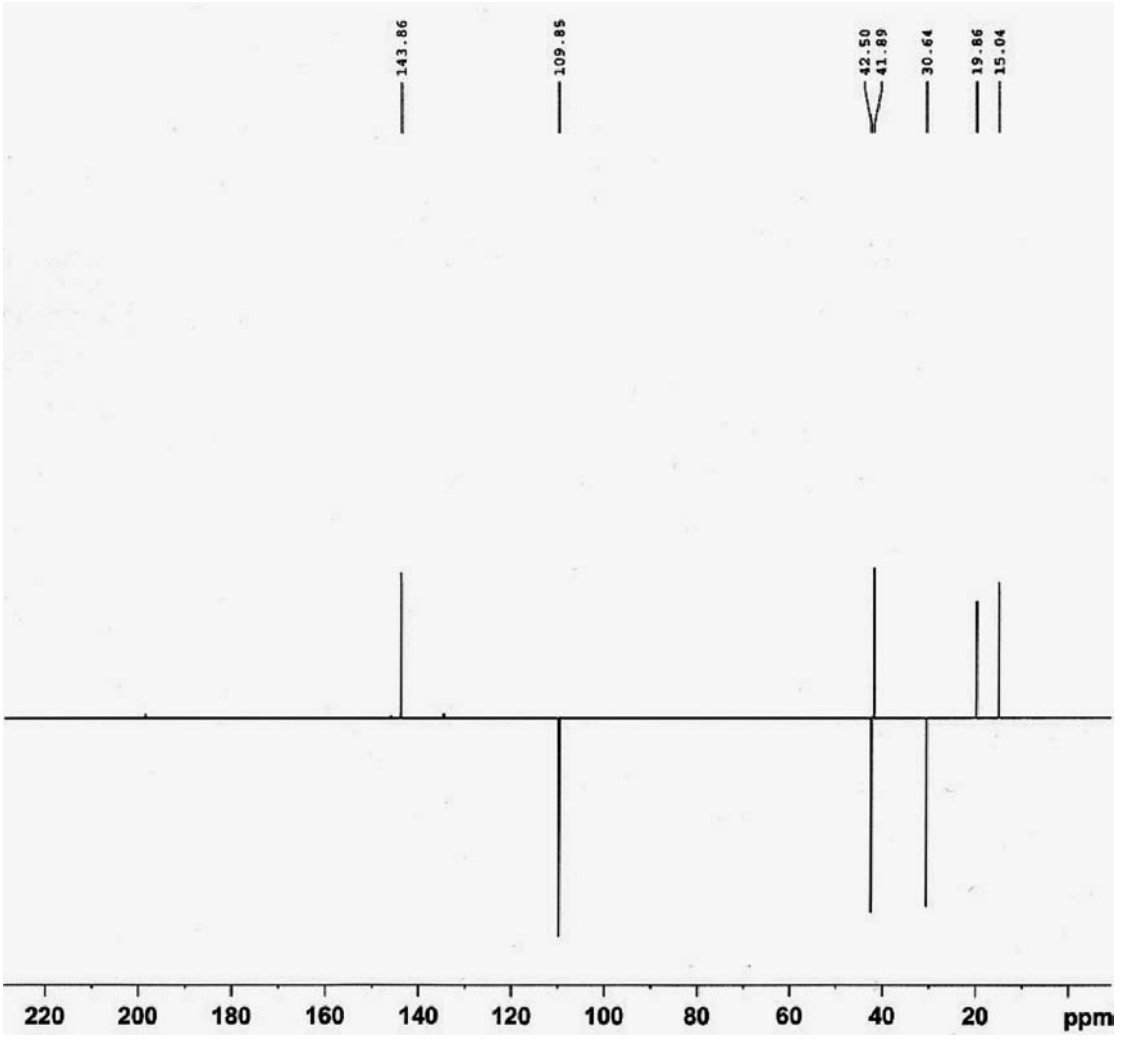

Figure 10.10. DEPT spectrum of carvone.

Fourier transform data processed spectrum technique that shows all methyl (CH_3) and methine (CH) protons above the baseline, all methylenes (CH_2) below the baseline and quaternary carbons disappear altogether. Thus, it provides valuable information about molecular structure. Figure 10.11 shows a 2-D proton COSY (Correlation Spectroscopy) spectrum of carvone. A 2-D proton and carbon correlation spectrum, which shows which protons are directly linked with which carbons in a molecule, is also obtainable.

Carbon-13 and deuterium are the most common nuclei from which NMR spectra are generated. However, by using a multinuclear probe, it is also possible to generate similar spectra for compounds containing ^{15}N, ^{17}O, ^{19}F and ^{31}P (Brown and others 1988).

A recent development in NMR techniques is Site Specific Natural Isotope Fractionation (SNIF-NMR). This technique has been used to determine the authenticity of foods and natural materials such as wines, fruit juices, and vanilla extracts. It is dependent upon the natural abundance and distribution of deuterium atoms within a molecule. The distribution of deuterium atoms has been found to differ depending upon the source of the molecule. Therefore,

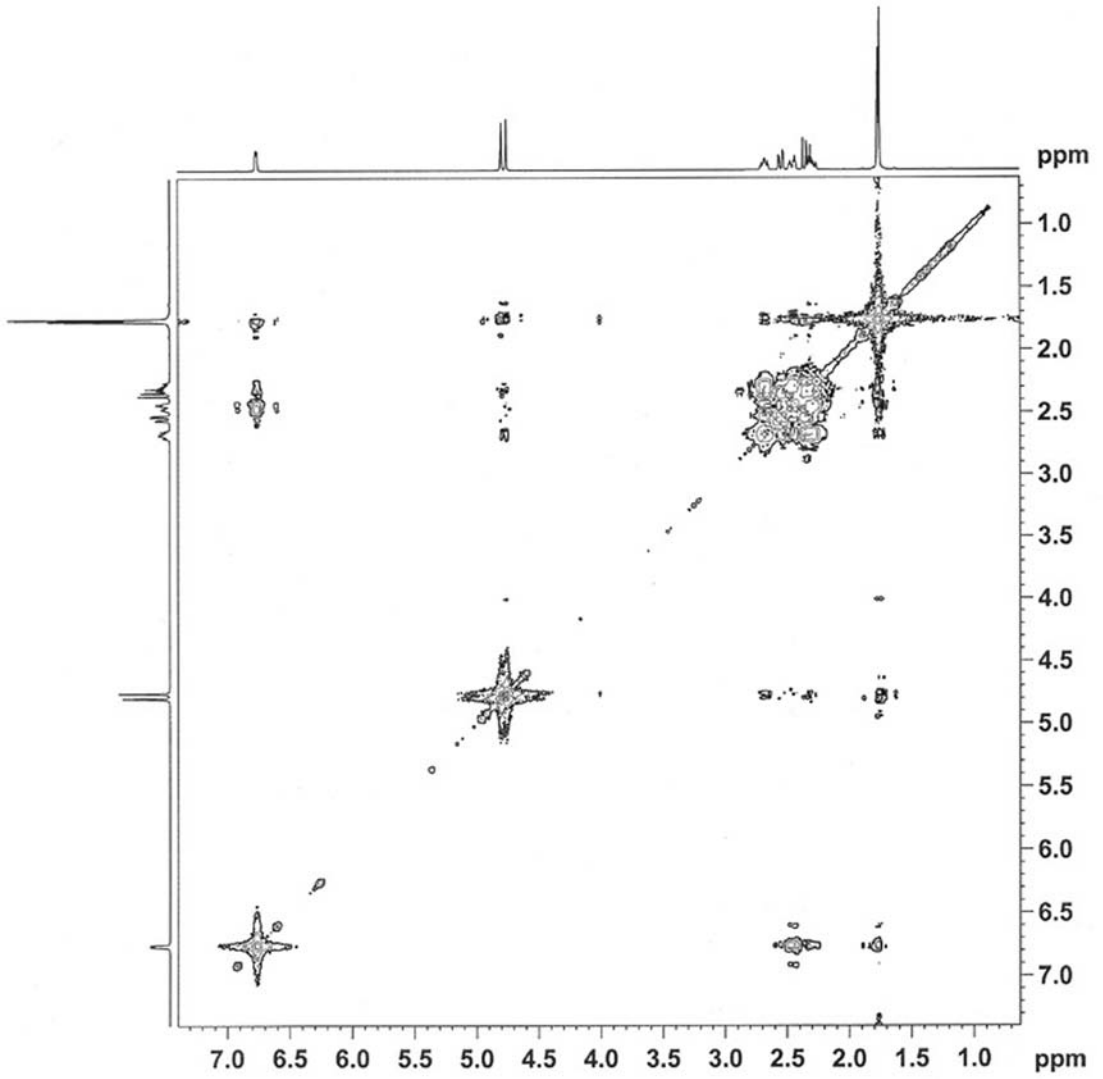

Figure 10.11. 2-Dimensional COSY NMR spectrum of carvone.

an isotopic fingerprint can give information about origin of the sample. A drawback is the relatively large amount of sample required for the analysis. The technique is described in greater detail by Martin and Martin (1999a, 1999b).

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Handbook of Meat, Poultry and Seafood Quality
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11
Chemistry, Technology, and Safety of Synthetic Flavors

Rakesh K. Singh and Epal Singh

Introduction
Synthetic Flavor Compounds
- Green Grassy Flavor
- Fruity Flavor
- Citrus Flavor
- Mint Flavor
- Floral Sweet Flavor
- Spicy Herbaceous Flavor
- Woody Smoky Flavor
- Roasty Burnt Flavor
- Caramel Nutty Flavor
- Bouillon Hydrolyzed Vegetable Protein Flavor
- Meaty Flavor
- Fatty and Cheesy Rancid Flavor
- Dairy Buttery Flavor
- Mushroom and Earthy Flavor
- Celery Flavor
- The Sulfurous Flavor

Flavor Quality
Safety
- JECFA Safety Evaluation Procedure for Flavoring Substances
- Safety Evaluation of Flavoring Substances in the U.S. and European Union
- Issues and Challenges

References

INTRODUCTION

Food flavoring substances are a unique class of food ingredients being an essential element in practically all foodstuffs whether prepared fresh or processed for sale in the marketplace. Whether we accept or reject food depends mainly on its flavor, which plays a very important role in the palpability of food and is one of the key parameters determining the overall quality of a food product. Flavor is defined as the combined perception of mouthfeel (texture), taste, and aroma (odor) (Ney 1988). It is a multidimensional attribute, which needs to be studied from various aspects including (a) analytical, synthetic, and organic chemistry of flavor compounds, (b) biochemistry of flavor formation, and (c) biology of flavor perception. Schrankel and others (2002) defined flavor as the sum total of the sensory responses of taste and aroma combined with the general tactile and temperature responses to substances placed in the mouth, and further stated that flavor can also mean any individual substance or combination of substances used for the principal purpose of eliciting these responses. The perception by the human sensory system and the measurement of this phenomenon are of interest not only to food scientists but also to manufacturers since it drives the consumer's preference for a product. Creation of a flavor that smells and tastes close to nature has been an ongoing challenge for decades. Flavorists strive to imitate nature in creating flavors that mimic the fresh, juicy, green, and ripe aroma and taste of the original food, spice, or herb. In the past, creation of "natural-like" flavors was not possible because of various technical challenges. These included the lack of sophisticated analytical instruments and techniques that could identify the true drivers needed to help flavorists re-create nature. The dilemma flavorists often faced was that their noses could identify aromas but the instrumentation was not capable of identifying those chemicals accurately. This prevented flavorists from

identifying those key ingredients and synthesizing the molecules that occur in nature.

Flavor compounds, substances stimulating taste and smell, are extremely important for food, animal feed, cosmetics, and pharmaceutical industries. The global food market is considerable and amounts to approximately 25% of the total food-additive market. Throughout history, men have sought to make their food more appetizing, first by using spices and herbs, and then by the spirits of fruits and aromatic plants or by essential oils. Supply of these commodities was obviously limited in terms of both availability and quality, especially as the size of markets increased in the nineteenth century with the growth of modern consumerism. Then, as a result of advances in chemical analysis and synthetic organic chemistry, came an increasing number of nature-identical and synthetic flavor chemicals, which allowed improved fidelity to the original materials and greater flavor intensity, stability, and reproducibility. Around 1985, a range of natural flavors chemically produced by enzymatic, microbial, or mild chemical processes became available so that good quality flavor formulations can be made whose compositions closely resemble the analysis of natural extracts of the fruit. A big advantage of this approach is that a range of new raw materials can be utilized that may be cheaper and more available than the traditional raw materials used to manufacture flavors. A main challenge in this approach remains the development of high-yielding and cost-effective processes. More recently, chemically synthesized flavors have made their appearance. Synthetic flavor substances are compounds that have not yet been identified in plant or animal products for human consumption. Alcohols, aldehydes, ketones, esters, and lactones are classes of compounds that are represented most frequently in natural and artificial fragrances. The need for synthetically prepared flavor compounds arises from the fact that during the storage of foodstuffs a certain loss of flavor is inevitable. These losses can be compensated for by adding synthetically produced flavor compounds. Besides this, synthetic flavor compounds have the great advantage of being available in the required quantity and quality irrespective of crop variation and season. A constant quality permits standardization of the flavorings. Synthetically produced compounds make it possible to vary the proportions of single components and thereby create new flavor notes.

The International Organization of the Flavor Industry (IOFI 2003) founded in 1969, in Brussels, Belgium, represents the global flavor industry and provides the industry, its customers, government agencies, and consumers with sound scientific information, education, and training to promote the benefits and safe use of flavors. The Global Scientific Manage-ment Committee (GSMC) of IOFI is responsible for the science program. One of its main objectives is in the area of science in which it maintains and supports a consistent global approach, based on sound science, for the safety assessment of flavoring ingredients. IOFI collects confidential information on a worldwide basis on the identity and use levels of flavoring substances and collects data on safety studies that are provided to scientific bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for use in evaluating the safety of flavorings.

SYNTHETIC FLAVOR COMPOUNDS

Synthetic flavor compounds (i.e., nature identical products) cover the whole range of organic substances but no obvious relationship has been established between structure and flavor properties. Some components of similar structure have broadly similar odors but there are many exceptions. One of the many possible ways to describe flavor substances or ingredients used in the process of flavor composition is to classify them in groups with parent flavor characteristics. Many key flavor notes are associated with a high-impact aroma chemical as shown in the flavor wheel (Figure 11.1). The same component that belongs to a typical group may be used in quite different flavors. This flavor wheel is not always representative since every flavorist has his own subjective perception of flavor notes.

There are approximately 2,500 chemically defined flavoring substances in use either in Europe or the U.S. Of these substances, approximately 1,500 have been evaluated by the Flavor and Extract Manufacturers Association (FEMA) Expert Panel and are legally recognized by the U.S. Food and Drug Administration (FDA) to be Generally Recognized As Safe (GRAS) substances, meaning that they are considered safe for their intended use. The majority of flavoring substances have simple, well-characterized structures with a single func-

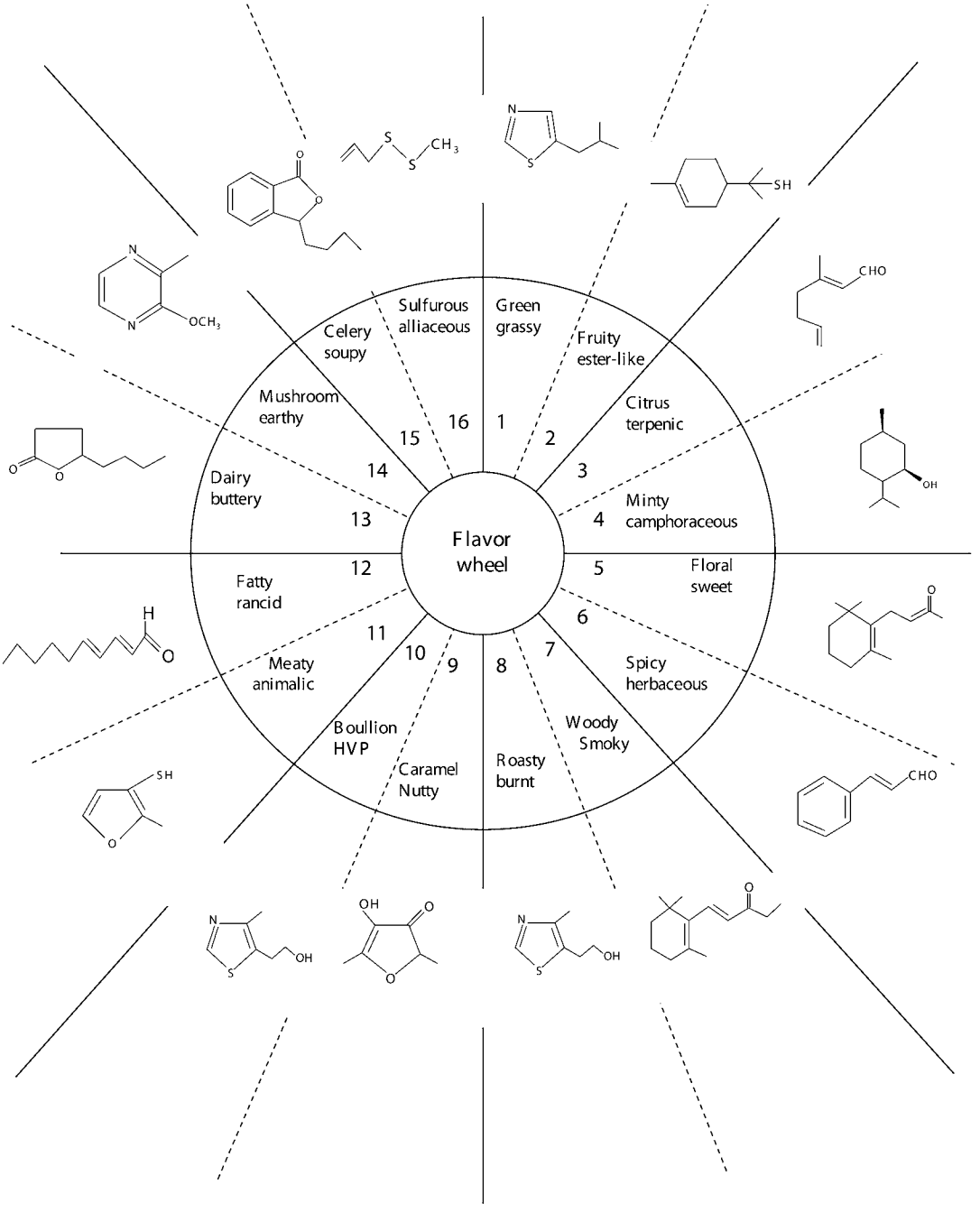


Figure 11.1. A flavor wheel of high impact aroma chemicals.

tional group and low molecular weight (<300 grams per mole [g/mol]). More than 700 of the 1,323 chemically defined flavoring substances used in food in the U.S. are simple aliphatic acyclic and alicyclic alcohols, aldehydes, ketones, carboxylic acids, and related esters, lactones, ketals, and acetals. Other structural categories include aromatic (e.g., cinnamaldehydes and anthranilates), heteroaromatic (e.g., pyrazines and pyrroles), and heterocyclic (e.g., furanones and alicyclic sulfides) substances with characteristic organoleptic properties. Incremental changes in carbon chain length and the position of a functional group or hydrocarbon chain typically describe the structural variation within groups of related flavoring substances. Within structural groups of flavoring substances, many substances have considerable toxicology data; repeat dose studies exist for many substances or their metabolic products, and several representative members of structural groups have chronic toxicity studies. At the 46th and 49th meetings of JECFA, the Committee (JECFA 1997, 1998) was able to use information on metabolism, toxicity, and intake of individual substances within a group to evaluate 263 flavoring substances.

The flavor wheel is a pictorial illustration of some basic flavor relations. At the center is the flavor matrix, which represents the body of the flavor to be created. It contains all the ingredients needed to support, dilute, enhance, and protect the single flavor components, which cannot be applied in a pure state. The flavor has sweet and fruity notes as well as savory and alliaceous notes. The range of chemical functionalities is also wide, with sulphur-containing molecules particularly prominent. The odor of the wheel is not random; the east-west division is largely between sweet and savory. Clockwise from mushroom to vegetable, the materials are formed by biogenesis in plants, and so are of particular importance in creating the flavors of fresh fruits and vegetables. By contrast, most of the others are commonly found as Maillard reaction products, and hence are of most interest in flavors for cooked foods. The material in the Eastern sector of the wheel, from green grassy to smoky are of interest to the perfumer as well.

Green Grassy Flavor

Substances representing this class are short chain unsaturated aldehydes and alcohols such as *trans*-2-hexenal (leaf aldehyde) and *cis*-3 hexenol (leaf alcohol) with odor thresholds of 17 and 70 parts per billion (ppb), respectively. Many other compounds like esters, acids, and terpenoids could be placed in this group (e.g., hexyl 2-methyl-butyrate, α-pinene). A fresh greenness is also associated with the more odorous 2-isobutylthiazole (e.g., tomatoes with an odor threshold of 3 ppb). A stereospecific synthesis of *cis*-3-hexen-1-ol starts with the ethylation of sodium acetylide to 1-butyne, which is reacted with ethylene oxide to give 3-hexyn-1-ol. Selective hydrogenation of the triple bond in the presence of palladium catalysts yields *cis*-3-hexen-1-ol. Leaf alcohol is used to obtain natural green notes. The annual world consumption is estimated to be about 8,000 pounds, much of it going into the production of esters, such as the acetate. *Trans-2*-hexenal is the simplest straight chain unsaturated aldehyde of interest for flavors. It occurs in essential oils obtained from green leaves of many plants. It is a colorless, sharp, herbal green-smelling liquid with a slight acrolein-like pungency. Upon dilution, however, it smells pleasantly green and apple like. It is commonly used in flavor compounding because of its stability and commercial availability. The aldehyde can be synthesized by reacting butanal with vinyl ethyl ether in the presence of boron trifluoride, followed by hydrolysis of the reaction product with dilute sulfuric acid (Figure 11.2). It has an intense odor and is used in fruit flavors for green nuances (Union Carbide 1953).

Fruity Flavor

Typical ingredients representing this group are esters and lactones, but ketones, ethers and acetals are also involved. The fruity ester-like compounds have low odor thresholds (ethyl butyrate 1 ppb, ethyl isobutyrate 0.1 ppb, ethyl 2-methylbutyrate 0.1 ppb, ethyl hexanoate 1–3 ppb). Isoamyl acetate is a strongly fruity-smelling liquid and has been identified in many fruit aromas. It is the main component of banana aroma and is, therefore, also used in banana flavors. 3-methylthiopropionic acid esters are characteristic for pineapple. A particularly important flavor component of pears is 2-*trans*-4-*cis*-decadienoic acid ethyl ester. 1-*p*-Hydroxyphenylbutan-3-one, which has a flavor threshold of 5 μg/kg, gives raspberries its characteristic flavor. 4-Hydroxy-2,5-dimethylfuran-3-one and *cis*-3-hexenol are important in determining strawberry flavor. Also contribut-

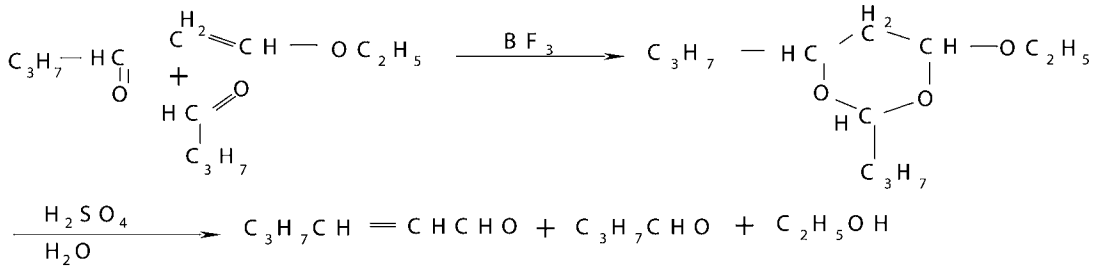


Figure 11.2. Synthesis of *trans*-2-hexenal (leaf aldehyde).

ing are methylbutanoate, ethyl 2-methylbutanoate, methyl-2-methylbutanoate, etic acid, 2,3-butanedione, and methyl and ethyl cinnamates. Various sulfur-containing flavor chemicals give the characteristic flavor of black currant. Examples are cat ketone (4-thio-4-methylpentan-2-one), black currant mercaptan (4-methoxy-2-methyl-2-butanethiol), and particularly 8-thio-*p*-menthan-3-one. Passion fruit and durian have shown the presence of many powerful sulphur compounds. The best known is tropathiane, 2-methyl-4-propyl-1,3-oxathiane (odor threshold approximately 3 ppb); 3 mercapto-1-hexanol and a number of acetylated derivatives. Sesquiterpene (*R*)-nootkatone has a potent grapefruit flavor character with a quite low odor threshold of 1 μg/l. More recently, it has been discovered that a quite different chemical, (R)-(+)-*p*-1-menthene-8-thiol (grapefruit mercaptan) also gives grapefruit character and has a remarkably low threshold of 0.00002 μg/l. Ethyl 2-*trans*-4-*cis*-decadienoate has been identified in pears and has the typical aroma of pears. It is believed to be produced in the fruit from linoleic acid by β-oxidation, isomerization of a double bond and further β-oxidation, following desaturation and esterification with ethanol. Synthesis of ethyl 2-*trans*-4-*cis*-decadienoate starts from *cis*-1-heptenyl bromide, which is converted into a 1-heptenyllithium cuprate complex with lithium and copper iodide. Reaction with ethyl propionate yields a mixture of 95% ethyl 2-*trans*-4-*cis* and 5% ethyl 2-*trans*-4-*trans*-decadienoate.

Anapear® is a new powerful odorant with a fruity green pear note. It is synthesized by an orthoester Claisen rearrangement of hexa-1,5-dien-3-ol as outlined in Figure 11.3 a (Kaiser 1995).

In peaches, the main flavor character is provided by lactones, especially γ-decalactones, and also other C_6 to C_{12} γ-lactones and C_{10} and C_{12} δ-lactones. The lactones are intermolecular esters of corresponding hydroxyl fatty acids. They are ubiquitous in nature and have been isolated from all major food systems. Due to their mostly low order thresholds averaging about 0.1 ppm, lactones often have a high flavor value. The naturally occurring sensorily important lactones generally have γ- or δ-lactone structures while a few are macrocyclic. The macrocyclic esters hold a special position among the industrially produced lactone fragrance compounds. γ-lactones tend to occur preferentially in plants, δ-lactones are mainly found in animal products. Precise odor description for these lactones is oily-peachy, creamy, fruity, nut like, coconut, honey, butter, and so on. The γ-lactones can be prepared in good yield in a one-step process by radical addition of 1-octanol to acrylic acid using di-tert-butyl peroxide as catalyst (Figure 11.3 b) (Ube Industries 1975). Another simple and efficient synthesis of γ-undecalactone is by treating undecylenic acid with 70–80% sulfuric acid. This causes shifting and hydration of double bond and subsequent lactonization of 4-oxyundecylic acid (Figure 11.3 c).

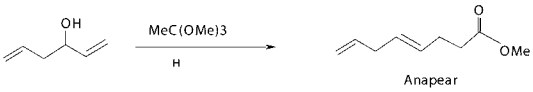


Figure 11.3 (a). Synthesis of Anapear® (Pear odorant).

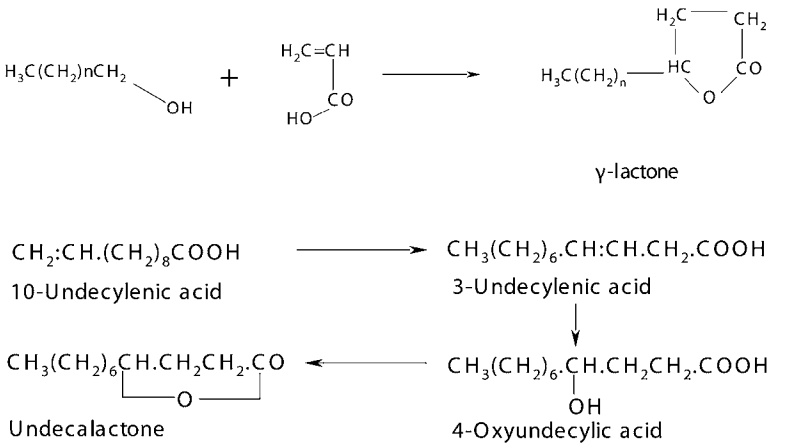


Figure 11.3 (b, c). Synthesis of undecalactone.

Citrus Flavor

Among the acyclic terpene aldehydes, citral holds the key position as fragrance and flavor chemical, as well as starting material for the synthesis of other terpenoids. Besides natural citral, which is frequently preferred for its harmony, standardized synthetic citral is generally used when large amounts and low costs are needed. Citral, which is a mixture of the two stereoisomers geranial and neral, is an important synthetic ingredient with an annual production of thousands of tons in the United States. Since citral is an α, β unsaturated aldehyde with an additional double bond, it is highly reactive and may undergo reactions such as cyclization and polymerization. Geraniol, citronellol, and 3,7-dimethyloctan-1-ol can be obtained from citral by stepwise hydrogenation. The condensation of citral with active methylene groups is used on an industrial scale in the synthesis of pseudoionones, which are starting material for ionones and vitamins. Figure 11.4 shows a technical synthesis of citral. 3-Methyl-3-buten-1-ol, obtained from isobutene and formaldehyde, isomerizes to form 3-methyl-2-buten-1-ol. However, it is also converted into 3-methyl-2-butenal by dehydrogenation and subsequent isomerization. Under azeotropic conditions in the presence of nitric acid, 3-methyl-2-buten-1-ol and 3-methyl-2-butenal form an acetal, which eliminates one molecule of 3-methyl-2-buten-1-ol at higher temperatures. The intermediate enol ether undergoes Claisen rearrangement followed by Cope rearrangement to give citral (Nissen et al. 1981).

Mint Flavor

In flavor chemistry, terpenoid hydrocarbons are of great importance. These compounds, which are found in so many of the essential oils, form a very distinctive group of chemicals that have carbon skeletons comprising isoprene units joined together in a regular head-to-tail configuration called isoprene rule. They may be open or closed chain or cyclic compounds. The compounds may be saturated or unsaturated. Cyclic terpene hydrocarbons occur in essential oils, sometimes in large amounts. Of various types of monocyclic terpene hydrocarbons, those with the *p*-menthadiene structure are the most important (e.g., Limolene, α-Terpinene, γ-Terpinene, and Terpinolene). Of the bicyclic terpene hydrocarbons, the pinenes are by far the most important industrially.

Cyclic terpene alcohols occur widely in nature, few have the physiological properties that make them important fragrance or flavor compounds. (−) Menthol is the isomer that occurs most widely in nature. Peppermint and cornmint oil are by far the most important since they contain large percentages of *l*-menthol. It has three asymmetric carbon atoms in its cyclohexane ring and, therefore occurs as four pairs of optical isomers. The configuration of four of

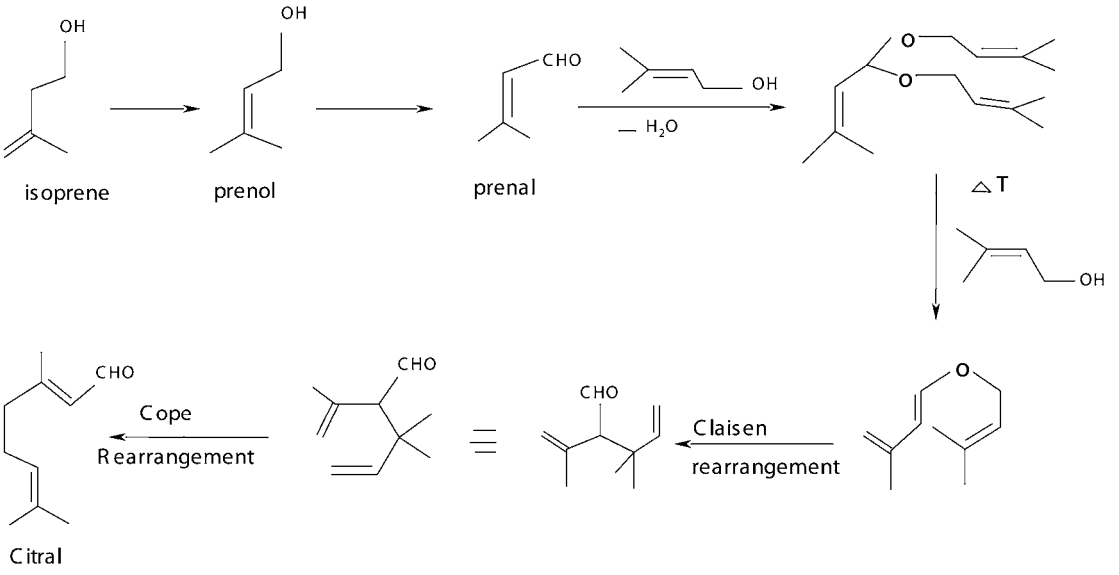


Figure 11.4. Synthesis of citral.

these isomers is given below (Figure 11.5); the other four are their mirror images. It is estimated that nearly 10 million pounds of menthol are used annually throughout the world. *l*-menthol is economically the most important synthetic ingredient; the main quantity is being used in nonfood applications. In most applications, *l*-menthol is the preferred isomer of menthyl alcohols.

In Figure 11.6, an industrial synthesis of *l*-menthol is shown, which produces specifically the desired isomer. The crucial step is the enantio selective isomerization of geranyl diethyl amine with a homogenous asymmetric rhodium catalyst (Noyori and Kitamura 1989). First, the triene is converted in a region and stereoselective manner to diethylgeranylamine by lithium catalyzed addition of diethylamine. The key step is the subsequent BINAP-Rh (I) catalyzed enantioselective isomerization of the allylic amine in THF giving (R)-citronellal enamine. Enantiomeric purity of the chiral aldehyde obtained by hydrolysis is much higher than the natural product, at most approximately 80%. Sterioselective cyclization of (R)-citronellal promoted by zinc bromide, giving isopulegol, followed by catalytic hydrogenation completes the synthesis of menthol.

Floral Sweet Flavor

The floral flavor can be defined as the odors emitted by flowers and contain sweet, green, fruity, and

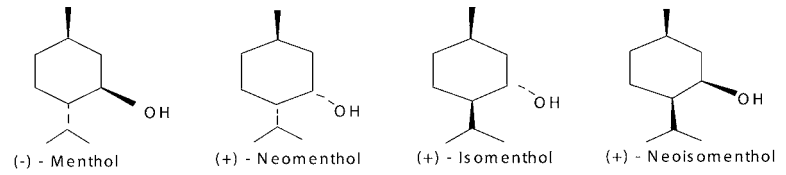


Figure 11.5. Menthol isomers.

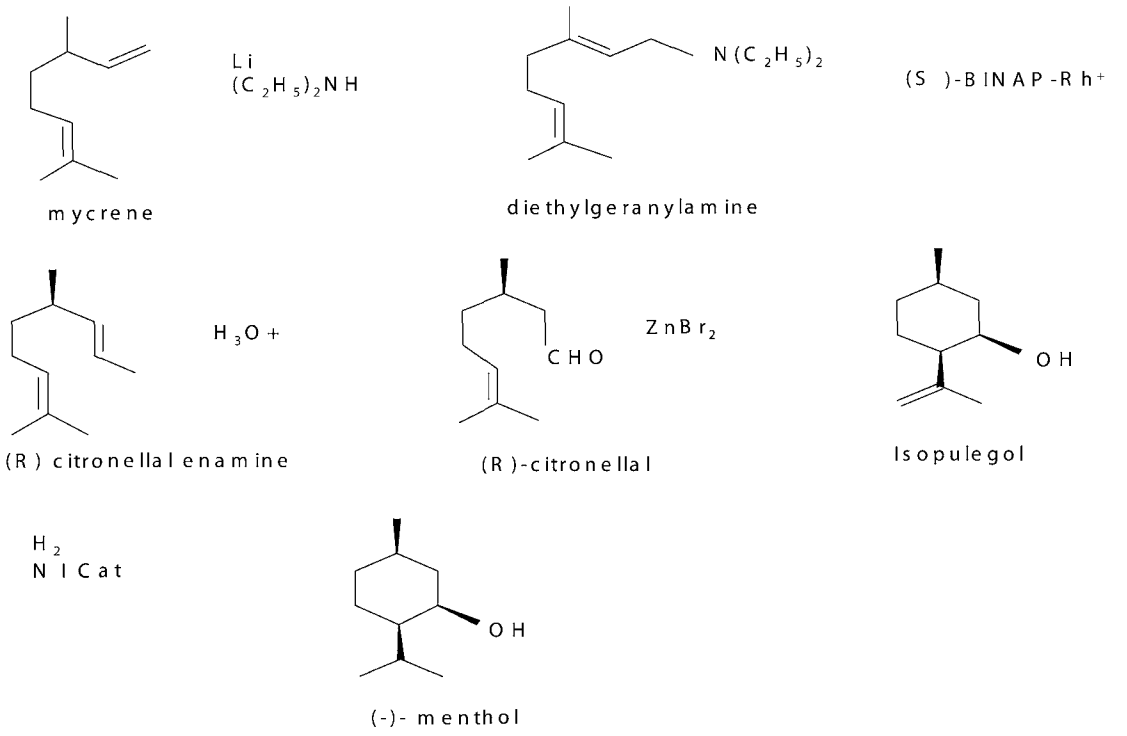


Figure 11.6. Synthesis of *l*-menthol.

herbaceous characters. Phenylethanol, geraniol, β-ionone and some esters (benzyl acetate, linalyl acetate) are important compounds in this group. Geraniol is an acyclic, doubly unsaturated alcohol. It can undergo a number of reactions, such as rearrangement and cyclization. Rearrangement in the presence of copper catalysts yields citronellal. In the presence of mineral acids, it cyclizes to form monocyclic terpene hydrocarbons, cyclogeraniol being obtained if the hydroxyl function is protected. Partial hydrogenation leads to citronellol, and complete hydrogenation of the double bonds yields 3,7-dimethyloctan-1-ol. Linalyl acetate is used extensively in perfumery. It is an excellent fragrance material. Smaller amounts are used in other citrus products. Since linalyl acetate is fairly stable toward alkali, it can also be employed in soaps and detergents. Figure 11.7 shows synthetic procedures for linalyl acetate and β-ionone based on methylheptenone, which is another very large volume commodity (Bedoukian 1986, Mayer and Isler 1971, Saucy and Marbeth 1967). In the acetylene process, dehydrolinalool (DLL) is acetylated and the pure ester, which is free of any isomeric alcohol esters, is partially hydrogenated to yield pure linalyl acetate. The elongation of DLL with a C_3-unit makes use of a modified Claisen rearrangement. Isomerization of the allenic systems and acid catalyzed cyclization conclude the synthesis of β-ionone.

Spicy Herbaceous Flavor

Spices encompass a huge range of taste and odor sensations, achieved using an equally wide range of chemicals. Aromatic aldehydes, alcohols, and phenolic derivatives are typical constituents with their strong flavor effect. Many impact character chemicals: dihydrocapsaicin (peppers), sotolone (fenugreek curry), zingerone (ginger), trans-2-dodecenal (coriander), 1,8-cymene (rosemary, cardomon, allspice and sage), methyl charvicol (basil), fenchone and anethole (fennel), anthranilic acid ester (man-

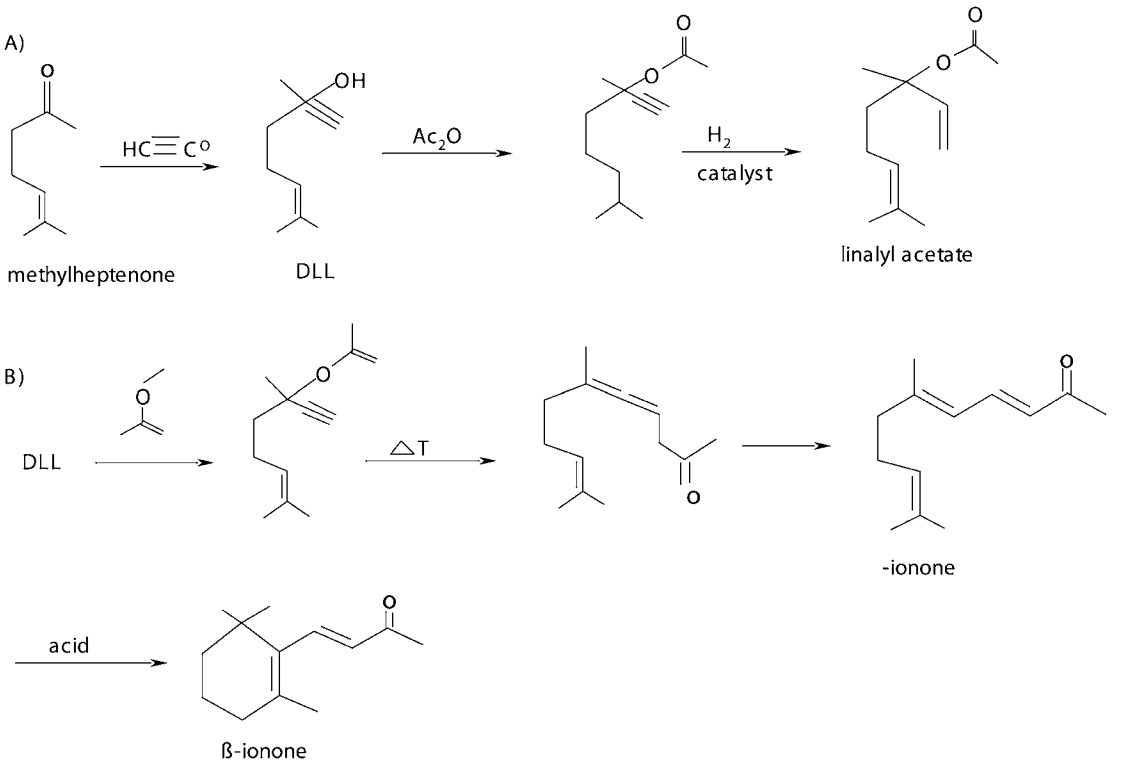


Figure 11.7. Synthesis procedures for (a) linalyl acetate and (b) β-ionone.

darin), α-phellandrene (dill), cinnamaldehyde, estragole, eugenol (clove and cinnamon), *d*-carvone and thymol, etc. The largest item used is cinnamaldehyde, which is synthetically produced on an industrial scale almost exclusively by alkaline condensation of benzaldehyde and acetaldehyde (Figure 11.8a). Self decomposition of acetaldehyde can be avoided by using an excess of benzaldehyde and by slowly adding acetaldehyde. The concentration of base solution may vary between 2 and 4% without affecting the yield. Contact with iron should be reduced to a minimum to avoid discolorations and polymerizations. Cinnamaldehyde is used in many compositions for creating spicy and oriental notes. It is the main component of artificial cinnamon oil.

Some spices and some spicy odorants also possess sweet aspects, close to vanilla. Methyl Diantilis (derivative of vanillin) with its clove-carnation-powdery note is one example. It is synthesized as presented in Figure 11.8 (b), by hydrogenation of ethyl vanillin and methylation of the benzylic hydroxyl function formed in intermediate (Ochsner 1985), and possess the sweet odor characteristic.

Woody Smoky Flavor

Woody smoky flavors are characterized by substituted phenols (guaiacol), methylated ionone derivatives (methylionone) and by some aldehydes (trans-2-nonenal). 4-Ethyl- and 4-methylguaiacols have rather phenolic, medicinal odors with threshold value of 90 and 50 ppb, respectively, but the more important guaiacol is 4-vinylguaicol (2-methoxy-4-vinylphenol, MVP). This has a spicy, clove-like smokiness particularly associated with smoked ham and a low odor threshold of only 3 ppb. The methylionones are among the most important fragrance substances and exist as α-, β, and γ-isomers. They are structurally very similar to the carotene degradation products, the ionones. The synthesis of

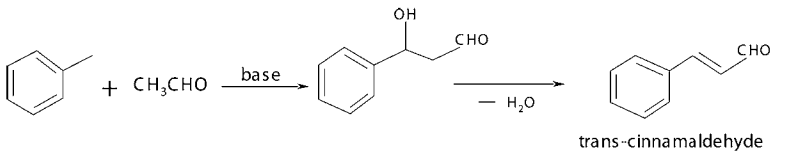


Figure 11.8 (a). Synthesis of cinnamaldehyde.

ionones involves two fundamental steps: the preparation of pseudoionone or pseudomethylionone and its cyclization to the ionones. In the methylionone synthesis, condensation of citral with methyl ethyl ketone results in a mixture of *n*-methyl- and isomethylpseudoionone, each of which may occur as one of four possible *cis-trans* isomers. The ratio of major isomers in the mixture depends on the conditions, catalysts, and the reaction conditions (The Givaudan Index 1962) (Figure 11.9).

Roasty Burnt Flavor

For roasted and burnt notes, derivatives of furfuryl mercaptan are paramount. The mercaptan itself, with an odor threshold of 0.005 ppb, was the first high impact aroma chemical. At low concentration (0.01–0.5 ppb) the material has roasted coffee aroma, becoming burnt and sulphurous in the range 1–10 ppb. The roasty burnt flavor is also associated with pyrazines. They are a class of nitrogen containing, six-membered ring heterocyclic compounds. They play an important role as intermediates for perfumes, pharmaceuticals, and agricultural chemicals because of their occurrence in nature and their unique odor characteristics at extremely low concentrations. Different substitution by alkyl, acyl, or alkoxy and combinations thereof induces a great diversity impression. Within this group of the roasty burnt flavor notes, alkyl and acetyl substituted pyrazines are the most important. 2-ethyl-3-methoxy pyrazine has been found effective in enhancing the flavor of dehydrated potatoes. 2-ethyl-2,5-dimethyl pyrazine is one component in the aroma of baked potatoes. Pyrazines also contribute to the nutty roasted aroma of cooked meat. 2,3-dimethyl pyrazine is useful in roasty meaty flavors, and 2,5-dimethyl pyrazine in chicken broth, grilled meat, and beef flavors. 2-acetyl pyrazines are desirable flavoring agents, imparting a popcorn-like flavor to food systems. 2-isobutyl-3-methoxy pyrazine is utilized whenever a fresh pepper note is desired.

Pyrazines can be prepared by the complex interaction of amino acids and sugar, or sugar degradation products. For example, five alkyl pyrazines were obtained by heating glucose with cysteine (Kato et al. 1973). Pyrolysis of alpha hydroxyl amino acids gave pyrazines and various alkylated derivatives (Wand and Odell 1973). Okada found, for the first time, that pyrazine compounds can be produced by the catalytic reaction of diamines with diols in a vapor-phase reaction in the presence of

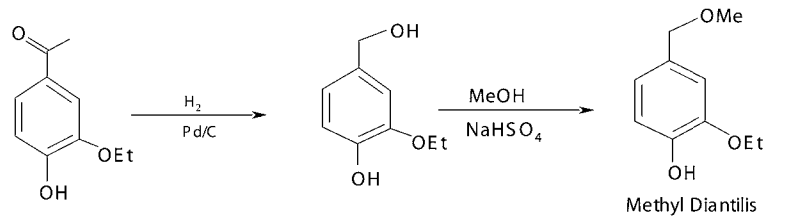


Figure 11.8 (b). Synthesis of Methyl Diantilis.

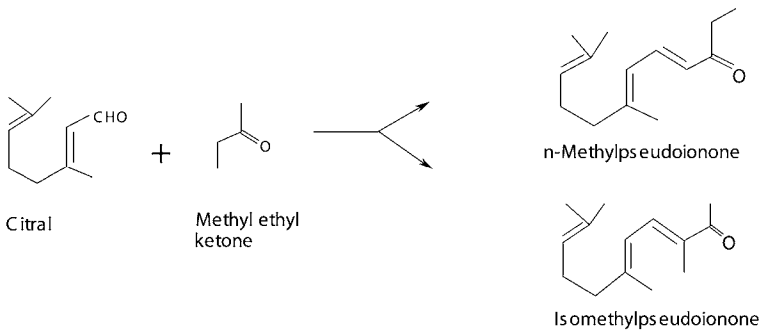


Figure 11.9. Synthesis of methylionones.

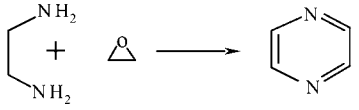
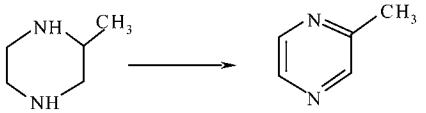
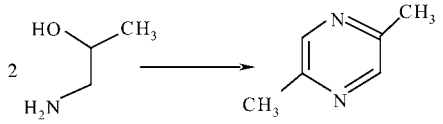
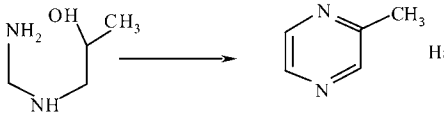
granular alumina (Okada 1974). Catalytic systems such as copper-chromium (Koei Chemical Co. 1978), copper-zinc-chromium (Korea Res. Inst. 1993), zinc-phosphoric acid-manganese (Tokai Electro-Chemical Co. 1980), and silver (Koei Chemical Co. 1997) are also patented as catalysts for preparation of 2-methylpyrazine (MP) from ethylene diamine (ED) and propylene glycol (PG) (Figure 11.10 a).

It is also possible to obtain pyrazines from condensation reaction of diamines and epoxides, condensation reaction between alkanolamines or cyclodehydrogenation of *N*-(-hydroxyalkyl) alkyl diamine on the same catalysts. In the presence of copper-zinc catalysts, dehydrogenation of piperazines gives corresponding pyrazines with high yield (Table 11.1).

Nippon Soda Co. (1986) established an industrial manufacturing process for the preparation of

Figure 11.10 (a). Synthesis of 2-methylpyrazine.

Table 11.1. Pyrazine condensation reactions.

Reaction	Company	Catalysts
	Koei Chemical Co.	$Cu-Cr_2O_3$
	BASF A.G.	$Pd-MgCl_2-Al_2O_3$
	Wyandotte Chemical Co.	$Cu-Cr_2O_3$
	Hasegawa T. Co.	$Cu-Cr_2O_3$

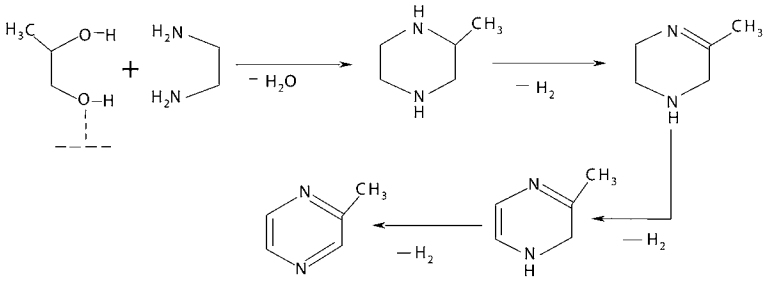


Figure 11.10 (b). Synthesis of Methylpyrazine.

pyrazines from diaminomaleonitrile (DAMN). Tetramerization of hydrogen cyanide yields DAMN. Pyrazines are produced from DAMN and glyoxals in the presence of oxalic acid. These cyano-substituted pyrazines play important roles as industrial intermediates because they are easily functionalized to amino, carboxyl, or amide group. Forni et al. (1991) studied the mechanism of the cyclization of ED and PG to give MP on zinc-chromium catalyst by means of TPD-TPR-MS (temperature programmed desorption-temperature programmed reaction-mass spectrometry) technique. They propose a mechanism that involves an intermediate (methylpyperazine) formation between adsorbed PG and gaseous ED, which in turn carry out dehydrogenation and aromatization to give methylpyrazine (Figure 11.10 b).

Caramel Nutty Flavor

The furanones and pyranones are oxygen-containing heterocyclic compounds associated with both caramelized and non-enzymatic browning (NEB) flavors. Besides corleone, maltol, furonol, etc., a special range of components like vanillin, ethylvanillin, benzaldehyde, phenylacetic acid, cinnamic alcohol, dehydrocoumarin, and trimethylpyrazine also belong to this group. They are characterized by a common structural unit, namely a cyclic enolone system, and also by a common flavor profile. These cyclic enolones are most valuable compounds for their strong impact character and for their distinct flavor-enhancing effect. These compounds are used in roasted and fruit flavors applications as well as flavor compositions with caramel, coffee, meat, or bread character. The ubiquitous hydroxydimethylfuranone has a sweet, cotton-candy aroma and a low odor threshold of 0.04 ppb. 2-Methyltetrahydrofuran-3-one (coffee furanone) is less odorous but has a very pleasant, sweet caramel character. The nuttiness is particularly associated with the higher pyrazines such as methyldihydrocyclopentapyrazine (Maple lactone pyrazine) and 5,6,7,8-tetrahydroquinoxaline (THQ). The synthetic concept for homofuronol allows easy variation of the starting materials so that with the same reaction sequence, other homologues can be prepared. Homofuronol can be prepared by the condensation of 2-pentene nitrile with ethyl lactate followed by oxidation of the intermediate 4-cyano-5-ethyl-2-methyldihydro-3(2H)-furanone with monoperoxy-sulfate (Huber and Wild 1977) (Figure 11.11).

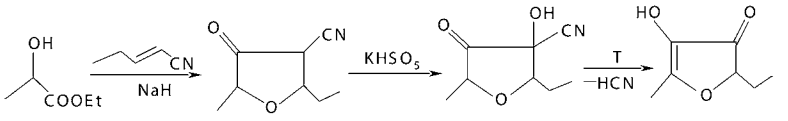


Figure 11.11. Synthesis of homofuronol.

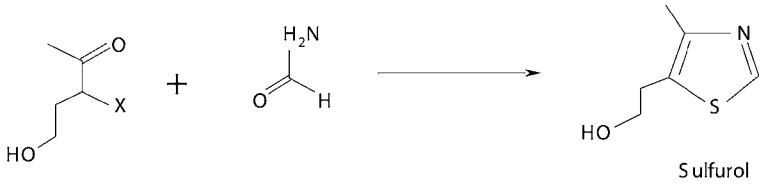


Figure 11.12. Synthesis of sulfurol.

Bouillon Hydrolyzed Vegetable Protein Flavor

The hydrolyzed vegetable protein (HVP) bouillon type flavors are complex and diffuse meat flavors that cannot easily be described in terms of a few flavor chemicals and that have a "warm" salty and spicy sensation. 5-Methyltetrahydrothiophene-3-thiol and 2-methyltetrahydrothiophene-3-thiol are top notes. Other flavor chemicals include 5-ethyl-4-methyl-3-hydroxy-2(*5H*)-furanone, methional and 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone, which gives a meaty, savory character. The most common aroma chemical associated with this HVP flavor is 4-methylthiazole-5-ethanole (sulfurol). The role of sulfurol is particularly interesting, as it has a relatively high flavor threshold of 10 mg/l, but the sulfurol note often increases in intensity with storage. The related molecule 2-methyltetrahydrofuran-3-thiol may contribute to this, as it has a much lower flavor threshold, and may be formed from sulfurol during cooking. Hantzsch's procedure is the most versatile for the preparation of thiazoles from alpha halocarbonyls and leads to a wide variety of thiazoles derivatives. Buchman (1936) obtained sulfurol (4-methyl-5-thiazole ethanol) by condensing 3-chloro-3-aceto propyl alcohol with thioformamide (Figure 11.12).

Meaty Flavor

Sulphur-containing components (mercaptans, thiozoles, thiophene, etc.) as well as nitrogen heterocycles (pyrazines, pyrroles, pyridines, oxazoles, etc.) are the most widely distributed heterocyclic compounds and find application in the formulation of both flavors and fragrances. They are present in traces in a variety of foods, but because of their powerful, strong odor and low threshold, play an important role in enhancing the basic aroma of flavors to which they add a natural character. These compounds have been found to possess a strong meat like odor. Nonvolatile derivatives of nucleotides and peptides as well as minerals are also responsible for typical meat mouthfeel. The compound 2-methylfuran-3-thiol (MFT) itself initially has a rather chemical odor, becoming more meaty on dilution. The thiol, its disulphide, mixed disulphide and thioether have all been found in beef. The odor threshold of the disulphide has been reported as being as low as $2x10^{-5}$ ppb. Other high impact chemicals, such as mercaptopropanone dimer, has an intense chicken broth odor and the unsaturated aldehyde trans-2-trans-4-decadienal is very reminiscent of chicken fat. Another compound with excellent pork character is pyrazineethanethiol, which has not been reported in nature. Figure 11.13 shows the chemical synthesis of 2,4,5-trimethylthiamine (3-thioacetone) by trimerization of acetone in the presence of hydrogen sulfide. It has been identified in meat aroma (Wilson et al. 1973) and chicken flavor. It occurs as a liquid having a very strong repulsive sulfurous odor and can be used in the formation of meat flavor.

Fatty and Cheesy Rancid Flavor

Fattiness is a key character in foodstuffs in terms of both flavor and mouthfeel. Cream and butter flavor depends greatly on free fatty acids and δ-lactones.

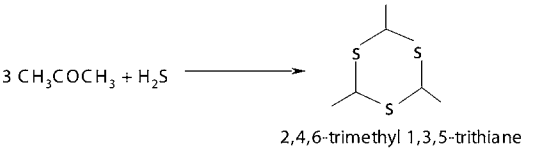


Figure 11.13. Synthesis of 3-thioacetone.

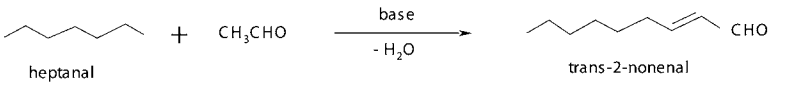


Figure 11.14. Synthesis of trans-2-nonenal.

The most potent fatty rancid flavors are butyric and isobutyric acids. Medium chain methyl branched fatty acids such as 4-methyl octanoic and nonanoic acids are responsible for off flavors of mutton fat. Aldehydes have very fatty notes, in particular trans-2-nonenal and trans-2-trans-4-decadienal with its specifically fatty odor character is indispensable in chicken meat flavor compositions. Free fatty acids make a great contribution to cheese flavors. Cheesy rancid flavor is associated with short chain fatty acids such as valeric acid. Unsaturated acids such as trans-2-hexonic acid have more powerful, acrid odors. Simple thioesters such as methyl thiobutyrate and methyl (2-methyl) thiobutyrate also have an intense cheesy-sweet-fruity odor. Synthesis of α, β-unsaturated aldehyde *trans*-2-nonenal is outlined in Figure 11.14 (Tressl et al. 1981).

Dairy Buttery Flavor

The dairy buttery flavor note varies from typical buttery notes (diacetyl, acetoin, pentadione) to sweet creamy fermented notes (acetoacetate, δ-decalactone, γ-octalactone). For instance, 6-*cis*-6-dodecene-γ-lactone is an important flavor component of butter. Several aliphatic aldehydes and acids generated by lipid oxidation reactions also contribute to the full flavor sensation. Acetoin (3-Hydroxy-2-butanone) is synthesized by partial oxidation of 2,3-butanediol and is obtained as a by-product in the fermentation of molasses. Diacetyl (2,3-Butanedione) is a constituent of many fruit and food aromas and also a constituent of butter. Many methods are known for its manufacture, e.g., dehydrogenation of 2,3-butanediol with a copper chromite catalyst. It is mainly used in aromas for butter and for flavoring margarine.

A number of methods of preparing gamma lactones are known. Many of them depend upon the formation of unsaturated or hydroxyl acids that are converted to the lactones by treatment with various reagents. Gamma lactones can be routinely synthesized as follows (Ohloff 1969) (Figure 11.15).

Mushroom and Earthy Flavor

The mushroom earthy flavor is mainly represented by 1-octen-3-ol, with an odor threshold of only 1 ppb and very characteristic of mushroom, which is reminiscent of a typical mushroom flavor and by geosmin, representing the earthy part. However, 1-octen-3-one has a threshold some 200 times lower at only 0.05 ppb and has a very fresh wild mushroom aroma. A range of C_8 components, saturated and unsaturated alcohols and carbonyl are also responsible for typical mushroom flavor. Other synthetic ingredients like 2-octen-4-one and 1-pentyl pyrrole are known to possess mushroom-like flavor notes. Earthiness is associated with some pyrazines, especially 2-methyl-3-methoxypyrazine. 4-Terpinenol, 2-ethyl-3-methylthiopyrazine, and resorcinol dimethyl ether also have earthy characters. Saito et al. (1996) reported an asymmetric synthesis of (-) geosmin via highly diasteroselective reduction of the enantiomerically pure title ketone [(+)-octalone]. Diisobutylaminum hydride reduction of (+)-octalone in a mixed solvent of tetrahydrofuran and 1,2-dimethoxyethane led to allylic alcohol. This was then converted to (-)-geosmin through a five-step reaction sequence (Figure 11.16).

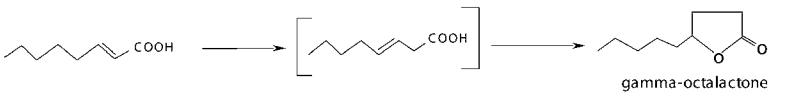


Figure 11.15. Synthesis of γ-octalactone.

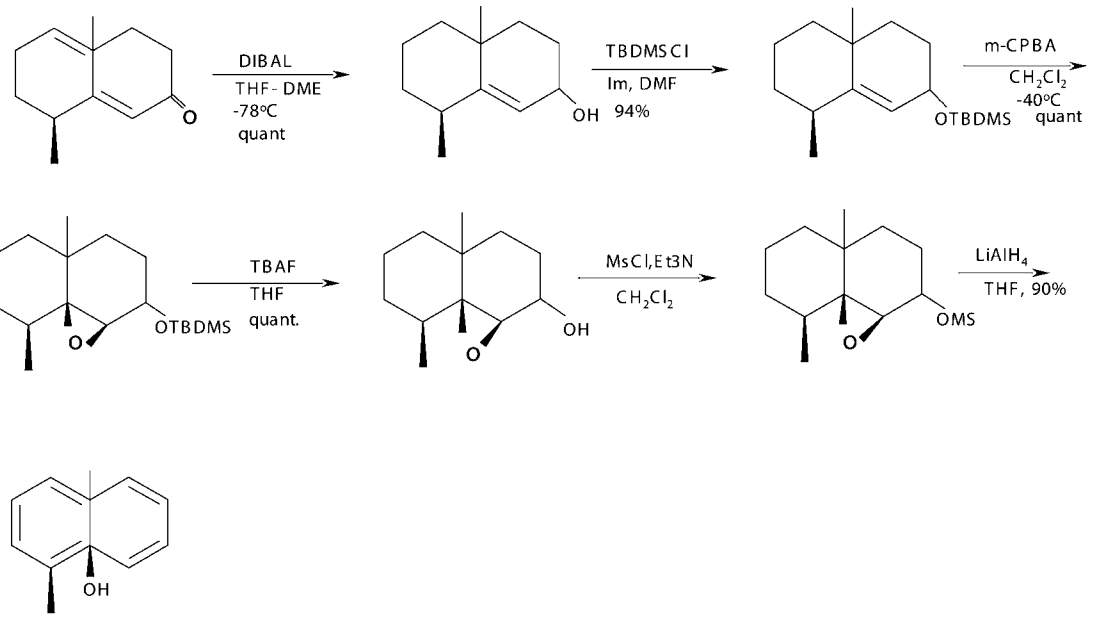


Figure 11.16. Synthesis of Geosmin.

Celery Flavor

Celery flavor note is described as a warm, spicy, rooty odor. Butylidene phthalide, butyl phthalide, 4,5-dimethyl-3-hydroxy-2[5H]-furanone, dihydrojasmone (3-methyl-2-pentyl-2-cyclopenten-1-one) and cis-jasmone are typical components of this flavor. A preferred method for the synthesis of dihydrojasmone is intramolecular aldol condensation of 2,5-undecanedione, which can be prepared from heptanal and 3-buten-2-one in the presence of thiazolium salt, such as 5-(2-hydroxyethyl)-4-methyl-3-benzylthiazolium chloride (Bayer 1974) (Figure 11.17 a & b).

A patented method for the synthesis for cis-jasmone3-methyl-2-(2-*cis*-penten-1-yl)-2-cyclopenten-1-one involves alkylation of 3-methyl-2-cyclopenten-1-one with cis-2-pentenyl chloride in an alkaline medium in the presence of a phase-transfer catalyst (e.g., tricaprylmethylammonium chloride) (International Flavors & Fragrances 1976).

The Sulfurous Flavor

Volatile organic sulfur compounds contribute to the aroma of many vegetables, fruits, and food products. In general, thiols and sulfides belong to the most intense and characteristic aroma substances, with sulfury, vegetable-like, and fruity notes perceived at low concentrations. The identification of thiols and sulfides is generally a challenging task due to their instability and low concentration. Therefore, sulfur compounds can easily be overlooked in complex

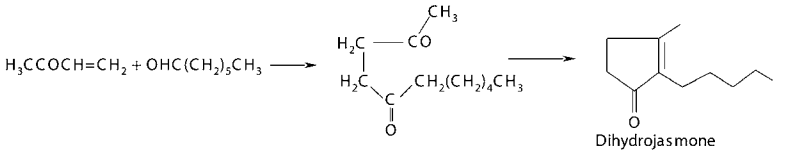


Figure 11.17a.

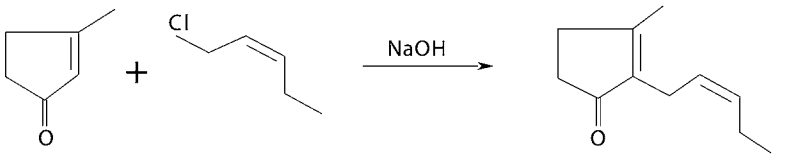


Figure 11.17b. Synthesis of (a) dihydrojasmone (b) *cis*-jasmone.

mixtures. The sensory relevance of such odorants is due to their low threshold values. For example, (2*R*, 3*S*)-3-mercapto-2-methylpentan-1-ol, a character-impact constituent of fresh onions, shows an odor threshold of 0.03 μg/kg of water. They need very special handling because they often show instability (e.g., oxidation) and interactive reactions with other compounds in the flavor mixture. Cooked onion flavor is given by dipropyl disulfide, and *cis*- and *trans*-2-propenylpropyl disulfides, which have odor thresholds of 3.2 and 2.0 μg/l, respectively, and also by various other sulfides such as trisulfides. In fried onions the characteristic flavor chemicals formed are 2-(propyldithio) dimethylthiophenes, which have odor thresholds of 0.01–0.05 μg/l (Kuo and Ho 1992). Garlic flavors are based on sulphur compounds such as allyl disulphide, mercaptan, trisulphides, mixed disulphide (allyl methyl disulphide), and (di) allyl disulfide (di-[2-propenyl] disulfide), which constitutes 90% of the active flavor of garlic oil. It is produced from the precursor alliin (s-allyl-L-cystein sulfoxide), via the intermediate allicin, by allinase enzyme, which is only released when the vegetable tissue is crushed. Vermeulen and coworkers (2001, 2005) and Vermeulen and Collin (2002) have prepared a series of sulfur-containing odorants by reacting various precursors. They synthesized mercaptoaldehyde by continuous bubbling of hydrogen sulfide through α,β-unsaturated aldehyde/piperidine/THF mixture (Figure 11.18a). In the second procedure, mixing of pure thioacetic acid with α,β-unsaturated aldehydes led to thioesters, subsequently subjected to basic hydrolysis to thiols and acetic acid (Figure 11.18b).

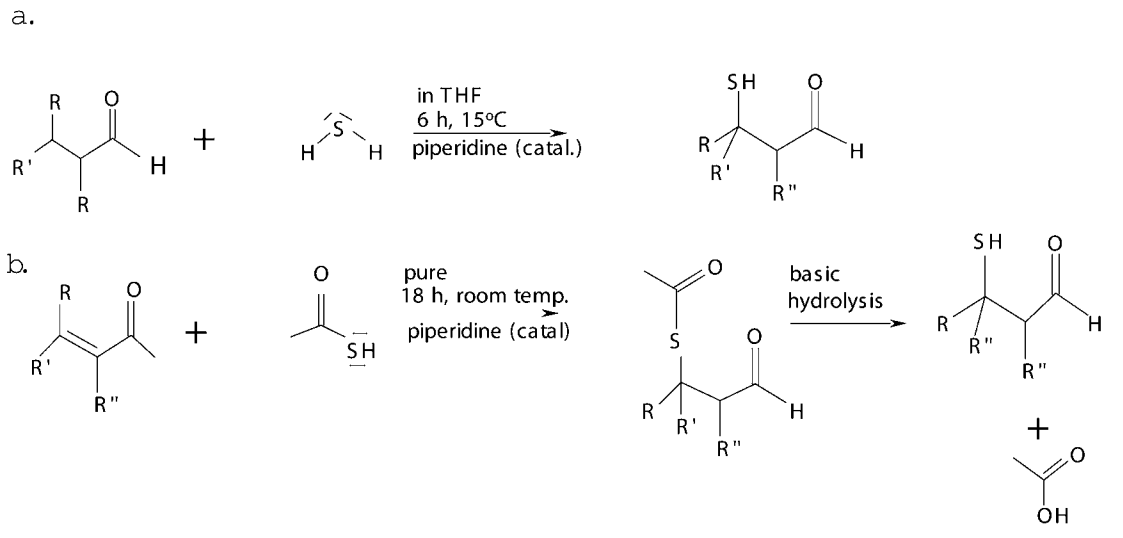


Figure 11.18. Synthesis of (a) mercaptoaldehyde and (b) thiols.

FLAVOR QUALITY

The purity of synthetic flavorants is a very important quality attribute. Chemicals are rarely so pure that they have simple odor and flavor profiles. Most contain sufficient trace impurities, stemming either from the start materials or processing conditions used in their manufacture, to display a spectrum of perceptible character notes. Sometimes it is the presence of these notes that characterize the compound and make it far more acceptable as a flavorant than the pure material itself. It is the complex odor and flavor characters that are recognized as normal and that lead to the chemically pure material being rated as unacceptable. From a quality point of view, the purchasing of synthetic organic flavoring materials calls for precise analysis of their physical characters, particularly the refractive index and specific gravity, coupled with a critical appraisal of their odor and flavor under controlled conditions against an acceptable reference sample. This latter evaluation is of far greater value in determining the suitability for use than insistence on rigid compliance with specified physical standards. The two methods of assessment should always be considered together and the organoleptic results given the greater emphasis in deciding acceptance or otherwise.

The evaluation of synthetic compounds to be used in flavorings calls for considerable care because of their extremely powerful aromatic effects. Flavor quality is comparatively easy to assess, although the detection and identification of individual flavor molecules present at very low concentrations can be challenging. Sensory evaluation is a widely used technique for identification and measurement of odor. For the assessment of the flavor profile, the chemical should be first diluted in a food-acceptable solvent such as propylene glycol or ethanol; with a bulking agent, such as maltodextrins, or even microencapsulated, such as cyclodextrins. Any comparative reference sample should be similarly diluted at the same time. Whatever comparison method is used it must be meaningful in terms of acceptability for use of the material under test and reproducible on successive occasions. For highly aromatic compounds, the material should be first diluted to a level at which the nose and palate are not swamped causing temporary anosmia. Secondly, sufficient time should be allowed between repeat tests for the senses to recover their initial acuity, and the tests should be repeated in reverse order of presentation to eliminate, or at least minimize, any carry over effects.

The advances in analytical methodology made possible the identification of numerous compounds with known flavor properties. In an increasingly competitive commercial environment, the food industry must satisfy consumer demands for high quality, palatable food and must strive to minimize adverse consumer reactions. The perceived quality and intensity of a flavor depends on the environment in which it is presented, its temperature, shear-forces during chewing, diffusion of molecules, binding, pH of the solution, and solubility of the flavor molecules. The studies on interaction of food constituents with flavor molecules have been done in model systems wherein one-to-one interactions are given more thrust. The effect of protein solutions on flavor release shows that as the protein concentration increases, the headspace concentrations of both allyl isothiocyanate and diacetyl decrease; and that different protein have different effects, presumably because they bind the flavor molecules to different extents. A particularly big influence comes from the fat content of the food, because of the ability of flavors to partition between the water and fat phases to different extents depending on their lipophilicity/hydrophilicity, requiring rebalancing of the flavor formulations to give the taste required by consumers. In-depth study of effects of all the factors on the perception of released and bound flavor molecules, under varying physicochemical conditions, will be useful for product development. Currently, the focus is on the combined instrumental and sensory approach. The potency of odorants in food extracts can be determined by Aroma Extract Dilution Analysis (AEDA), a quantitative gas chromatography-olfactometry (GC-O) method. The importance of a flavor compound in a particular food is expressed as the odor activity value (OAV) calculated as the ratio of concentration to threshold. Stable isotope dilution assays have been developed for most of the odorants by adopting techniques of high performance liquid chromatography (HPLC), capillary gas chromatography (GC), and GC-mass spectrometry (MS). Odorants with high OAVs are considered as indicator substances for the objective determination of flavor differences in foods.

Despite the developments in analytical equipment, sensory techniques, and data processing, qual-

itative and quantitative flavor analyses still remains a challenging area. Therefore, only in-depth studies utilizing a combination of sensory and analytical techniques along with greater focus on the various aspects of release and perception will eventually help in formulating products for ultimate consumer preference.

SAFETY

Safety is an important aspect of flavor manufacture. Flavoring substances in food formulations do not merit the same degree of attention as other food ingredients because of low exposure, innocuous simple structures, low toxicity, natural occurrence, and the large number of individual substances with these characteristics and thus, are exempt from specific labeling by name. They are labeled generically as either "contains flavor" or "contains flavoring." Lists of approved flavoring substances have been complied in the United States since 1959. According to the American Federal Food, Drug, and Cosmetic Act, every artificial flavoring substance has to be approved by the U.S. FDA or a reliable expert panel. Lists of approved and Generally Recognized As Safe (GRAS) flavor substances are published in the *Code of Federal Regulations* (CFR) of the U.S. Government Printing Office, Washington, DC and by the Flavor and Extract Manufacturers Association (FEMA) of the United States, 1620 Eye Street, NW, Washington, DC. Several safety assessment procedures have developed and are currently in place to assure regulators and consumers that food additives flavoring agents are safe for human consumption (JECFA 1968, 1996, 1998, 1999, 2000; NAS 1970, 1980; Oser and Hall 1977; FSC 1980; FDA 1982, 1993; WHO 1987; SCF 1991; Hallagan and Hall 1995; Munro et al. 1999). One new approach to ensuring the safety of foods is through the control and monitoring of the production processes using Hazard Analysis and Critical Control Points (HACCP) (Ropkins and Beck 2000).

JECFA Safety Evaluation Procedure for Flavoring Substances

The Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additive (JECFA 1997) applied a new safety evaluation procedure to the evaluation of flavoring substances based in part on the scientific principles laid down by Munro and others (1999). The procedure is a series of steps-wise sequential decision trees (Figure 11.19) beginning with the placement of the substance under evaluation in one of three structural classes, I, II or III, based on structural characteristics and metabolism (Cramer et al. 1978, Munro et al. 1999). Class I is the presumptively least toxic. Class III is the presumptively most toxic, or least confidently classified, with no lower limit for no-observed-effect levels (NOEL). Class II is smaller, narrower, and intermediate in its span of NOELs.

The safety evaluation sequence contains a number of questions on structure, metabolism, in-take data, and toxicity and provides an integrated mechanism to evaluate the safety of flavor ingredient. The effective application of this safety evaluation procedure depends on a substantial knowledge of toxicology, chemistry, metabolism, and intake of flavoring substances. It can be applied most effectively when groups of structurally related flavoring substances are evaluated together. For example, the results of the evaluation for butyl butyrate should be consistent with results for other esters formed from aliphatic acyclic linear saturated alcohols and acids having similar levels of intake. In the first step of the safety evaluation procedure, assign a decision tree structure class (Cramer et al. 1978) to the substance, followed by a question on metabolic fate. This question identifies those substances that are anticipated to be efficiently metabolized to innocuous products (e.g., 1-butanol) versus those that are transformed to more toxic metabolites (e.g., estragole) or have limited information on which to predict confidently the metabolic fate (e.g., 2-phenyl-3-carbethoxy furan). Once a substance has been sorted according to structure class and knowledge of metabolic fate, the next question compares the substance's daily intake from use as a flavor ingredient to the human exposure threshold for the same structure class. If the substance is metabolized to innocuous products (Step No. 2) and has an intake less than the human exposure threshold for the structure class (Step No. A3), the substance is considered safe (e.g., 1-octanol). If the intake is greater than the human exposure threshold (Step No. A3) and the substance or its metabolites are endogenous (Step No. A4), the substance is also considered safe, even though the intake is greater than the human exposure threshold (e.g., bu-

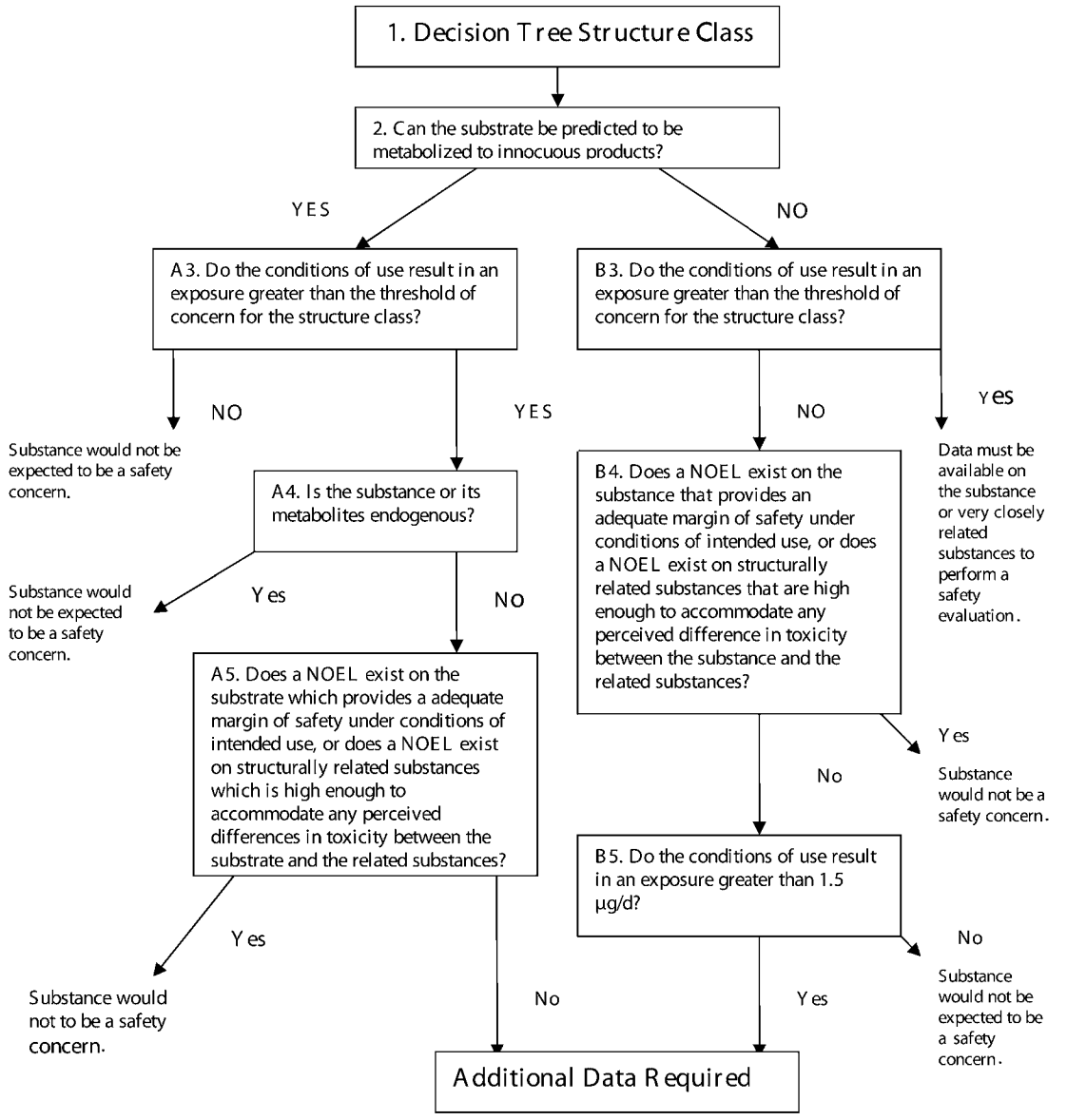


Figure 11.19. Safety evaluation sequence.

tyric acid). If the substance is not endogenous, then the substance or related substances must have a NOEL (Step No. A5) significantly greater than the intake of the substance in order to be considered safe (e.g., citral). If no such data exist or the NOEL is not significantly greater than the intake for the substance, then additional data are required to complete the safety evaluation. If metabolic fate cannot be confidently predicted and the intake (Step No. 2) is greater than the human exposure threshold (Step No. B3), additional data on metabolic fate or toxicity on the substance or structurally related substances are required to complete the safety evaluation (e.g., dihydrocoumarin). If the intake is less than the thresh-

old of concern for the structural class, the substance or structurally related substances must have a NOEL that provides an adequate margin of safety under conditions of intended use (Step No. B4) for the substance to be considered safe (e.g. 2-ethyl-4-hydroxy-3[2H]-furanone). If an adequate toxicity study is not available and the substance has an intake less than 1.5 μg/day (Step No.B5), the substance is considered not to present a safety concern (e.g., 3-acetyl-2,5-dimethylthiophene). Otherwise additional data are required to complete the safety evaluation (e.g., 2-ethylfuran). The principal objective of the safety evaluation procedure is to identify two groups of flavoring substances: (1) those substances whose structure, metabolism, and relevant toxicity data clearly indicate that the substance would be expected not to be a safety concern under current conditions of intended use; and (2) those substances that may require additional data to perform an adequate safety evaluation.

The adoption and application of the above safety evaluation method resulted in a practical and efficient scientific procedure for the evaluation of large numbers of flavoring substances, and it uses criteria similar to those used by the FEMA Expert Panel. To date, JECFA has evaluated 1,282 or 76% of the FEMA GRAS chemically identified materials (through GRAS list 21) using the procedure adopted at their 44th Meeting, and further revised at their 46th and 49th Meetings. It is anticipated that JECFA evaluated approximately 400 flavoring substances at the 2004 meeting. The goal of the safety evaluations is to obtain a JECFA-approved global positive list of flavoring substances that can be adopted by Codex and included in the General Standard for Food Additives (GSFA). Codex is currently focusing on food additives in the GSFA but will eventually incorporate approved flavoring substances that can be used by all Codex member states.

Safety Evaluation of Flavoring Substances in the U.S and European Union

The Food Additives Amendment in the US in 1958 introduced the concept of generally recognized as safe (GRAS) by an independent panel of qualified experts in the field of flavor safety assessment. The U.S. FDA recognizes all of the FEMA GRAS substances as permitted substances (*Federal Register 1997*). The GRAS assessment performed by the Expert Panel includes a rigorous evaluation of all the available data on flavor ingredients and structurally related substances. The analyses include a comprehensive evaluation of the potential exposure to the flavor ingredients through food compared with toxicologic and pharmacokinetic characteristics. Between 1965 and 1985, the first comprehensive and systematic scientific literature reviews (SLR) of flavoring substances were completed by FEMA. These SLRs served as the basis for a comprehensive review of substances already designated as FEMA GRAS. This GRAS status reassessment program was known as "GRAS affirmation" or "GRASa" and was completed in 1985. In 1994, the Expert Panel initiated a second comprehensive reassessment program known as "GRAS reaffirmation" or "GRASr." This reaffirmation program was completed in 2005. As part of the GRASr program, the Expert Panel regularly publishes key scientific data on structurally related groups of flavoring substances on which GRAS decisions are based. FEMA GRAS assessments of alicyclic substances, furfural, lactones, and trans-anethole have been published as part of the GRASr program. The fifth in the series, on pyrazine compounds, and the sixth, on methyl eugenol and estragole, have been accepted for publication (Adams et al. 1996, 1997, 1998; Newberne et al. 1999; Smith et al. 2002a,b). To date, information on 21 GRAS lists comprising 2,058 substances have been approved by the FEMA Expert Panel, provided to the FDA, and published by FEMA (Smith et al. 2003). The work of the Expert Panel continues by not only reevaluating substances and reaffirming their GRAS status as new data become available, but also evaluating new substances and adding to the list of approved flavoring substances in the U.S.

At the European Union level, the Scientific Committee on Food (SCF) established in 1974 (EC 1974) provided scientific advice on matters of protection of public health and safety to the European (EU) Commission. When the Commission reorganized its Scientific Committees in 1997, one of the eight new committees was the Scientific Committee on Food, which was given the mandate to "Provide advice on scientific and technical questions concerning consumer health and food safety associated with the consumption of food products, and in particular, questions relating to toxicology and hygiene in the entire food production chain, nutrition, and applications of agri-food technologies, as well as those

relating to materials coming in contact with foodstuffs, such as packaging" (EU Commission 1997). The European Council took the first step toward a EU harmonized approach to the regulation of flavoring substances in 1988 with the publication of EC Directive 88/388 (European Council 1988). By October 1996, the EU had decided upon a positive list approach and detailed its requirements in Regulation (EC) No. 2232/96 of the European Parliament and of the Council laying down a Community procedure for flavoring substances used or intended for use in or on foodstuffs (European Parliament and Council 1996). This regulation consisted of several phases: (1) compilation of a register of flavoring substances that may be used in or on foodstuffs marketed within the EU; (2) request for a recommendation for a safety evaluation procedure that should be carried out by the SCF although it would be left to the Commission to determine the procedure to be adopted; (3) safety evaluations would be carried out by the SCF; and (4) a positive list of flavoring substances permitted at the exclusion of all others would be established 5 years after the establishment of a safety evaluation program. In 1999 the EU Commission adopted a register of flavoring substances to be used in or on foodstuffs. After amendment, the final register contains all FEMA GRAS substances (European Commission 2000b). Also in 1999, the SCF published its opinion on a program for the evaluation of flavoring substances (SCF 1999), and the program was adopted by the European Commission in 2000 (2000a). The Commission showed wisdom in its adoption of an evaluation program by stating that there would be no unnecessary duplication of the evaluations of flavoring substances carried out by the Joint FAO/WHO Expert Committee of Food Additives (JECFA) (EC 1565/2000). Other substances previously evaluated and accepted by the Council of Europe and the SCF were deemed acceptable without further evaluation (EC 1565/2000). Due to the concern about food safety and the need to regain the confidence of consumers that the food supply is safe, the European Parliament and Council (2002) established the European Food Safety Authority (EFSA), which has responsibility for all matters of food safety. Under EFSA, it is the Scientific Committee and permanent Scientific Panels that are responsible for providing the scientific opinions of EFSA. The Scientific Committee is composed of the chairs of the eight Scientific Panels and six independent scientific experts who do not belong to any of the Scientific Panels. Finally, it is the Panel on Food Additives, Flavorings, Processing Aids and Materials in Contact with Food, under the chairmanship that will carry out the final phase of Regulation (EC) No. 2232/96 in cooperation with the member states with the aim of establishing a positive list of approved flavoring substances in 2005.

Issues and Challenges

However, safety evaluation is not a static process; it is dynamic and must take into account new scientific developments that may improve and facilitate the assessment process. The important issue is how best to assess exposure to food flavor materials, and clearly this remains a matter of debate. For establishing "margins of safety," it is essential to have reliable and robust data on levels of exposure. Over the years, several procedures have been proposed and utilized: (a) disappearance from the market place; (b) food surveys; (c) stochastic approaches; and (d) theoretical average maximum daily intake (TAMDI) and possible average daily intake (PADI). There remains debate as to which provide the best estimates of exposure. What may best be said at this point is that quite probably all approaches are conservative in approach and generate "over-estimates" of intakes. One reason for this is that they all fail to take into account the actual "losses" that can occur during exposure and whether or not there is an "additive effect" when several flavors of the same structural class are consumed together and may therefore reduce the calculated margins of safety. It is also needed to give consideration to the existence of population subgroups and whether or not the margins of safety established for the population at large are adequate for these special groups.

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12
Process Flavors

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Introduction
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INTRODUCTION

Process flavor is a food flavoring manufactured by heating various precursors together under controlled reaction conditions, such as temperature, reaction time, pH, and moisture content, which mimic conventional cooking process. The term "reaction flavor" (instead of "process flavor") has been widely used since its introduction to the food industry. However, process flavor is a more preferred term in the flavor industry because it is more related to a thermal process than to chemical synthesis or to reaction chemistry (Manley and Ahmedi 1995). Process flavoring, thermal process flavoring, and processed flavor (flavoring) are also interchangeably used (May 1991). The International Organization of the Flavor Industry (IOFI) defines process flavor as a thermal process flavoring prepared for its flavoring properties by heating food ingredients and/or ingredients permitted for use in foodstuffs or in process flavorings. The basic theory involved in process flavor is a Maillard reaction. In addition to browning, reduction in nutritional value (for example, loss of lysine), toxicity, and antioxidant properties, this reaction is of great significance in food processing regarding flavor generation. Reaction mechanism and application of this reaction to the process flavor have been the main topics in food processing (MacLeod 1986, Nagodawithana 1995). In general, the term "process flavor" is used in describing flavors produced by (1) processing (for example, cocoa/chocolate), (2) enzymatic modification or fermentation, or (3) Maillard reaction (Heath and Reineccius 1986, Manley 1994).

Numerous reviews on process flavors have already been published (Heath and Reineccius 1986, MacLeod 1986, May 1991, Manley 1994, Manley and Ahmedi 1995, Nagodawithana 1995, Weenen and de Rooij 1998, Manley and others 1999). Process flavor is important in imparting savory or meaty flavor to ready-to-eat foods. Meat process flavors are manufactured by reacting precursors, hydrolyzed vegetable protein (HVP), meat extracts, and yeast extracts under controlled conditions, and then by adding spices and flavor enhancers. Enzymatically hydrolyzed vegetable protein (EVP) is used as an alternative to HVP due to possible concern on monochloropropanediols MCPD) or dichloropro-panols (DCP). Process flavor can be developed by heating EVP (Aaslyng and others 1999a, 1999b; Wu and others 2000). Inexpensive

protein sources from seafood by-products can also be used as precursors to develop process flavor (Baek and Cadwallader 1995, 1999).

Enzymatic modification of butter, coffee, chocolate, cereal products, and roasted nuts are also considered process flavors, but meat flavor is the most important among the process flavors. In this chapter, meat flavors will be discussed extensively because the flavors of cooked meats are typical examples of process flavor.

AROMA-ACTIVE COMPOUNDS FOUND IN MEATS AND PROCESS FLAVORS

To date, many hundreds of volatile flavor compounds have been identified in beef, pork, chicken, and lamb (Mottram 1991, Shahidi 1998). Early research on volatile flavor compounds of cooked meats was not successful in characterizing the meaty flavor because these compounds do not necessarily represent the character-impact compounds or aroma-active compounds of cooked meats. Most important meaty flavors were in low thresholds, which made them difficult to be identified as potent aroma compounds by instrument analysis. Recent development of gas chromatography-olfactometry (GC-O) techniques revealed important aroma-active compounds from cooked meats (Gasser and Grosch 1988, 1990; Guth and Grosch 1994; Kerscher and Grosch 1997). Flavor chemistry of meat flavors has recently been reviewed intensively (Shahidi 1998). Lists of significant aroma-active components frequently found in cooked meats and meatlike process flavors are given in Tables 12.1 and 12.2. Sulfur- and nitrogen-containing (or heterocyclic) compounds as well as furanones and lipid-derived compounds are important in meats and process flavors. Of these, sulfur-containing compounds contribute most significantly to process flavors. Classes of heterocyclic aroma-active compounds found in meats and process flavors are shown in Figure 12.1.

Sulfur-containing Compounds

Sulfur-containing (or heterocyclic) compounds are most important in cooked meat flavors (Mussinan and Keelan 1994). Sulfur-containing aroma-active compounds found in beef, chicken, and process flavors are listed in Table 12.1. MacLeod (1986) found that most meaty aromas were sulfur-containing compounds. 2-Methyl-3-furanthiol (2-MF), 2-furfurylthiol, and 2,5-dimethyl-3-furanthiol were identified as primary odorants of chicken broth (Gasser and Grosch 1990). 2-MF and its oxidative dimer bis-(2-methyl-3-furyl) disulfide play very important roles in cooked beef (Gasser and Grosch 1988). These compounds are formed by thiamine degradation and reactions involving cysteine (Zhang and Ho 1991b, Hofmann and Schieberle 1995a). These sulfur-containing compounds have been identified from process flavors made from enzyme-hydrolyzed soybean protein (Baek and others 2001, Wu and Cadwallader 2002). Odor thresholds of 2-MF and bis-(2-methyl-3-furyl) disulfide were known to be 0.005–

Table 12.1. Potent sulfur-containing aroma-active compounds found in cooked meats and meatlike process flavors.

Compounds	Occurrence	Reference
2-methyl-3-furanthiol	B, C, P	2–4, 6–9
3-mercapto-2-pentanone	B, P	2–4, 6, 9
dimethyl trisulfide	B, C, P	2, 5–8
2-furfurylthiol	B, C, P	2–5, 7, 9
methional	B, C, P	1, 3–9
2,5-dimethyl-3-furanthiol	C	9
2-acetylthiazole	B, P	1, 2, 8, 9
2-acetyl-2-thiazoline	B, C, P	1–5, 7,
bis-(2-methyl-3-furyl) disulfide	B, P	2, 4, 9

B = beef, C = chicken, P = process flavor

References: 1: Cerny and Grosch 1992; 2: Hofmann and Schieberle 1995a; 3: Wu and Cadwallader 2002; 4: Kerscher and Grosch 1997; 5: Guth and Grosch 1994; 6: Baek and others 2001; 7: Kerler and Grosch 1997; 8: Gasser and Grosch 1988; 9: Gasser and Grosch 1990.

Table 12.2. Potent aroma-active compounds frequently found in cooked meats and meatlike process flavors.

Compounds	Occurrence	Reference
3-methylbutanal	B, C, P	3, 4, 5, 7
2,3-butanedione	B, P	1–6
Hexanal	B, C, P	1, 3–5, 7
1-octen-3-one	B, C, P	1, 3–5, 7
2-acetyl-1-pyrroline	B, P	3, 4
(*E,E*)-2,4-decadienal	B, C	1, 4, 5, 7
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	B, C, P	1–5, 7
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	B, C, P	1, 2, 4, 5, 7
γ-dodecalactone	C	9

B = beef, C = chicken, P = process flavor

References: 1: Cerny and Grosch 1992; 2: Hofmann and Schieberle 1995a; 3: Wu and Cadwallader 2002; 4: Kerscher and Grosch 1997; 5: Guth and Grosch 1994; 6: Baek and others 2001; 7: Kerler and Grosch 1997; 8: Gasser and Grosch 1988; 9: Gasser and Grosch 1990.

0.01 and 0.00002 ppb, respectively (Gasser and Grosch 1988, Buttery 1999). Furans with a sulfur atom at the 3- or 4-position are known to have a particularly strong meat character (Weenen and de Rooij 1998). A number of sulfur-containing furans have been previously identified in yeast extracts (Ames and MacLeod 1985, Ames 1994).

The aroma difference between beef and chicken might be explained by the fact that bis-(2-methyl-3-furyl) disulfide and methional predominated in beef whereas lipid-derived (E,E)-2,4-decadienal and γ-dodecalactone were more potent in chicken (Gasser and Grosch 1990).

2-Acetyl-2-thiazoline is considered to be a key contributor to meat process flavors with roasty and popcorn-like aroma-note (Hofmann and Schieberle 1995b). This heterocyclic compound has also been identified as a potent aroma-active compound in beef, chicken, and process flavors.

Nitrogen-containing Compounds

Pyrazines are one of the major groups of nitrogen-containing volatile compounds generated by Maillard reaction, which are responsible for the roasted aroma of meats. These compounds also contribute to the roasted aroma note of process flavors. Few pyrazines including 2-ethyl-3,5-dimethylpyrazine have been identified in process flavors from enzyme hydrolyzed soybean protein (Wu and others 2000, Baek and others 2001). 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine have odor thresholds of 0.04 ppb and 8.6 ppb, respectively (Buttery and Ling 1997).

2-Acetyl-1-pyrroline (2-AP) is an important aroma-active compound in heated foods. L-Proline is a responsible precursor for this compound (Roberts and Acree 1994). The formation of 2-AP from L-proline was affected by pH, which prefers neutral and slightly alkaline conditions (Blank and others 2003).

Furanones

Furanones, such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), were identified as key aroma-active compounds of stewed beef (Guth and Grosch 1994). Enolones, such as maltol, cyclotene, and furanones, are important contributors to meat flavors to give rise to sweet and caramel-like aromas (Belitz and Grosch 1999). 4-Hydroxy-5-methyl-3(2*H*)-furanone (HMF) and HDMF were first isolated from beef broth (Tonsbeek and others 1968). These two furanones play an important role in meat flavor as precursors for beef flavor (Shu and others 1985, Shu and Ho 1988, Whitfield and Mottram 1999).

Lipid-derived Compounds

The Maillard reaction products can be classified into four groups, which include sulfur-, oxygen-, and nitrogen-containing heterocyclic compounds, cyclic

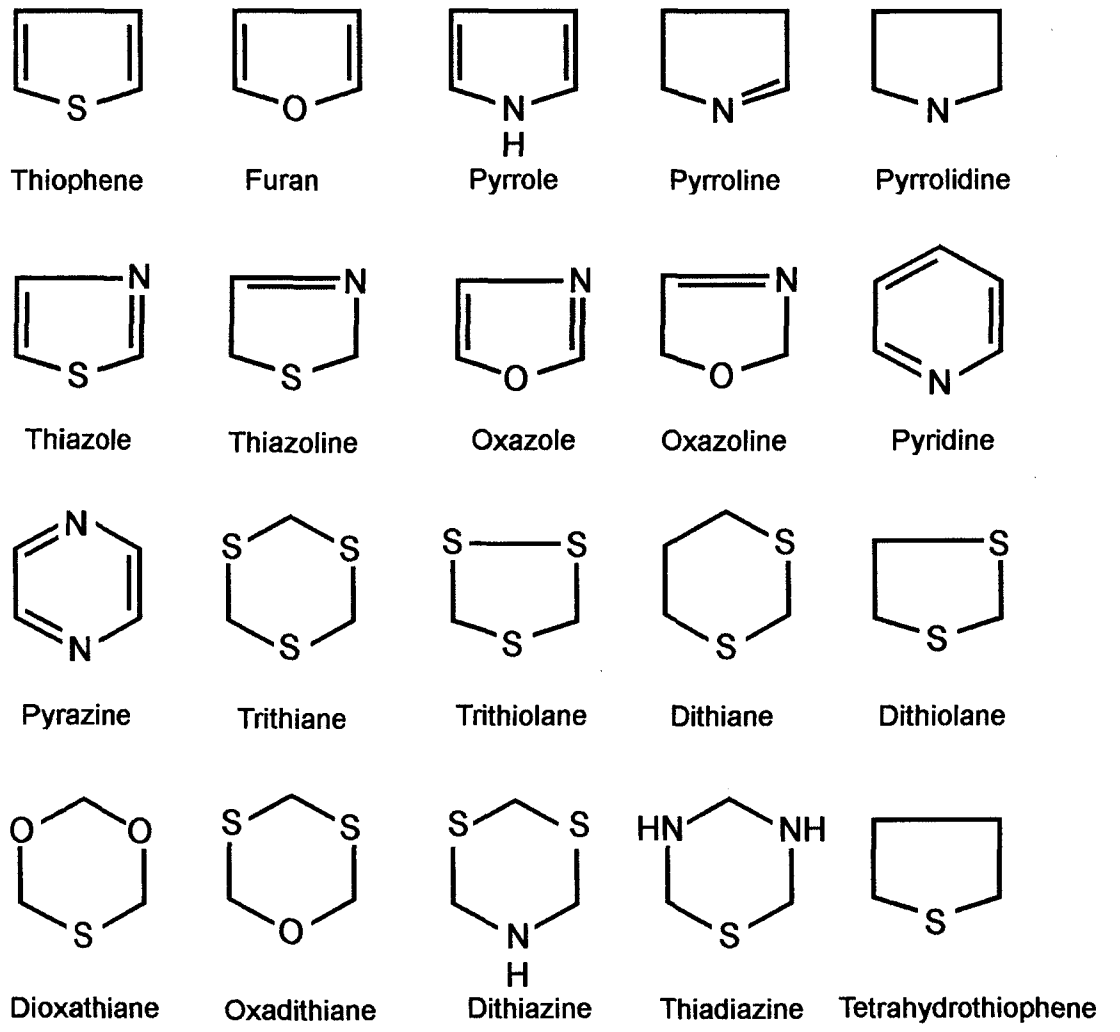

Figure 12.1. Classes of heterocyclic aroma-active compounds found in meats and process flavors.

enolones, polycarbonyls, and monocarbonyls, depending on their aroma types, chemical structures, molecular shapes, and processing parameters (Heath and Reineccius 1986). Fat degradation products appear to determine the species-specific flavor of meat. (*E,E*)-2,4-Decadienal, which is formed by autoxidation of linoleic acid, is known to be a characteristic aroma of chicken (Gasser and Grosch 1990, Chen and Ho 1998b). However, this lipid-derived compound was of minor importance in beef broth (Gasser and Grosch 1990).

Regardless of meat species, characteristic meaty aroma is associated with all meats. Species-specific aroma-active compounds of meats are believed to be derived from lipid sources. 12-Methyltridecanal has been identified as a species-specific odorant of stewed beef (Guth and Grosch 1993).

OTHER VOLATILE COMPOUNDS FOUND IN MEATS AND PROCESS FLAVOR

Thiophenes, thiazoles, thiazolines, dithianes, dithiolanes, trithiolanes, and trithianes are other important meat volatiles found in meat flavors or process flavors (Shu and others 1986, Mottram 1991, Zhang

and Ho 1991b, Chen and Ho 1998a). These sulfur-containing heterocyclic compounds are considered to contribute to the meat aroma. Of these 3-thiophenethiol having meaty aroma note plays an important role in meat flavor. Thiophenes with long alkyl chains are believed to arise from lipid sources or phospholipids by the reaction of hydrogen sulfide with unsaturated fatty acids (Whitfield and others 1988, Ho and others 1989). Large numbers of thiazoles have been identified from cooked meat flavors (Mottram 1991). Of these, 2-acetylthiazole was identified as an aroma-active compound of meats.

Oxazoles and oxazolines have been found in boiled beef, pork, and fried chicken (Mottram 1991). Although 2,4,5-trimethyl-3-oxazoline and other alkyl substituted oxazoles are found in meats, the contribution of these oxygen-containing heterocyclic compounds to meat aroma is probably not as important as sulfur- and nitrogen-containing heterocyclic compounds. Some important heterocyclic compounds of cooked meats and meatlike process flavors are shown in Figure 12.1.

Heterocyclic compounds with long-chain alkyl substitution are formed from the reaction of lipid or lipid oxidation products, for which the formation mechanism has been proposed (Ho and others 1989). The formation of 2,5-dimethyl-3-pentylpyrazine can be explained by the reaction of 2,5-dimethyldihydropyrazine with pentanal. 2-Pentylpyridine is formed by the interaction of 2,4-decadienal and amino acids.

PRECURSORS USED IN THE MANUFACTURE OF PROCESS FLAVORS

Water-soluble components from meat extracts contain a complex mixture of precursors including nucleic acids, nucleotides, peptides, amino acids, sugars, lipids, and vitamins. When these precursors reacted, meat flavor would be theoretically well duplicated. However, because of the cost, availability, or labeling restrictions, other precursors have also been considered.

Amino Acids

Amino acid is the most important factor determining aroma property for process flavor. Lane and Nursten (1983) reported that thermally generated aromas are basically the same for each particular amino acid on the basis of the assessment of aroma properties generated from more than 400 model systems.

Cysteine is the most important amino acid as a source of sulfur in the generation of meat flavor (Figure 12.2). Precursor mixtures used in the industrial production of process flavors usually contain cysteine. Numerous research has proved that this amino acid is essential in the production of process flavors (Hofmann and Schieberle 1995a; Schieberle and Hofmann 1996, 1998; Wu and others 2000). More than 200 volatile compounds have been identified from the reaction of cysteine and reducing sugar (Schieberle and Hofmann 1998). Cysteine reacted with ribose to produce meat flavor comprising mainly furanthiols (Hofmann and Schieberle 1995a).

Other sulfur-containing amino acids, such as methionine and cystine, and glutathione (Glu-Cys-Gly) are also important precursors. From a practical point

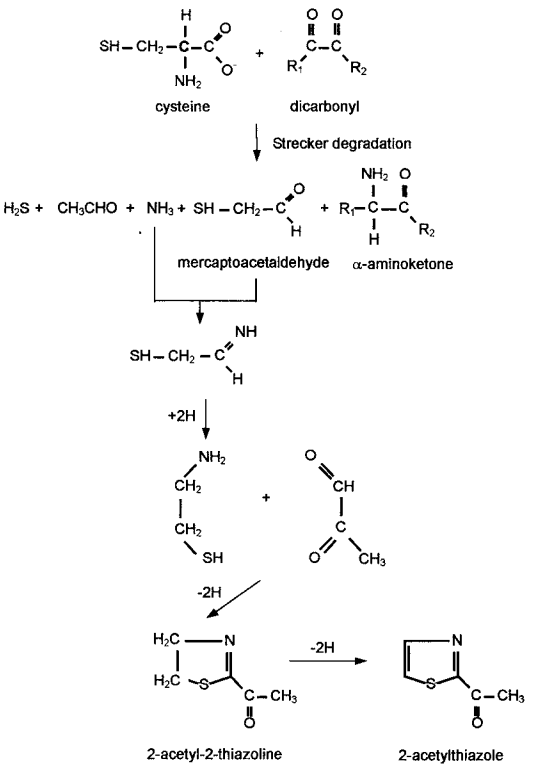

Figure 12.2. The formation of sulfur-containing heterocyclic compounds via Strecker degradation of cysteine. (Adapted from Nagodawithana 1995)

of view, cysteine is the most satisfactory sulfur compound in manufacturing process flavor.

Proline, which is an amino acid, reacts with reducing sugars to produce 2-acetyl-1-pyrroline (Roberts and Acree 1994). Proline does not produce pyrazines because this amino acid cannot release ammonia in Strecker degradation (Nursten 1986). Maillard reaction products differ depending on 1,2- and 2,3-enolization, which is dependent on pH. At lower pH, 1,2 enolization occurs, and at higher pH, 2,3-enolization occurs. In contrast, hydroxyamino acids, such as serine and threonine, produce pyrazines (Nursten 1986). Glutamic acid and monosodium glutamate (MSG) are also known for their ability to serve as precursors for process flavors as well as flavor enhancer (Nagodawithana 1995).

Reducing Sugars

Ribose and ribose-5-phosphate increased during aging from inosine-5′-monophosphate (IMP) decomposition. The most significant loss occurred for cysteine and ribose during heating of meats, which led to the first process flavor patent by Morton and others (1960). A reaction mixture of cysteine and ribose is generally accepted to produce meat flavor.

Previously it is known that overall odor of Maillard reaction products is not influenced by sugar moiety (Lane and Nursten 1983). However, recently it was reported that the sugar moiety significantly influenced the volatile pattern formed (Hofmann and Schieberle 1997, 1998; Schieberle and Hofmann 1998). When cysteine is reacted with different reducing sugars, specific aroma-active compounds are formed depending on the source of reducing sugar. For example, rhamnose produced 5-methyl-2-furfurylthiol and 3-hydroxy-6-methyl-2(2H) pyranone, whereas 2-(1-mercaptoethyl) furan was exclusively formed from glucose. In addition to monosaccharides, reducing disaccharides, such as maltose and lactose, can participate in Maillard reaction (Hurrel 1982). Pentoses are more reactive than hexose with xylose being the most reactive.

Thiamine

Thiamine (vitamin B1) is a very important nonvolatile flavor precursor, which decomposes thermally to give rise to sulfur-containing compounds important to meat flavor. Reaction mechanism of thermal degradation of thiamine can be found elsewhere (MacLeod 1986, Nagodawithana 1995, Weenen and de Rooij 1998). Thermal degradation of thiamine depends on pH (Figure 12.3). Thermal degradation at neutral or slightly alkaline pH appears to favor the breakdown of thiazole ring, which leads to the formation of H_2S. Breakdown of the methylene bridge is predominant under acidic conditions (Nagodawithana 1995).

Ribonucleotides

Ribonucleotides are known to serve as a source of ribose as well as flavor enhancers. Nucleotides, such as IMP, which is formed from adenosine-5′-triphosphate (ATP) during aging, are the most important aroma precursors in the development of cooked meat flavor.

IMP increased the meaty note of process flavor by thermal reaction (Zhang and Ho 1991b, Farmer and others 1996). The role of IMP is more than just a ribose source. By thermal reaction of cysteine and IMP, a lot of sulfur-containing compounds including 2-methyl-3-furanthiol and 2-furfurylthiol were formed (Zhang and Ho 1991b). IMP is considered as an important precursor to generate sulfur-containing compounds responsible for meat flavor. Possible mechanism for the formation of 2-methyl-3-furanthiol from thermal reaction of IMP with cysteine has been proposed (Zhang and Ho 1991b). The major compounds were furfural and 2-furfurylthiol, indicating that liberation of ribose from IMP is the main pathway and these sulfur compounds were subsequently produced by dehydration and oxidation at acidic conditions.

Phospholipid

Mottram and Edwards (1983) reported that the characteristic aroma of cooked meat disappeared by the removal of phospholipid from meat, indicating that phospholipids played a significant role in the generation of cooked meat flavor. Phospholipids contribute to the aroma of cooked meat through thermally induced lipid oxidation and interaction of lipid with Maillard reaction products (Farmer and Mottram 1990; Mottram and Whitfield 1995a, 1995b). Phospholipid inhibited the formation of heterocyclic compounds, such as alkylpyrazines, by participating in Maillard reaction in the aqueous

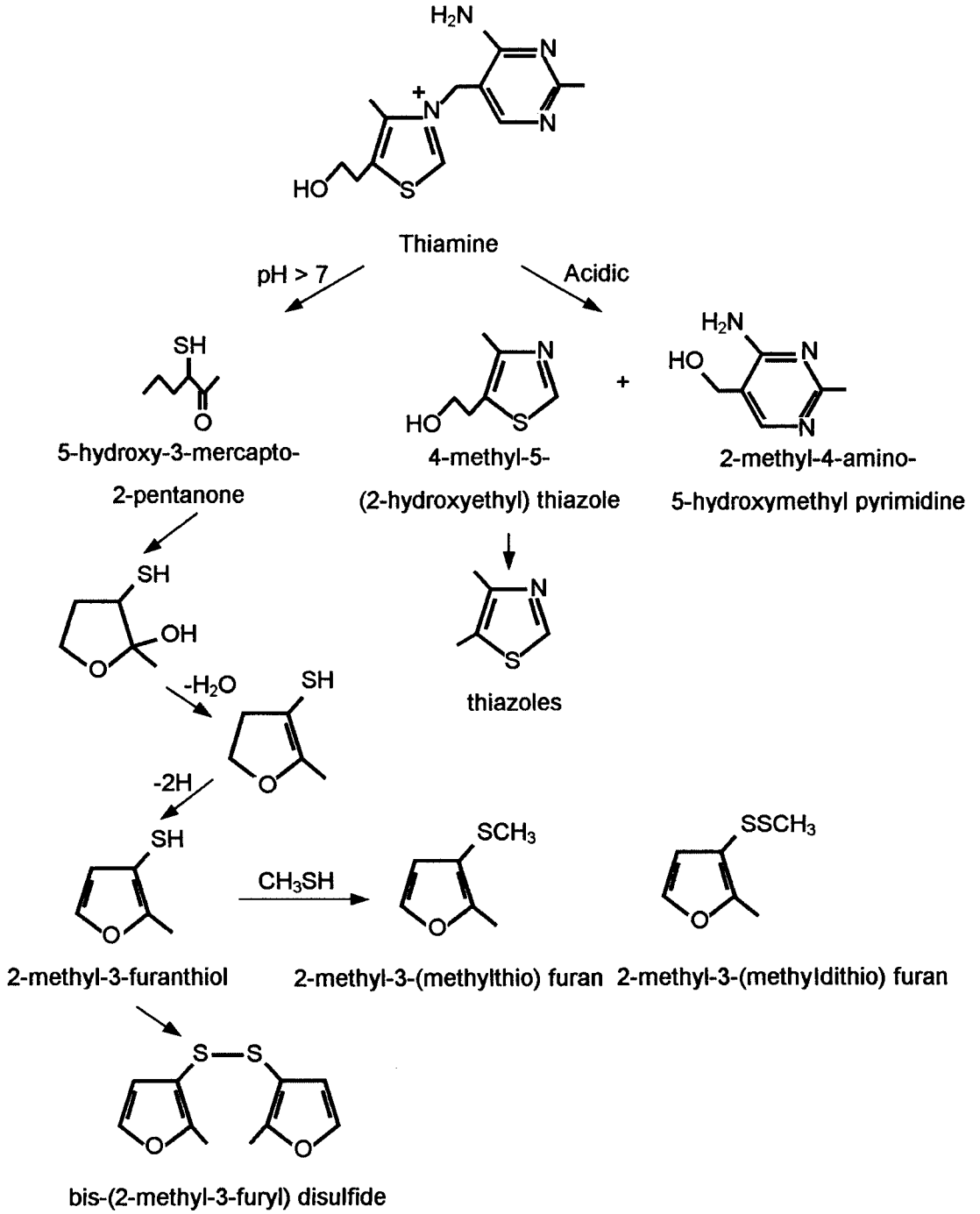

Figure 12.3. The formation of sulfur-containing heterocyclic compounds from thermal degradation of thiamine at different pH levels. (Adapted from Nagodawithana 1995)

system (Whitfield and others 1988, Farmer and others 1989). Sulfur-containing compounds, such as methylthio-substituted furans and thiophenes, were generated by the addition of phospholipid in nonaqueous systems (Mottram and Whitfield 1995a). Water contents also influenced volatiles produced in Maillard reaction systems with phospholipid (Mottram and Whitfield 1995b).

Aromas generated by thermal degradation of phospholipids are mainly due to lipid-derived volatiles, such as trans-4,5-epoxy-(E)-decenal and (E,E)-2,4-decadienal (Lin and Blank 2003). Recently, it was hypothesized that molecular organization of phospholipids played an important role in thermal generation of aromas (Vauthey and others 2000, Lin and others 2004).

Others

Meat extracts, which contain precursor amino acids, are usually major ingredients in many process flavors. Protein hydrolysates are good sources of amino acids for process flavors. HVP is an economical source; however, the use of HVP has decreased in light of safety concerns. Flavors without HVP have been regarded as "clean-label" flavors in the United States (Manley and others 1999). EVP can be used as an alternative to HVP. Autolyzed yeast extract is a very useful reactant in creating process flavors. Yeast extract generates sulfur-containing compounds, such as 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) disulfide, which impart meatlike aroma (Ames and MacLeod 1985). Glutathione is also a good source for process flavor (Zhang and Ho 1991a).

Peptides can react with reducing sugars via complex reactions. Taking into consideration the raw material price, meat by-products are good sources of amino acids and peptides. Protein hydrolysates from plants or animal origins provide a good nitrogen source to process flavor. HVP and autolyzed yeast extract are widely used as nitrogen sources. Incorporation of fats into the mixture before reaction gives species-specific process flavors, such as, mutton, chicken, and pork.

HMF and HDMF are considered to be involved in the meaty flavors through their reactions with either hydrogen sulfide or sulfur-containing amino acids (Shu and others 1986, Shu and Ho 1988, Whitfield and Mottram 1999). 3,5-Dimethyl-1,2,4-trithiolane and other heterocyclic compounds described as roasted and meaty were formed by the thermal reaction of HDMF and cysteine. The formation of HDMF from rhamnose has been proposed.

Maillard reaction intermediates, such as acetaldehyde, pyruvaldehyde (2-oxopropanal), and 2,3-butanedione, have been used as precursors for the development of process flavors (MacLeod 1986).

FORMATION MECHANISM

Numerous reviews on the formation mechanisms of meat flavor have been published (MacLeod 1986, Nursten 1986, Nagodawithana 1995). Since sulfur-containing compounds play a critical role in the perception of meat flavor, a possible source of sulfur-containing compounds and their formation mechanisms have been extensively reviewed (Mussinan and Keelan 1994). Hydrogen sulfide evolved from Strecker degradation of cysteine plays a central role in the generation of meaty flavor. In addition, mercaptoacetaldehyde, acetaldehyde, and ammonia produced from Strecker degradation of cysteine serve as intermediates in the generation of sulfur-containing heterocyclic compounds, such as 2-acetyl-2-thiazoline and 2-acetylthiazole (Figure 12.2).

Basic chemistry of process flavors has been reviewed in recent articles (Mottram 1998, Weenen and de Rooij 1998, Manley and others 1999). The most important reactions involved in process flavor are Maillard reaction, Strecker degradation, sugar degradation/fragmentation, thermal degradation of thiamine, and lipid oxidation (Nagodawithana 1995, MacLeod 1986). Formation mechanism relating to process flavor has been reviewed elsewhere (Nagodawithana 1995, MacLeod 1986). Figure 12.3 shows the thermal degradation of thiamine at different pHs to generate aroma-active compounds important to process flavors, such as 2-methyl-3-furanthiol, 2-methyl-3-(methylthio) furan, 2-methyl-3-(methyldithio) furan, and bis-(2-methyl-3-furyl) disulfide.

Thermal breakdown mechanisms of sugars and amino acids have been described in detail (Scarpellino and Soukup 1993). 5-Hydroxymethyl-2-furfural and 2-furfural are formed under acidic conditions through 1,2-enolization of Amadori compounds derived from thermal breakdown of pentose and hexose, respectively. Under alkaline conditions, the formation of HMF and HDMF are through 2,3-enolization of Amadori compounds derived

from the thermal breakdown of pentose and hexose, respectively. Sulfur substituted furans and thiophenes were formed as main volatile compounds from the reaction of HMF and hydrogen sulfide (van den Ouweland and Peer 1975). Amino acids are thermally degraded through Strecker degradation, which is a reaction of an amino acid with a dicarbonyl to produce Strecker aldehyde that has one less carbon than original amino acid. Strecker aldehydes play important roles in process flavors as aroma-active compounds as well as precursors for further reactions.

REACTION PARAMETERS

The aroma property of process flavor depends on (1) the nature and ratio of amino acids/protein and reducing sugars present, (2) the time/temperature conditions of the reaction, and (3) the nature of any thermal degradation reactions. Free amino acids are presumably more important than peptides or proteins. Of these, temperature has the greatest influence on the reaction.

Lipid oxidation products play an important role in the development of some thermally generated aromas as described by Ho and others (1989).

Moisture content is one of the important reaction parameters in the generation of aromas from process flavors. Volatile profiles of process flavors showed significantly different patterns depending on moisture contents of reactants. Dry heating of precursor mixtures generated higher yield of aroma-active compounds than heating under the aqueous system (Schieberle and Hofmann 1998). Some odorants increased in dry heating, whereas some decreased under dry heating. For example, 2-furfurylthiol, 3-mercapto-2-pentanone, 2-methyl-3-furanthiol, and 5-acetyl-2,3-dihydro-1,4-thiazine were the most intense odorant generated in aqueous solution (Hofmann and Schieberle 1995a). 2-Furfurylthiol and 2-acetyl-2-thiazoline were considered the most potent aroma-active compounds in dry heated mixture, whereas 5-acetyl-2,3-dihydro-1,4-thiazine and 3-mercapto-2-pentanone significantly decreased (Schieberle and Hofmann 1998). Also, a comparison has been made in dry heated and the aqueous rhamnose/cysteine system (Hofmann and Schieberle 1997). Caramel- and seasoning-like aroma notes prevailed in the aqueous system, whereas roasty note predominated in dry heated.

The pH effect on the volatile formation in process flavors is important. Cysteine produces meat flavor with the reaction of ribose at acidic pH. In the reaction of cysteine-ribose, pH remarkably influenced the overall aroma (Schieberle and Hofmann 1996). As pH increased from 3.0 to 7.0, 2-methyl-3-furanthiol and 2-furfurylthiol decreased significantly, whereas the 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) increased, which resulted in significant changes of overall aroma. pH plays a key role in manipulating the volatile compositions in producing process flavors. The production of sulfur-substituted furans with meatlike aromas from the reaction of cysteine is dependent on pH, which is favored at acidic pH (Zhang and Ho 1991a, 1991b; Whitfield and Mottram 1999). pH influenced the volatile formation from the reaction of cysteine and 4-hydroxy-2,5-dimethyl-3(2H)-furanone with the generation of the best meat flavor at pH 2.2 (Shu and Ho 1988). The formation of pyrazines was favored at alkaline pH due to the increased reactivity of amino groups (Hwang and others 1994). In general, browning is more favored at alkaline pH.

SAFETY CONCERN

Heterocyclic amines formed by heat treatment and monochloropropanediols (MCPD) or dichloropropanols (DCP) in HVP are the major concern of safety in process flavors. However, these harmful materials can be reduced by processing conditions and technological achievements. Furthermore, the levels of process flavors used in foods are below levels of safety concern.

Recently, acrylamide has been detected in heated foodstuffs including potato chips and meats. Moderate levels of acrylamide (5–50 μg/kg) were measured in heated protein-rich foods, such as boiled beef, whereas higher contents (150–4,000 μg/kg) were measured in carbohydrate-rich foods (Tareke and others 2002). Acrylamide is formed by the reaction of asparagines and glucose (Yaylayan and others 2003). However, process flavor is used as an ingredient in small amounts, normally less than 2%, so it does not seem to be harmful to food. Process flavors are on the list of generally recognized as safe (GRAS) (Manley and others 1999).

The IOFI guideline defines process flavor as a product or a mixture prepared for its flavoring

properties and which is produced from ingredients or mixtures of ingredients that are permitted for use in foodstuffs, or are present naturally in foodstuffs, or are permitted for use specifically in process flavorings by a process used for the preparation of foods for human consumption. The ingredients can include (a) a protein nitrogen source, (b) a carbohydrate source, (c) a fat or fatty acid source, and (d) other ingredients including herbs and spices; thiamine; organic acids; sodium chloride; and lecithin. The processing conditions should not exceed 15 minutes at 180°C or proportionately longer at lower temperatures. The pH should not exceed 8.0.

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Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

13
Savory Flavors

Christoph Cerny

Meat Flavor
    Beef
    Chicken
    Pork
    Sheep
Fish and Seafood Flavor
    Fish
    Seafood
Taste Compounds
References

Savory tasting food like meat and fish form a substantial part of our diet and are important nutrient sources for biologically high-grade proteins, iron, and vitamins. Vegetables add minerals, vitamins, dietary fibers, and antioxidants to our diet. The pleasure of eating savory dishes depends on good quality of the foodstuffs as well as on a well-balanced and stimulating recipe and finally on the perfectly executed preparation and cooking. The resulting flavor is not caused by a single compound or a single compound class, but by the sensory impression stimulated by a multitude of differently structured chemicals. These components can be divided into three categories (Dwivedi and Snell 1975):

- Aroma compounds, which are volatile and perceived by the sense of smell through the olfactory receptors in the nasal mucosa
- Nonvolatile compounds, which contribute the five basic tastes (sweet, sour, salty, bitter, and umami) as well as tactile properties by interacting with receptors in the oral cavity
- Synergistic compounds intensifying the effect of other flavor sensations

More than 7,000 volatiles have been identified in food to date (Boelens 2000), whereof only a fraction actually contributes to the aroma. Analytical techniques, which combine instrumental analysis with sensory methods, are suitable to distinguish the aroma-active volatiles from the others. Most of these techniques are based on the aroma value concept (Rothe and Thomas 1963) and use gas chromatography-olfactometry (GC-O). Aroma extract dilution analysis (AEDA) is a further development of GC-O (Gasser and Grosch 1988). Successively diluted aroma extracts are analyzed by GC-O until the odor is no longer perceived. The flavor dilution (FD) factor indicates the highest dilution at which a compound is still detected at the sniffing port. The aroma value or odor activity value (OAV) is defined as the quotient of concentration and odor threshold of a compound in a given matrix.

Table 13.1 compares the identified volatiles in boiled beef (Boelens 2000) with the aroma impact compounds (Gasser and Grosch 1988, Konopka and Grosch 1991, Kerscher and Grosch 1997, Kerscher and Grosch 1998, Kerscher 2000). It becomes obvious that the volatiles outnumber the aroma-active ones among them and that not all chemical classes are equally important. The objective of this chapter is to concentrate the discussion on the aroma-active volatiles, and in addition on the nonvolatiles that contribute to the taste of meat, fish, and seafood.

MEAT FLAVOR

Meat flavor has been the subject of many review articles (Dwivedi and Snell 1975, Ohloff and Flament 1978, MacLeod and others 1981, Baines and Mlotkiewicz 1984, Werkhoff and others 1993, Mottram 1998) and books dedicated to this subject

Table 13.1. Identified volatiles and aroma compounds in boiled beef.

	Volatiles[a]	Aroma compounds[b]
Hydrocarbons	73	0
Alcohols	32	1
Aldehydes	57	20
Ketones	49	11
Acids	9	5
Esters and lactones	11	0
Bases	91	1
Sulfur compounds	124	19
Ethers	5	0
Halogens	6	0
Nitriles and amides	1	0
Phenols	3	3
Furans	22	2
Oxazoles	5	0

[a]Number of identified volatiles according to Boelens (Boelens 2000).

[b]Number of aroma-active compounds identified by GC-O (Gasser and Grosch 1988; Konopka and Grosch 1991; Kerscher and Grosch 1997, 1998; Kerscher 2000).

(Shahidi 1994, 1998). The most popular and most commonly produced meats worldwide are pork (86 million tons per year), poultry (58 million tons), beef (54 million tons), and lamb (7 million tons) (Belitz and others 1999).

The precursors responsible for the basic aroma of meat are generally water-soluble and found in the lean part of the meat, whereas the species-specific aroma compounds stem mainly from the lipids (Hornstein and Crowe 1960, Hornstein and others 1963). Table 13.2 shows the concentrations of potential water-soluble aroma precursors in beef (top round) meat. Besides the species of the meat, the way of cooking has a great influence on the aroma. While, for example, acetaldehyde, methylpropanal, methional, octanal, nonanal, and 2(Z)-nonenal dominate the aroma of roasted chicken meat (Kerscher 2000), the odor of boiled chicken is caused mainly by 2-furfurylthiol, hexanal, 2(E)-nonenal, 2,4(E,E)-nonadienal, 2,4(E,E)-decadienal, and 2,4(E,Z)-decadienal (Kerler and Grosch 1997). In chicken skin, 3-mercapto-2-pentanone, acetaldehyde, methylpropanal, 2-methylbutanal, 3-methylbutanal, and methional show the highest OAVs (Kerscher 2000). Methanthiol is a key aroma compound in all three types of chicken aroma. The cooking time equally affects the formation of aroma compounds. The concentration of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) in beef increases continuously up to 1.6 mg/kg during 15 minutes of roasting, and even reaches 11.6 mg/kg when stewed for 4 hours (Grosch and others 1993).

Beef

Table 13.3 lists the volatiles that have been shown by GC-O methods to be aroma-active in roasted, boiled, and stewed beef, respectively. Figure 13.1 shows some of their structures. The quantitative data were obtained by stable isotope dilution assay (Sweeley and others 1966, Schieberle and Grosch 1987), which is a reliable method to analyze delicate

Table 13.2. Free amino acids, thiamine, and sugars in beef.

	Beef (mg/kg)[a, b]
Alanine	313
Arginine	76
Aspartic acid	7
Cysteine	37
Glutamic acid	103
Glycine	96
Histidine	48
Isoleucine	44
Leucine	80
Lysine	56
Methionine	23
Phenylalanine	52
Proline	39
Serine	48
Threonine	32
Tyrosine	48
Valine	80
Glutamine	706
Thiamine	0.8[c]
Glucose-6-phosphate	1,834
Glucose	1,072
Fructose-6-phosphate	503
Fructose	283

[a]Based on moist mass of raw meat; beef: top round; chicken: breast.

[b]Grosch and coworkers (Grosch and others 1993).

[c]Zeiler-Hilgart (Zeiler-Hilgart 1994).

Table 13.3. Aroma-active compounds in beef, chicken, and pork.

No.		Beef		Chicken			Pork	
		Roasted[a]	Boiled[b]	Stewed[c]	Boiled[d]	Roasted[e]	Boiled[f]	Stewed[g]
	Aldehydes							
9	Acetaldehyde	+[h]	1,817	6,400	4,700	3,815	3,953	1,500
11	Methylpropanal	+	117		39	83	90	
13	2-Methylbutanal	+			+			
12	3-Methylbutanal	16	26	10	5	17	27	21
6	Methional	9–33	36	13	15	53	11	23
15	Phenylacetaldeyhde	+	+	+	+			
	Pentanal				+			
23	Hexanal	269	+	72	1,596	283		15
	2(E)-Hexenal		+					
	Heptanal	+	+		+			
	2(E)-Heptenal		+		+			
25	Octanal	+	382	+	148	190	154	+
	2(E)-Octenal		+					
26	Nonanal	+	1,262	+	+	534	643	
27	2(E)-Nonenal	42	32	+	75	23	15	
	2(Z)-Nonenal	+	6	+		6	1	+
	3(Z)-Nonenal	+						
28	2,4(E,E)-Nonadienal	+	+	+	57			
30	2,6(E,Z)-Nonadienal	+	+			3		
	Decanal			+	+			
	2(E)-Decenal				+			
	2(Z)-Decenal		+					
	4,5-Epoxy-2(E)-decenal	1.5		+	+			+
31	2,4(E,E)-Decadienal	+	+	12	302	11	7	10
	2,4(E,Z)-Decadienal		27		31		7	
	2-Undecenal				+			
	2(E)-Dodecenal	+						
32	12-Methyltridecanal		962	52				
	Ketones							
14	2,3-Butanedione	+	+	46				38
	2,3-Pentanedione	+						
	2-Heptanone		+					
	2-Octanone		+					
33	1-Octen-3-one	+	9	+	6	7	5	+
34	1,5(Z)-Octadien-3-one	+	+		+			
	2-Nonanone		+					
	1-Nonen-3-one				+			
	2-Decanone		+					
	2-Undecanone		+					
	2-Dodecanone		+					
	2-Tridecanone		+					
	β-Ionone		+		+			

(Continues)

Table 13.3. Continued

No.		Beef		Chicken			Pork	
		Roasted[a]	Boiled[b]	Stewed[c]	Boiled[d]	Roasted[e]	Boiled[f]	Stewed[g]
	Alcohols							
	1-Octen-3-ol		+					
	Tridecanol				+			
	Acids/Phenols							
	Acetic acid	+	+	200,000	22,100			270,000
	Butyric acid	+	7,074	6,100	3,800	8,119	17,200	3,900
	2-/3-Methylbutyric acid		+	74	+			95
	Valeric acid		+					
	Phenylacetic acid		+					
	Guaiacol	2–8	+		+			
	p-Cresol		+		+			
	p-Vinylguaiacol			+				+
	Vanilline		+					
	Sulfur compounds							
	Hydrogen sulfide				+	290		
3	Methanthiol	+	311	300	1,456	202	278	500
4	Dimethylsulfide	+	105					
5	Dimethyl trisulfide	+	+	+	0.8			+
1	2-Methyl-3-furanthiol		7–28		1–5	0.4–4	6–9	
	2,5-Dimethyl-3-furanthiol				+			
	2-Methyl-3-(methylthio)furan		+		+			
	2-Methyl-3(methyltrithio)furan				+			
8	3-Mercapto-2-pentanone		55–73	+	23–100	27–31	66–117	
7	2-Mercapto-3-pentanone		20–44		2–13	0.5–3.4	11–14	
2	Furfurylthiol		13–42	0.5	0.4–5	0.1–1.9	8–10	0.6
	Benzyl mercaptane		+					
	Bis(2-methyl-3-furyl)disulfide		+	+				
	Bis(2-furfuryl)disulfide				+			
	2-Methylthiophene				+			
	2-Formylthiophene				+			
	2-Formyl-5-methylthiophene		+		+			
	2-Acetylthiophene		+		+			
	3-Acetyl-2,5-dimethylthiophene		+					
	2,4,6-Trimethyltetrahydro-1,3,5-thiadiazine				+			
	3,5-Dimethyl-1,2,4-trithiolane				+			
	5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine				+			
	Lactones							
	γ-Octalactone	+						
	γ-Decalactone				+			
	γ-Dodecalactone			+	+			
17	4,5-Dimethyl-3-hydroxy-2(5H)-furanone	9	+	5	5			3

Table 13.3. Continued

No.		Beef		Chicken			Pork	
		Roasted[a]	Boiled[b]	Stewed[c]	Boiled[d]	Roasted[e]	Boiled[f]	Stewed[g]
	Other Heterocycles							
	Trimethylpyrazine	+						
20	2-Ethyl-3,5-dimethylpyrazine	2–5		+	+			
21	2,3-Diethyl-5-methylpyrazine	1–27		+				+
	2-Acetylthiazole	+	+		+			
19	2-Acetyl-2-thiazoline	12–28	+	1	14			5
	2,4,5-Trimethylthiazole				+			
	Benzothiazole		+					
18	2-Acetyl-1-pyrroline	+	+	+	+			
	Indol				+			
16	2,5-Dimethyl-4-hydroxy-3-(2H)-furanone	659–1,108	9,075	8,000	1,232	50	2,170	2,700
	4-Hydroxy-5-methyl-3(2H)-furanone				+			

[a]Literature data (Cerny and Grosch 1992, 1993; Kerler and Grosch 1996).
[b]Literature data (Gasser and Grosch 1988; Kerscher and Grosch 1997, 1998; Kerscher 2000).
[c]Literature data (Guth and Grosch 1993, 1994, 1995).
[d]Literature data (Kerscher and Grosch 1997, 1998; Gasser and Grosch 1990; Farmer and others 2000; Farkas and others 1997).
[e]Literature data (Kerscher and Grosch 1998; Kerscher 2000).
[f]Literature data (Kerscher and Grosch 1998; Kerscher 2000).
[g]Literature data (Guth and Grosch 1995).
[h]"+": detected by GC-olfactometry; concentration in (μg/kg), as determined by isotope dilution analysis (IDA).

aroma compounds, which are unstable or occur just in minute concentrations.

Sulfur-containing compounds have been thought to contribute to the meaty character of cooked beef (Wilson and others 1973), in particular sulfides like 2-methyl-3-furanthiol (compound 1 in Figure 13.1), 2-furfurylthiol (2) and methanthiol (3) (Gasser and Grosch 1988, Guth and Grosch 1994). According to van den Ouweland and coworkers (van den Ouweland and others 1989), compounds with meaty character possess a five-membered ring, a double bond, and a methyl and a thiol group. While the role of sulfur compounds for the aroma of boiled beef is important, roasted beef is more dominated by roasted notes deriving from certain pyrazines together with aldehydes from lipid degradation (Cerny and Grosch 1992, Kerler and Grosch 1996).

Key reactions for the generation of 1 and 2 in meat are, on the one hand, the Maillard reaction between the amino acid cysteine and the reducing sugar ribose (Farmer and others 1989, Farmer and Mottram 1990), and on the other hand, the degradation of thiamine (Arnold and others 1969, Dwivedi and Arnold 1973, van der Linde and others 1979). Aldehydes are mainly formed via oxidation of unsaturated fatty acids like oleic, linoleic, and linolenic acid present in the lipid fraction. Strecker degradation of valine, isoleucine, leucine, methionine, and phenylalanine causes the formation of methylpropanal (11), 2-methylbutanal (13), 3-methylbutanal (12), methional (6), and phenylacetaldehyde (15). The long-chain branched aldehyde 12-methyltridecanal (32) was considered a species-specific aroma compound in boiled and stewed beef (Guth and Grosch 1993). The compound 32 occurs also in higher concentrations in lamb, springbok, and deer, but only at a very low level in pork and chicken, and hence seems to be characteristic for ruminant animals. Possibly it is formed by the rumen micro flora, absorbed, and later incorporated into the plasmalogens of the muscle membranes from where it is released during longer cooking (Guth and Grosch 1993).

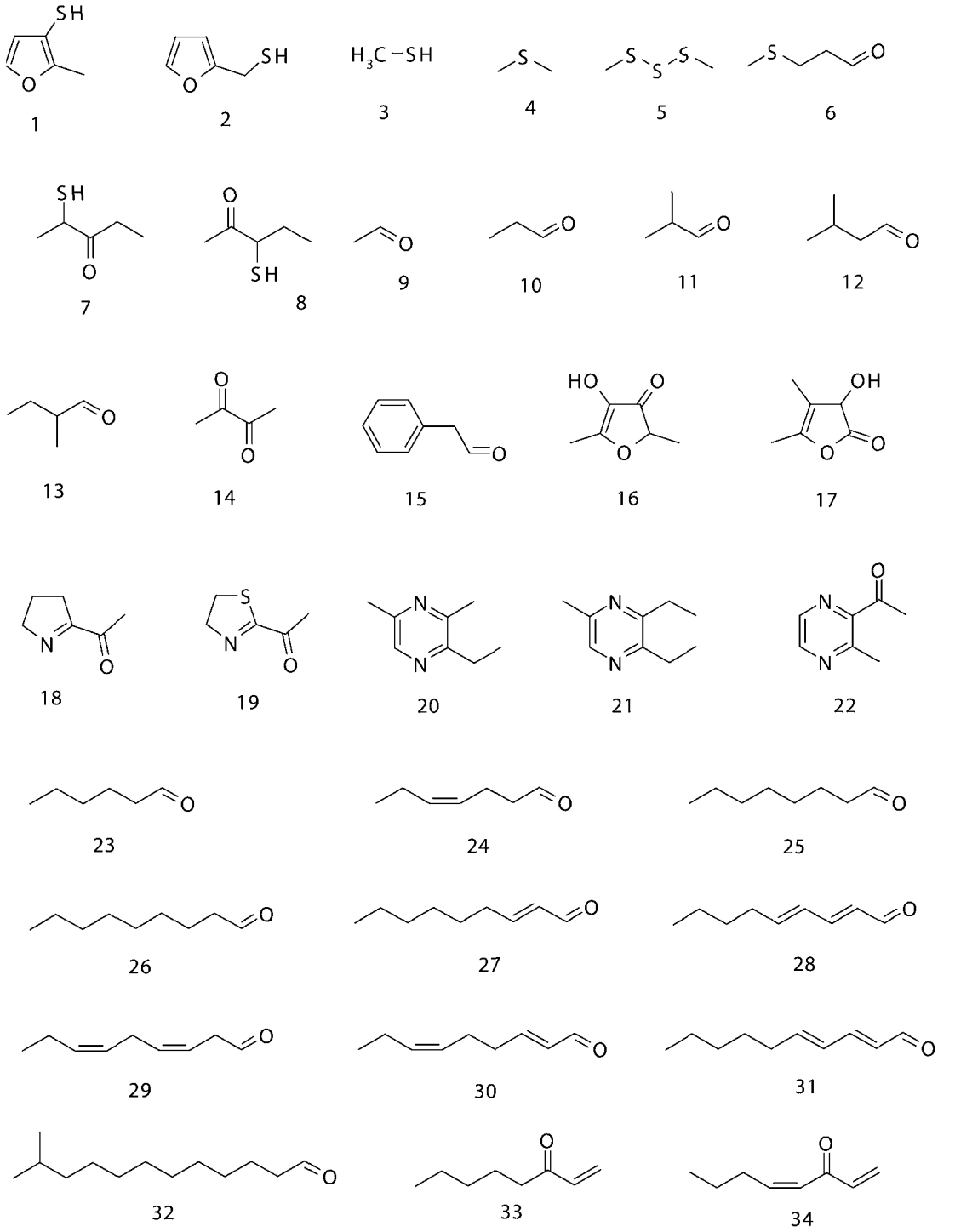

Figure 13.1. Chemical structure of some key aroma compounds of meat, fish and seafood.

Kerscher (2000) has, based on the concentrations of the key aroma compounds found in boiled beef, prepared a synthetic recombination model mixture of the aroma (Table 13.4) and found it highly similar compared to the original beef sample. In subsequent omission tests, the absence of 32 did not change the aroma character significantly indicating that its contribution to beef flavor is much lower than previously assumed (Kerscher 2000).

Chicken

Chicken meat has in general a higher fat content than beef (Belitz and others 1999). Chicken fat contains more unsaturated fatty acids, especially linoleic acid (19.5%) than both beef (3.1%) and pork fat (9.5%) (United States Department of Agriculture 2003). The fatty acids, which are present in chicken fat and in the phospholipids in chicken meat, undergo partial oxidation during cooking. Aldehydes and ketones belong to the major volatiles formed. Consequently, numerous review articles have assigned carbonyl compounds an important role in chicken aroma (Wilson and Katz 1972, Schliemann and others 1988, Shi and Ho 1994), together with sulfur-containing compounds, which are believed to be another important chemical class.

Sulfur-containing compounds are more important for the aroma of boiled chicken than for roasted chicken. The concentrations of 1-3 and 3-mercapto-2-pentanone (8) are overall higher in boiled chicken (1–5 μg/kg; 0.4–5 μg/kg; 1,456 μg/kg; 23–100 μg/kg) than in roasted chicken meat (0.4–4 μg/kg; 0.1–1.9 μg/kg; 202 μg/kg; 27–31 μg/kg) (Kerscher and Grosch 1998, Kerscher 2000). When compared to boiled beef, the concentrations of 1 and 2 are generally lower in boiled chicken (See Table 13.3).

Compounds 23 and 31 belong to the primary volatile products that are formed by autoxidation of linoleic acid at elevated temperatures (Grosch 1987). While 23 results from the breakdown of the corresponding 13-monohydroperoxide, 31 stems from the degradation of the 9-monohydroperoxide. Due to their low odor thresholds (23: 10 μg/L water; 31: 0.2 μg/L water) (Grosch and others 1993) both compounds contribute to the flavor of boiled and roasted chicken. Pippen and Nonaka (1958) recognized the contribution of decadienal to chicken flavor. Roasted chicken skin, which is much more greasy, contains even higher levels than the meat with a concentration of 893 versus 283 μg/kg for 23

Table 13.4. Recombination model mixture of boiled beef aroma (Kerscher 2000)[a].

No.[b]	Odorant	Odor	μg/kg	Odor activity value[c]
32	12-Methyltridecanal	tallowy, fatty	962	9,620
1	2-Methyl-3-furanthiol	meaty	24	3,429
2	2-Furfurylthiol	sulfury, roasted	29	2,900
3	Methanthiol	sulfury, rotten	311	1,555
26	Nonanal	fatty	1,262	1,262
16	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	caramel	9,075	908
25	Octanal	citrus	382	546
4	Dimethylsulfide	cooked asparagus	105	350
	2(Z)-Nonenal	fatty, green	6	310
33	1-Octen-3-one	mushroom	9	188
9	Acetaldehyde	fruity, pungent	1,817	182
6	Methional	boiled potato	36	180
11	Methylpropanal	malty	117	167
31	2,4(E,E)-Decadienal	fatty, fried	27	135
27	2(E)-Nonenal	fatty, cardboard	32	128
8	3-Mercapto-2-pentanone	sulfury, catty	69	99

[a]Values taken from Kerscher 2000.

[b]Numbering corresponds to Figure 13.1.

[c]Odor activity values were calculated by dividing the concentration of the odorants by their nasal odor threshold in water.

and a content of 711 versus 11 μg/kg for 31 (Kerscher 2000). Other volatiles with high impact on chicken aroma include the oleic acid degradation compounds heptanal, octanal (25), nonanal (26), the linoleic and arachidonic acid oxidation products 2(E)-nonenal (27), 2,4(E,E)-nonadienal (28), 1-octen-3-one (33), and 2,4(E,Z)-decadienal as well as 2,6(E,Z)-nonadienal (30) and 1,5(Z)-octadien-3-one (34), which originate from linolenic acid oxidation.

Besides carbonyls from lipid autoxidation and sulfur compounds from Maillard reaction, aldehydes from the Strecker degradation of valine, leucine, isoleucine, and methionine contribute with high odor activity values to the aroma of chicken: 6 and 11–13. Gasser and Grosch (Gasser and Grosch 1990) identified 14 primary odor compounds in chicken broth by aroma extract dilution analysis (AEDA): 1, 2, 6, 26-28, 31, 2,4,5-trimethylthiazole, 2-formyl-5-methylthiophene, p-cresol, 2-undecenal, β-ionone, γ-decalactone and γ-dodecalactone. Kerler (Kerler and Grosch 1997) who quantified chicken volatiles and determined their OAVs found additionally 23, 25, 33, 34, acetic acid, butyric acid, 2/3-methylbutyric acid, 2,4(E,Z)-decadienal, 2-acetyl-2-thiazoline (19), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (16), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (17), as well as the very volatile odorants hydrogen sulfide, methanthiol and acetaldehyde to be contributing to chicken flavor. The key odorants in roasted chicken skin were analyzed by Kerscher (2000), who prepared a model solution of 20 aroma compounds with high odor activity values in oil/water emulsion, which agreed well with the aroma of the original chicken skin (See Table 13.5). Volatile trithiolane derivatives, which have been identified in chicken flavor by different authors (Tang and others 1983, Hartman and others 1984), did not show up as aroma impact compounds in Kerscher's study. As with beef aroma, the very volatile compounds methanthiol and acetaldehyde had a high impact on the aroma of chicken.

Pork

Pork is the most consumed meat worldwide, particularly in China (Belitz and others 1999), with negligible consumption in countries with a large Muslim or Jewish population. Nonetheless, its aroma has been studied much less than beef. While fat degradation products form the majority of the volatile fraction of ground pork meat (Mottram 1982), on the other side pyrazines are dominating in grilled pork.

Gasser and Grosch (1991) found compounds 1 and 2 to be key aroma compounds of boiled pork, and the earthy, roasted smelling 2,4-dimethyl-5-ethylthiazole to be a pork-specific odorant. Later, Kerscher (2000) confirmed the importance of 1 and 2, and added 3, 9, 11, 16, 25, 26, 33, and 2,4(E,Z)-decadienal as key odorants. Pork aroma lacks the beef-specific aldehyde 32, but apart from that, differences between beef and pork aroma are more due to differences in the concentration of Maillard reaction products (Kerscher 2000). The differences in the aroma of braised pork and beef can be ascribed to the absence of 32 in pork and a considerably higher concentration of 16 in stewed beef (Guth and Grosch 1995).

In cured pork, the concentration of carbonyl compounds in the volatile fraction is drastically reduced versus uncured pork, for example hexanal occurring at 12.66 mg/kg versus 0.03 mg/kg (Ramarathnam and others 1991). The typical aroma note of cured pork is not the result of a single odorant, but the interaction of several aroma compounds. In Parma-type ham, 6, 15–18, 27, p-cresol, and phenylacetic acid belong to the relevant aroma compounds (Blank and others 2001). Interestingly, thiols like 1 and 2 as well as the fatty smelling lipid degradation product 31 did not seem to play a role for this flavor. Stahnke (2000) identified earlier the heterocyclic, popcorn-like smelling odorant 2-acetyl-1-pyrroline (18) in several sausages. She could show that the molded surface of sausages contained higher amounts than the center indicating that the compound was microbially formed.

Sheep

In many countries sheep meat has a low consumer acceptance due to an objectionable smell. Branched and unsaturated fatty acids with 8–10 carbon atoms have been identified in cooked mutton mince, and, because of their odor properties were thought to contribute to the undesirable flavor (Wong and others 1975). Metabolic processes in the rumen of the animals are probably involved in their formation. These acids include 4-methyloctanoic and 4-ethyloctanoic acids, both of which possess low odor thresholds and were therefore regarded as important contributors of sheep flavor (Brennand and Lindsay

Table 13.5. Recombination model mixture of chicken skin aroma[a].

No.[b]	Odorant	Odor	μg/kg	Odor activity value[c]
9	Acetaldehyde	fruity, pungent	3,287	14,941
3	Methanthiol	sulfury, rotten	164	2,733
6	Methional	boiled potato	97	485
13	2-Methylbutanal	malty	455	207
11	Methylpropanal	malty	538	158
8	3-Mercapto-2-pentanone	sulfury, catty	27	142
12	3-Methylbutanal	malty	668	124
18	2-Acetyl-1-pyrroline	roasted	2.9	29
16	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	caramel	395	16
25	Octanal	citrus	535	9.6
17	3-Hydroxy-4,5-dimethyl-3(2H)-furanone	seasoning-like	1.9	9.5
	2-Propionyl-1-pyrroline [d]	roasted	0.8	8
1	2-Methyl-3-furanthiol	meaty	4.1	7.3
21	2,3-Diethyl-5-methylpyrazine	roasted	2.5	5
2	2-Furfurylthiol	sulfury, roasted	1.9	4.8
28	2,4(E,E)-Decadienal	fatty, fried	711	4
19	2-Acetyl-2-thiazoline	roasted	5.8	3.2
20	2-Ethyl-3,5-dimethylpyrazine	roasted	4.3	2
26	Nonanal	fatty	832	<1
27	2(E)-Nonenal	fatty, cardboard	147	<1

[a]Values and odor descriptors taken from Kerscher 2000.
[b]Numbering corresponds to Figure 13.1.
[c]Odor activity values were calculated by dividing the concentration of the odorants by their nasal odor threshold in water.
[d]2-Propionyl-1-pyrroline was substituted by 2-acetyl-1-pyrroline on the basis of odor activity values.

1992, Brennand and others 1989). Sutherland and Ames (1996) analyzed the level of branched-chain fatty acids in the adipose tissue of lambs and found 3–5 mg/kg 4-methyloctanoic acid. Ha and Lindsay (1990) made the higher level of 4-ethyloctanoic acid in the meat of older animals responsible for the stronger characteristic mutton odor. Other authors suggest aldehydes and ketones, which were identified in the neutral volatile fraction of ovine adipose tissue, as important contributors to mutton flavor (Caporaso and others 1977). Buttery and others (1977) suspect alkyl pyridines, found in roasted lamb fat, to be the reason for its rejection by some consumers.

A more recent study (Schieberle and Rota 2003) analyzed raw and cooked sheep meat using GC-O and isotope dilution analysis to quantify the aroma-relevant compounds. According to their OAVs, compounds 3 and 34 contribute most to the aroma of the cooked meat. Compound 34 was already present before cooking and was, together with nonanal, the most potent odorant of the raw meat. Interestingly, the level of the lipid degradation-derived aroma compounds did not change significantly during cooking. The role of the intensely sheep-like smelling 4-methyloctanoic and 4-ethyloctanoic acids could be confirmed. Both acids, which are located in the membrane lipids, are already present in the raw meat.

FISH AND SEAFOOD FLAVOR

Fish

Fish has a high nutritional value because it is rich in polyunsaturated fatty acids (PUFA), especially ω-3-fatty acids, has high value protein and iodine (50–200 μg/100 g in seafish, also known as saltwater fish), and in general contains less fat than meat.

Raw fresh fish usually has a green, seaweed-like aroma (Josephson 1983). In the headspace of fresh whitefish 2(E)-nonenal (27), 2,6(E,Z)-nonadienal (30), 6-nonen-1-ol, 1-octen-3-ol, 1-octen-3-one (33), 1,5-octadien-3-ol, 1,5-octadien-3-one (34),

2,5-octadien-1-ol, and other compounds were identified by GC-O. The single compounds were described as having cucumber, melon-like, mushroom-like, and plant-like aromas (Josephson 1983). Hirano and others (1992) analyzed the aroma of fresh Ayu fish, a small Japanese river trout. GC-O analysis revealed 27, 30, and 3,6-nonadien-1-ol to be important for the cucumber and melon-like flavor of the fish. Another unsaturated aldehyde, 4(Z)-heptenal (24), was found to be important for the aroma of cold-stored cod (McGill 1974, 1977). The authors explained its formation by the oxidation of PUFA present in the fish phospholipid fraction. The 12-lipoxygenase, present in fish tissues, essentially in gills, is able to generate carbonyl compounds from arachidonic and eicosapentaenoic acids (Hsieh and Kinsella 1989) yielding 1-octen-3-ol, 2-octenal, 2-nonenal, 2,6-nonadienal, 1,5-octadien-3-ol, and 2,5-octadien-1-ol as the main volatiles. Distinct differences in flavor exist between different fish species and even between fish of the same species but different geographic origin (Prell and Sawyer 1988). Sérot and others (2001) could show that the diet also affected the aroma composition of carnivore fish. Turbot under a high PUFA diet contained more 30, 2(E)-pentenal, and 1,3,5(E,E,Z)-octatriene, whereas a vegetable oil diet resulted in a higher hexanal and decanal level in the fish. Trimethylamine has often been related to fish odor, especially to the amine-like note. However, it is less present in fresh fish, but formed by bacterial spoilage from its N-oxide at temperatures above 0°C. The precursor trimethylamine oxide level in the serum of marine fishes is between 0.2 and 47 millimols per liter (mmol/L), depending on the species (Shibamoto and Horiuchi 1997).

Cooking changes the flavor profile of fish. Milo and coworkers have carried out several studies to identify and quantify the volatile compounds that are relevant for the aroma of boiled salmon, cod, and trout (Milo and Grosch 1993, 1995, 1996, 1997; Milo 1994). Their data are summarized in Table 13.6. The most important aroma compounds in cooked salmon are 34 (OAV 750), 30 (OAV 485), 10 (OAV 240), 9 (OAV 230), 6 (OAV 200), and 31 (OAV 120) (Milo and Grosch 1996, Milo 1994).

The key odorants of cooked salmon, with the exception of 3,6(Z,Z)-nonadienal are already present in the raw fish. The concentration of 6 increases by a factor of 2–3, the concentration of compounds 23 and 24 doubles. Compound 10 is reduced to half its concentration, but the other odorants are less affected. Most of the important aroma compounds in trout are also found in salmon. Exceptions are 31, which adds less to the aroma of trout, but is important for the fatty note of salmon, and 12 (OAV 400), which plays a major role in trout flavor (Milo and Grosch 1993, 1995; Milo 1994). The sulfur compounds 3 (OAV 50) and 4 (OAV 39) are involved in the aroma of boiled cod but not of salmon or trout, in addition to 6 (OAV 275), 34 (OAV 250), 30 (OAV 175), 12 (OAV 204), and 9 (OAV 130). Recombination studies have shown that already an aqueous solution containing only two aroma compounds, namely 6 (10 μg/kg) and 34 (0.16 μg/kg), has the ability to evoke a fresh fish-like odor, along with potato and geranium-like notes (Belitz and others 1999). Thiols such as 1 and 2, which represent key odorants of boiled meat, seem to be unimportant for the aroma of fish. Only tuna, which contains 1, is an exception in this respect (Withycombe and Mussinan 1988).

The most potent aroma compounds in ripened anchovy are 6, 9, 11, 12, and 34 (Triqui and Guth 1997). In addition to these, Cha and coworkers (1997) found high odor values in salt-fermented anchovy for 6, 24, 30, and 34, as well as miscellaneous esters and 3-methyl-1-butanol and 2-phenylethanol, which obviously stem from fermentation.

Seafood

Crustaceans and mollusks are rich in protein. Crab, lobster, and crayfish, respectively, contain 15–19% protein; this is more than 80% of the dry matter content. Table 13.7 lists the relevant aroma compounds in cooked crab, crayfish, and lobster, which have been identified by the research group of Cadwallader (Chung and Cadwallader 1994, Cadwallader and others 1995, Chung and others 1995, Cadwallader and Baek 1998, Lee and others 2001) using GC-O. Similar to most fish, degradation products of PUFA, contribute to the aroma of seafood. On the other hand, 2-acetyl-1-pyrroline (18), a known Maillard degradation product of the amino acid proline with a cracker-like odor, is an important aroma impact compound and plays a major role for the aroma of seafood products, which is not the case with fish.

Together with 6, 14, and 24, 18 was found to be the most important odorant in cooked crabmeat

Table 13.6. Aroma-active compounds in cooked fish.

No.		Salmon (boiled)[a]	Cod (boiled)[b]	Trout (boiled)[c]
	Aldehydes			
9	Acetaldehyde	2,300	1,300	3,600
10	Propionanldehyde	1,700		2,300
11	Methylpropanal		27	
13	2-Methylbutanal		20	
12	3-Methylbutanal		51	100
6	Methional	1	11	6
23	Hexanal	58	115	37
25	Octanal	+[d]		+
	3(Z)-Hexenal	4	1	2
24	4(Z)-Heptenal	1	2	3
	2(E)-Octenal			+
27	2(E)-Nonenal	2.7		3
	2(Z)-Nonenal	+		+
	4,5-Epoxy-2(E)-decenal	+	+	+
	2,4(E,E)-Heptadienal			+
28	2,4(E,E)-Nonadienal	3	3	5
30	2,6(E,Z)-Nonadienal	10	4	8
29	3,6(Z,Z)-Nonadienal	6	1	+
31	2,4(E,E)-Decadienal	6	4	
	2,4(E,Z)-Decadienal			+
34	1,5(Z)-Octadien-3-one	0.3	0.1	0.4
	3-Methylnonane-2,4-dione			+
	Ketones			
14	2,3-Butanedione	52	200	268
	2,3-Pentanedione	234	86	140
33	1-Octen-3-one	0.4	0.7	0.6
	Acids			
	Butyric acid	1,137		
	2-/3-Methylbutyric acid	122		
	Sulfur compounds			
3	Methanthiol		100	
4	Dimethylsulfide		77	
5	Dimethyl trisulfide	+	0.2	
	Dimethyl tetrasulfide		+	
	Heterocycles			
19	2-Acetyl-2-thiazoline			+
18	2-Acetyl-1-pyrroline	+	+	+

[a]Literature data (Milo and Grosch 1996; Milo 1994).
[b]Literature data (Milo and Grosch 1995; Milo and Grosch 1996; Milo 1994).
[c]Literature data (Milo and Grosch 1993; Milo and Grosch 1995; Milo 1994).
+: detected by GC-olfactometry; concentration in (μg/kg), as determined by isotope dilution analysis.

(Cadwallader and others 1995, Chung and Cadwallader 1994). Likewise, in crayfish 18 and 6 dominate the aroma, together with 19, 28, 30, and 31 (Cadwallader and Baek 1998). In the headspace above the cooked crayfish tail meat, 3, 33, 34, hydrogen sulfide, trimethylamine, and dimethyltrisulfide were detected as aroma-active compounds.

In cooked lobster meat, again 18 was the most intense odorant (Cadwallader and others 1995). Other dominant aroma compounds are 6, 14, 24, 33, 2-acetyl-3-methylpyrazine, and trimethylamine. The authors held the latter three volatiles responsible for a negative impact on lobster aroma. Lee and coworkers (2001) confirmed the role of 6, 14, 18, and 33 for lobster aroma. In addition 12, 33, 34, and the very volatile sulfur compounds 3 and 4 belonged to the predominant odorants of lobster.

TASTE COMPOUNDS

Taste compounds, molecules that cause a taste sensation when interacting with receptors on the tongue and in the mouth cavity, are in general nonvolatile, polar, and water-soluble. The existence of five basic tastes is broadly agreed: sweet, sour, salty, bitter, and umami. The word "umami" originates from the Japanese language and means "delicious." Umami taste can be described as glutamate-like or bouillon-like. A short time ago, a corresponding receptor was identified in tongue tissue thereby confirming that the umami is actually a basic taste (Chaudhari and others 2000). As with aroma compounds, the analysis of taste compounds requires a combination of instrumental and sensory methods. A systematic approach uses the quantification of potentially taste-active water-soluble compounds in the respective food, recombination trials, and sensorial comparison with the original food to verify if all major taste compounds are included, followed by omission tests to narrow down the number of relevant tastants (Hayashi and others 1981, Warendorf and others 1992, Warmke and others 2004, Schlichtherle-Cerny and Grosch 1998).

Amino acids and peptides are regarded as important taste compounds in meat (van den Ouweland and others 1978, Mabrouk 1976). They show a ternary synergism—that is, they have no characteristic taste on their own, but enhance the basic taste in combination with other compounds like glutamic acid and 5′-nucleotides. Alongside amino acids, other organic acids play an essential role (van den Ouweland and others 1978). Warendorf and coworkers (1992) analyzed taste-active compounds in a beef bouillon and found that only low-molecular compounds (<500 dalton [Da]) were taste active, the most important ones being glutamic acid (39), 5′-adenosine monophosphate (45, AMP), 5′-inosine monophosphate (48, IMP), aspartic acid (40), the dipeptides carnosine (42) and anserine, lactic acid (43), carnitine and sodium, potassium, magnesium, calcium, chloride, and phosphate ions. In beef juice only 47 low-molecular weight compounds were taste-active (Schlichtherle-Cerny and Grosch 1998). Their number could be narrowed down by screening trials to 17 compounds, which are listed in Table 13.8. Figure 13.2 shows the corresponding chemical structures.

Table 13.7. Aroma-active compounds of cooked seafood.

No.		Crab (cooked)[a]	Crayfish (cooked)[b]	Lobster (cooked)[c]
	Aldehydes			
9	Acetaldehyde		+[d]	+
11	Methylpropanal		+	+
13	2-Methylbutanal			+
12	3-Methylbutanal		+	+
6	Methional	+	+	+
23	Hexanal			+
	Heptanal			+
24	4(Z)-Heptenal	+	+	+
25	Octanal			+
	2(E)-Octenal		+	+
27	2(E)-Nonenal		+	+
	4(E)-Decenal	+		

Table 13.7. Continued

No.		Crab (cooked)[a]	Crayfish (cooked)[b]	Lobster (cooked)[c]
28	2,4(E,E)-Nonadienal		+	
30	2,6(E,Z)-Nonadienal		+	+
29	3,6(Z,Z)-Nonadienal			+
31	2,4(E,E)-Decadienal		+	+
	Ketones			
14	2,3-Butanedione	+	+	+
	2-Heptanone			+
	1-Hexen-3-one			+
33	1-Octen-3-one		+	+
34	1,5(Z)-Octadien-3-one		+	+
	β-Damascenone			+
	o-Aminoacetophenone			+
	3-Methyl-1-butanol			+
	2-Methylisoborneol		+	
	Phenols, amines			
	Guaiacol			+
	p-Cresol		+	
	Trimethylamine	+	+	+
	Sulfur compounds			
	Hydrogen sulfide		+	+
3	Methanthiol		+	+
4	Dimethylsulfide			+
5	Dimethyl trisulfide		+	+
	Dimethyl tetrasulfide		+	
1	2-Methyl-3-furanthiol		+	+
	Heterocycles			
	Trimethylpyrazine			+
20	2-Ethyl-3,5-dimethylpyrazine			+
	2-Acetylpyrazine			+
22	2-Acetyl-3-methylpyrazine			+
	2-Acetylpyridine			+
	2,4,6-Trimethylpyridine			+
	2-Acetylthiazole			+
19	2-Acetyl-2-thiazoline		+	
	2,4,5-Trimethylthiazole			+
	Benzothiazole			+
18	2-Acetyl-1-pyrroline	+	+	+
	Pyrrolidine	+		
	3-Methylindol		+	+
	Benzothiophene			+
16	2,5-Dimethyl-4-hydroxy-3-(2H)-furanone			+

[a]Literature data (Chung and Cadwallader 1994; Chung and others 1995).
[b]Literature data (Cadwallader and Baek 1998).
[c]Literature data (Cadwallader and others 1995; Lee and others 2001).
[d]"+": detected by GC-olfactometry.

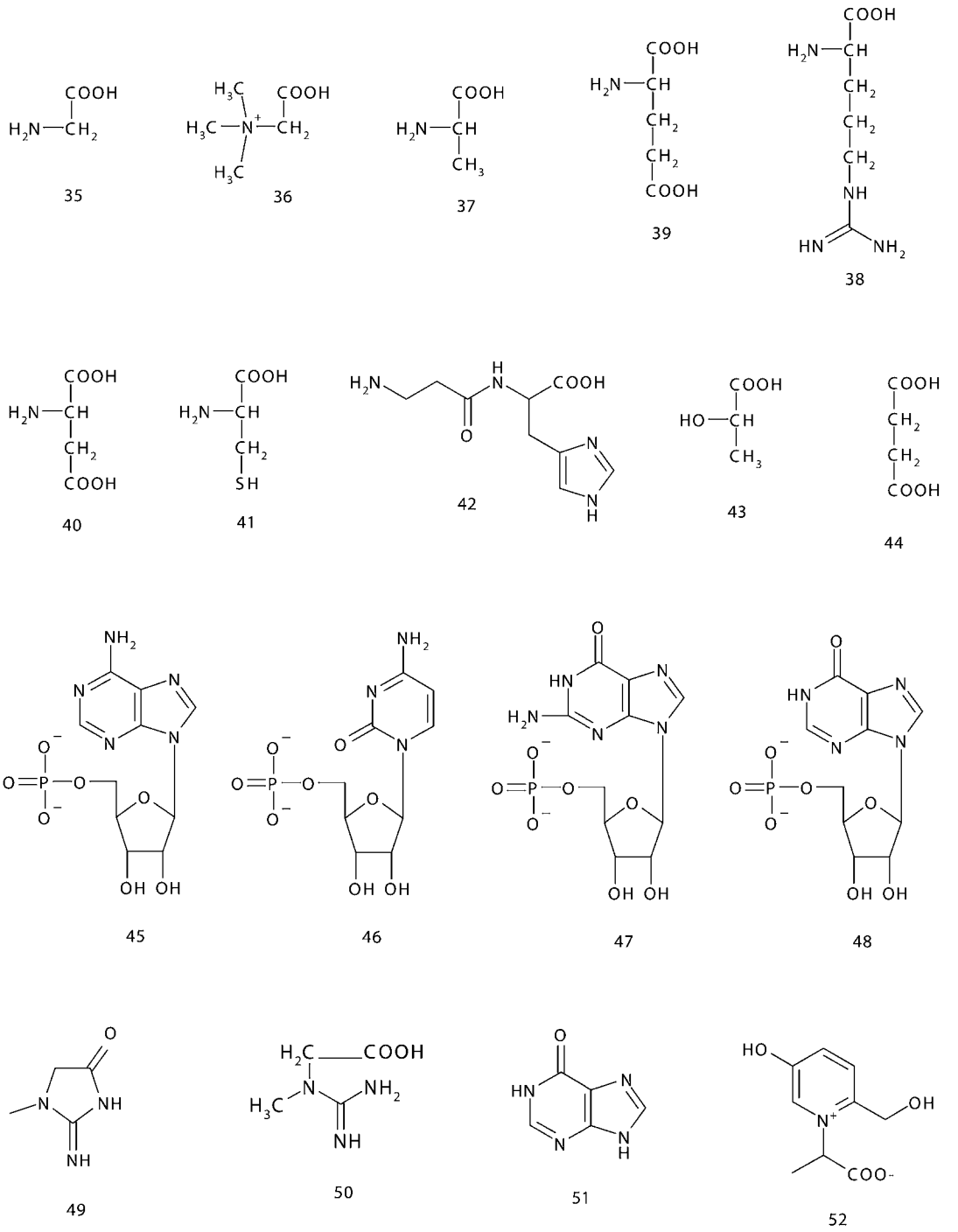

Figure 13.2. Chemical structure of taste compounds in meat and seafood.

Table 13.8. Taste-active compounds in beef juice and boiled crab extract.

No.	Concentration in mg/kg	Stewed beef juice[a]	Boiled crab extract[b]
35	Glycine		6,230
36	Glycine betaine		3,570
37	Alanine	840	1,870
38	Arginine		5,790
39	Glutamic acid	250	190
40	Aspartic acid	20	
41	Cysteine	60	
42	Carnosine	5,280	
43	L-Lactic acid	14,000	
44	Succinic acid	260	
45	5′-AMP	240	320
46	5′-CMP		60
47	5′-GMP		40
48	5′-IMP	2,800	
49	Creatinine	4,900	
50	Creatine	3,030	
51	Hypoxanthine	500	
	K^+	6,020	1,971
	Na^+	940	993
	Mg^{2+}	410	
	$PO4^{3-}$	5,670	2,169
	Cl^-	670	1,946

[a]Literature data (Schlichtherle-Cerny and Grosch 1998).
[b]Literature data (Hayashi and others 1981; Konosu and Yamaguchi 1987).

The two 5′-nucleotides 45 (240 mg/kg) and 48 (2,800 mg/kg), 39 (250 mg/kg), and 40 (20 mg/kg) were responsible for the bigger part of the brothy, umami taste of beef juice. Compound 39 had been already identified in 1908 by Ikeda (1909) as the taste-active principle in Konbu seaweed (*Laminaria japonica*). It is present in relatively high concentrations in Parmesan cheese (12,000 mg/kg), peas (2,000 mg/kg), tomatoes (1,400 mg/kg), and cured ham (3,370 mg/kg) ((Loeliger 2000, Ninomiya 2002). Compound 48 was for the first time isolated from dried bonito (Katsuobushi) by Kodama (1913). It shows synergism with 39 (Kuninaka 1960), small amounts of 48 (1–10%) relative to 39 already provoking strong taste enhancement (Yamaguchi 1967). The 5′-guanosin monophosphate (47, GMP), which has a similar effect as 48, is even 2.3 times more potent (Yamaguchi and others 1971). Examples of foods rich in 5′-nucleotides are tuna (2,860 mg/kg IMP), pork (2,000 mg/kg IMP), chicken (2,010 mg/kg IMP), and dried shiitake mushrooms (1,500 mg/kg GMP) (Ninomiya 1998).

The group of 43 and 44, potassium, sodium, magnesium, chloride, and phosphate salts contributed to the umami, salty, and sour taste of beef juice (Schlichtherle-Cerny and Grosch 1998). Sodium succinate on its own tastes glutamic acid-like, but also bitter and salty at the same time (Velisek and others 1978). The dipeptide 42 adds sourness and mouthfeel to beef juice. At a pH value of 5.7, which corresponds to the pH in meat, carnosine has a distinct sour note, whereas at higher values of 6.8 to 7.6, its taste becomes sweet and shows stronger mouthfeel (Suyama and Shimizu 1982). Creatine (50), creatinine (49), hypoxanthine (51), alanine (37), and cysteine (41) have less impact on the taste of beef juice than the other tastants mentioned above. Compounds 49–51 have a bitter taste, 37 a sweet savor, and 41 a meaty note (Schlichtherle-Cerny and Grosch 1998). Recently the new compound alapyridaine (52) was identified in beef broth (Ottinger and Hofmann 2003). It is claimed to enhance both sweet and umami character in beef broth.

The taste-relevant compounds in snow crab extract have been investigated by Hayashi, Konosu, and Yamaguchi (Hayashi and others 1981, Konosu and Yamaguchi 1987). They quantified 44 nonvolatiles and, using sensory studies including omission tests, found only 12 of these compounds to contribute to the characteristic taste (See Table 13.8): 35–39, 45–47, potassium, sodium, chloride, and phosphate ions. A synthetic extract of the 12 compounds simulated fairly well the taste profile of the original extract. Omission of 35 led to reduced sweet and umami taste. Umami taste was largely lost without 39, as well as sweetness. When the nucleotides were left out, the umami taste also decreased distinctively, the taste became flat, and the boiled crab taste disappeared completely. Likewise, without 38, the recombination solution tasted flatter and less crab-like. Omission of the minerals caused a decrease in the overall taste, sweetness, and umami and resulted in an inharmonic taste. Interestingly, already with a recombination of the taste compounds alone, the typical crabmeat character could be perceived, which underlines the importance of taste compounds for the flavor of a food. Most probably, due to better and more readily available analytical methods, such as high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), more taste-relevant compounds will be identified in our food.

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14
Natural Flavors

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INTRODUCTION

Acceptability of food is determined by its appearance, smell, taste, texture, and chewing sound. We use all the senses of sight, smell, taste, touch, and hearing for organoleptic evaluation of food quality. Flavor is defined as the complex sensation derived from the characteristics of food taken in the mouth and is principally perceived by the senses of both smell and taste. It may be influenced by sensations of pain, heat, and cold, and by tactile sensations (Amerine et al. 1965, Hall 1968, Thomson 1986). Flavor substances perceived by the senses of smell and taste are limited because they need to be accepted by the sensory receptors. Despite this limitation, a myriad of flavors occur in foods. They are generally ascribed to either natural or synthetic flavors. Natural flavors originate in the natural materials produced by enzymatic or voluntary reactions during processing and storage. Synthetic flavors are produced intentionally by chemical reactions from simpler starting substances. Although the defined "flavor" is perceived by mouth feeling and/or by the sense of taste, this chapter focuses on natural volatile flavors that mainly stimulate the sense of smell. There are generally hundreds or even more volatile compounds in each food, and a mixture of them composes its characteristic flavor note. It is difficult to express a flavor note of any particular food by a few substances. However, there sometimes exists a key compound in terms of a character-impact substance that almost precisely represents the flavor note of a food. This chapter pinpoints each key compound as well as overviews major compounds occurring in some characteristic food materials.

THE *ALLIUM* PLANTS

The *Allium* plants belong to the *Liliaceae* family (Lily family) and are characterized by their strong sulfuric odor. Responsible odorants are enzymatically produced by damaging the plant tissues. The

precursor, *S*-(+)-alk(en)yl-L-cysteine sulfoxide, exists in the cytosol of mainly mesophyll cells, while the enzyme, C-S lyase (alliinase), exists in the vacuoles of the vascular bundle sheath cells around the veins or phloem (Lawson 1996). *S*-(+)-Alk(en)yl-L-cysteine sulfoxide itself does not have any specific odor, but it can be converted into volatile flavors by C-S lyase when the plant is cut or crushed (Figure 14.1). Cultivated species of *Allium* plants include garlic (*Allium sativum* L.), onion (*Allium cepa* L. var. *cepa*), shallot (*Allium cepa* L. var. *ascalonicum*), great-headed garlic (*Allium ampeloprasum*

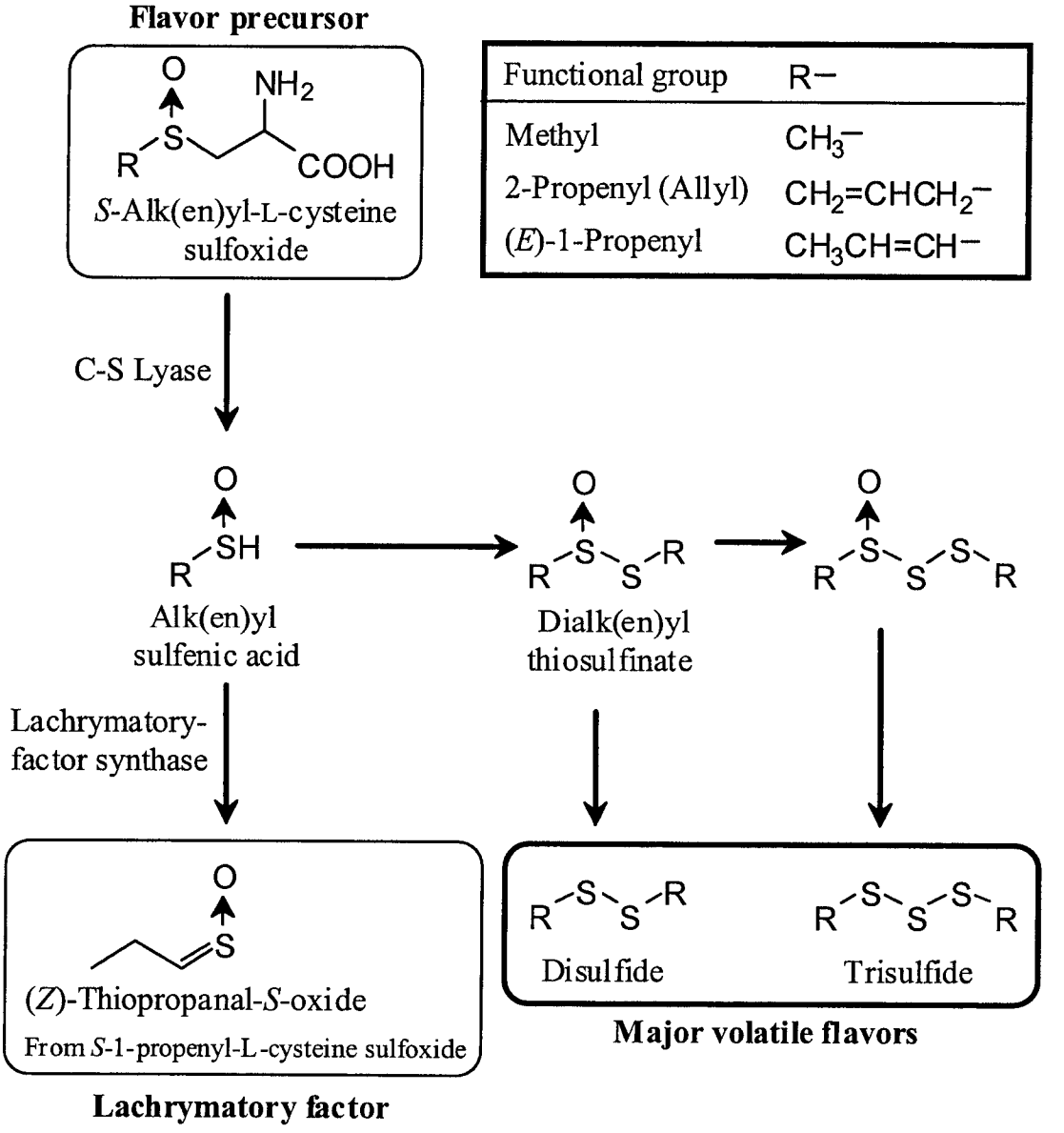


Figure 14.1 Formation of sulfuric flavors from *S*-(+)-alk(en)yl-L-cysteine sulfoxide in *Allium* plants.

L. var. *holmense*), leek (*Allium ampeloprasum* L. var. *porrum*), Japanese bunching (Welsh) onion (*Allium fistulosum* L.), Rakkyo (*Allium chinese* G. Don), chives (*Allium schoenoprasum* L.), and Chinese chives (Nira) (*Allium tuberosum* Rottl. ex Sprengel) (Fenwick and Hanley 1985). Different flavors are produced depending on the composition of the alk(en)yl group of the flavor precursors.

Garlic (*Allium sativum* L.)

S-Allyl-L-cysteine sulfoxide (alliin) accounts for about 85% of the total sulfoxides in garlic (Lawson 1996). The others are *S*-methyl-L-cysteine sulfoxide (methiin) and *S*-1-propenyl-L-cysteine sulfoxide (isoalliin) amounting to 10% and 5%, respectively. When the plant cell is damaged by cutting or crushing, C-S lyase immediately converts these sulfoxides into alk(en)yl sulfenic acid such as allyl sulfenic acid and methyl sulfenic acid (Figure 14.1). Two molecules of alk(en)yl sulfenic acid produce dialk(en)yl thiosulfinate. Diallyl thiosulfinate is produced from two molecules of allyl sulfenic acid, and allyl methyl thiosulfinate is produced from allyl sulfenic acid and methyl sulfenic acid. Diallyl thiosulfinate is also known as allicin, which has a potent antibiotic activity (Reuter et al. 1996). Dialk(en)yl thiosulfinate is reactive and rapidly converted into volatile compounds. Since *S*-allyl-L-cysteine sulfoxide is the most abundant among the sulfoxides, most of the volatile compounds possess an allyl group in their structure and give off a characteristic sulfury odor of garlic. Diallyl disulfide, diallyl trisulfide, allyl methyl disulfide, and allyl methyl trisulfide are the principal flavors produced from garlic (Whitaker 1976). These sulfides have been reported to have an anticancer effect (Reddy et al. 1993, Ariga and Seki 2005) and an antiplatelet effect (Ariga et al. 1994).

Onion (*Allium cepa* L.)

(*E*)- *S*-1-Propenyl-L-cysteine sulfoxide (isoalliin) is the most abundant sulfoxide in onion, making up about 70% of the total sulfides (Ueda et al. 1994). The rest is mostly *S*-methyl-L-cysteine sulfoxide (methiin). Isoalliin is converted into 1-propenyl sulfenic acid by C-S lyase in the same way as the production of allyl sulfenic acid (Figure 14.1). However, most of the 1-propenyl sulfenic acid produced is further converted into (*Z*)-thiopropanal-*S*-oxide as a lachrymatory factor of onion by the lachrymatory-factor synthase (Imai et al. 2002). Therefore, the production of di(1-propenyl) thiosulfinate would not be large, and this may be the reason why di(1-propenyl) disulfide is scarcely found in onion. Other alk(en)yl sulfenic acids are converted into dialk(en)yl thiosulfinate and then into various sulfides. Dipropyl disulfide, 1-propenyl propyl disulfide, methyl propyl disulfide, methyl propyl trisulfide, and dipropyl trisulfide are the principal flavor compounds in onion (Whitaker 1976). The reason why the sulfides with a propyl group are produced more in spite of the low existence of *S*-propyl-L-cysteine sulfoxide in onion remains to be clarified.

MUSHROOMS

Mushrooms are edible fungi, and most of them have been famous for their specific flavor. The mushrooms that are well-known as having a strong flavor note are Matsutake (*Tricholoma matsutake* Sing.) and Shiitake (*Lentinus edodes* Sing.). Other cultivars include the common mushroom (*Agaricus bisporus* Lange Sing.), hen of wood (*Grifola frondosa* S. F. Gray), oyster mushroom (*Pleurotus osteatus* Kummer), and Shimeji (*Lyophyllum Shimeji* Hongo). The flavor components are enzymatically produced from the tissues of the fruiting bodies when damaged.

Matsutake Mushroom (*Tricholoma matsutake* Sing.)

Matsutake mushroom is called the king of mushrooms, and its flavor is highly evaluated organoleptically. The key compound in the Matsutake mushroom is 1-octen-3-ol and is commonly called "matsutake-ol." Most of the other 60 compounds are alcohols such as 3-octanol and *cis*-2-octenol, and carbonyls such as 3-octanone and 1-octen-3-one (Yajima et al. 1981). 1-Octen-3-ol is also the principal flavor compound in other mushrooms (Rapior et al. 1996, Venkateswarlu et al. 1999), and is produced from linoleic acid by lipoxygenase and hydroperoxide lyase (Figure 14.2).

Shiitake Mushroom (*Lentinus edodes* Sing.)

There are two major pathways for the production of flavors from the Shiitake mushroom. One is the

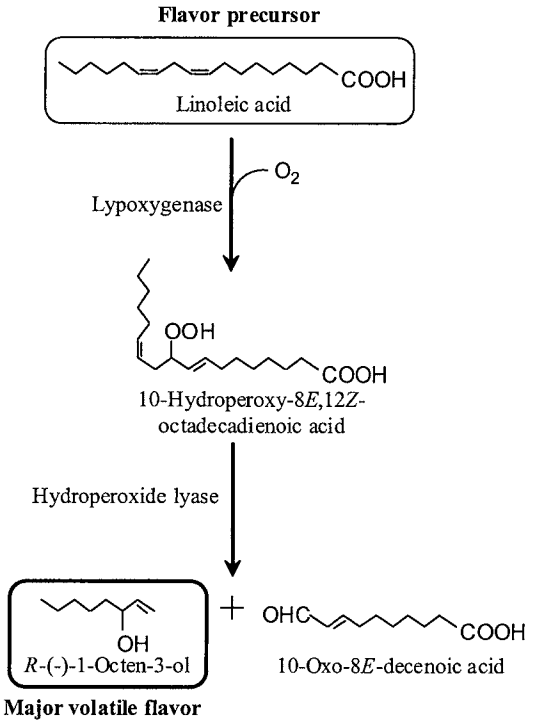


Figure 14.2 Formation of 1-octen-3-ol from linoleic acid in mushrooms.

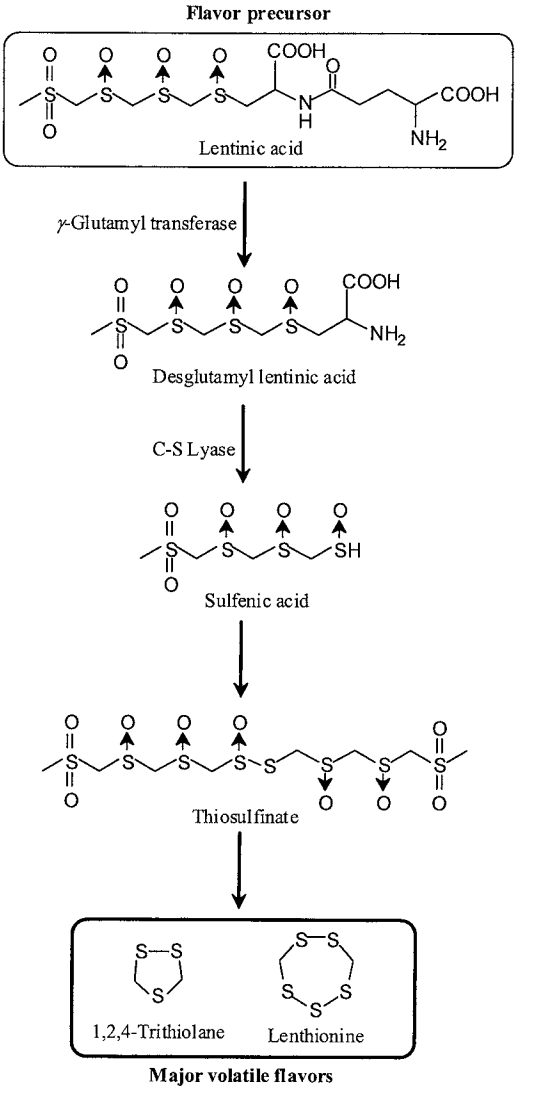


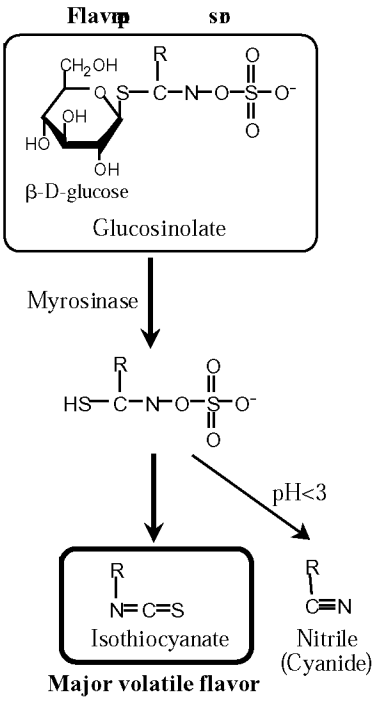
Figure 14.3 Formation of cyclic sulfur compounds from lentinic acid in Shiitake mushroom.

pathway to produce C8 compounds such as 1-octene-3-ol from linoleic acid by lipoxygenase and hydroperoxide lyase, and the other is to produce cyclic sulfuric compounds such as 1,2,3,5,6-pentathiepane (lenthionine) and 1,2,4-trithiolane from lentinic acid by γ-glutamyl transferase and C-S lyase (Figure 14.3). The optimum pH of lipoxygenase and hydroperoxide lyase is reported to be around pH 5–7 (Chen et al. 1984, Mau et al. 1992) and that of C-S lyase is around 9 (Iwami et al. 1975). Then, 1-octen-3-ol is effectively produced under acidic and neutral pH conditions, while the effective production of lenthionine is attained under an alkaline pH condition. The yield of 1-octen-3-ol produced around neutral pH is about 42–63% (Cho et al. 2003). However, the flavor component that characterizes the Shiitake mushroom is lenthionine. The production of lenthionine from the Shiitake mushroom is similar to that of sulfides from the *Allium* species, because C-S lyase is involved in both cases (Kumagai et al. 2002). Lenthionine is reported to have an antiplatelet effect (Shimada et al. 2004) as well as a characteristic sulfury flavor note.

THE *CRUCIFERAE* PLANTS

The characteristic flavor components in the *Cruciferae* (Mustard family) plants are isothiocyanates, most of them having a pungent flavor.

Isothiocyanates are enzymatically produced by damaging the plant tissues. The flavor precursor, glucosinolate, exists in the vacuoles of various cells but not in the myrosin cells (Andréasson and Jørgensen 2003). The precursor contents of the root cap cells and cortex cells near the root tip are high (Wei et al. 1981), while myrosinase (thioglucosidase) is localized exclusively in the myrosin cells that are special idioblasts (Andréasson et al. 2001). Glucosinolate can be converted into volatile flavors by myrosinase released when the plant is cut or crushed (Figure 14.4). Isothiocyanates characterized by having the common structure -N=C=S are the most abundant volatile flavors produced from the glucosinolates. Nitriles (-CN) are formed at low pH, while thiocyanates (-SCN) are rarely formed (Takeoka 1999, Wittstock and Halkier 2002). There are about 100 glucosinolates with different functional groups (Fenwick et al. 1983, Harborne et al. 1998), and the produced isothiocyanates vary depending on the difference in their functional groups. The cultivated species of the *Cruciferae* plants include cabbage (*Brassica oleracea* L. var. *capitata* L.), brussel sprouts (*Brassica oleracea* L. var. *gemmifera* DC. Zenk.), broccoli (*Brassica oleracea* L. var. *italica* Plenck), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), kale (*Brassica oleracea* L. var. *acephala* DC.), turnip (*Brassica rapa* L.), rape



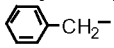
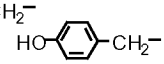
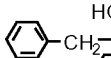
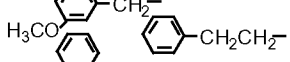
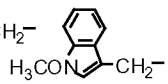
Functional group	R–	Glucosinolate
Methyl	CH_3–	Glucocapparin
Ethyl	CH_3CH_2–	Glucolepidiin
Isopropyl	$(CH_3)_2CH$–	Glucoputranjivin
2-Butyl (*sec*-Butyl)	$CH_3CH_2CH(CH_3)$–	Glucocochlearin
2-Hydroxy-2-methylpropyl	$CH_3C(OH)(CH_3)CH_2$–	Glucoleomin
2-Hydroxy-2-methylbutyl	$CH_3CH_2C(OH)(CH_3)CH_2$–	Glucoconringiin
2-Propenyl (Allyl)	$CH_2=CHCH_2$–	Sinigrin
3-Butenyl	$CH_2=CH(CH_2)_2$–	Gluconapin
2-Hydroxy-3-butenyl	$CH_2=CHCH(OH)CH_2$–	Progoitrin
4-Pentenyl	$CH_2=CH(CH_2)_3$–	Glucobrassicanapin
2-Hydroxy-4-pentenyl	$CH_2=CHCH_2CH(OH)CH_2$–	Gluconapoleiferin
3-Methylthiopropyl	$CH_3S(CH_2)_3$–	Glucoiberverin
3-Methylsulfinylpropyl	$CH_3SO(CH_2)_3$–	Glucoiberin
3-Methylsulfonylpropyl	$CH_3SO_2(CH_2)_3$–	Glucocheirolin
4-Methylthiobutyl	$CH_3S(CH_2)_4$–	Glucoerucin
4-Methylsulfinylbutyl	$CH_3SO(CH_2)_4$–	Glucoraphanin
4-Methylsulfinyl-3-butenyl	$CH_3SOCH=CH(CH_2)_2$–	Glucoraphenin
4-Methylsulfonylbutyl	$CH_3SO_2(CH_2)_4$–	Glucoerysolin
5-Methylthiopentyl	$CH_3S(CH_2)_5$–	Glucoberteroin
5-Methylsulfinylpentyl	$CH_3SO(CH_2)_5$–	Glucoalyssin
Benzyl		Glucotropaeolin
p-Hydroxybenzyl		Glucosinalbin
m-Metoxybenzyl		Glucolimnanthin
Phenylethyl		Gluconasturtiin
3-Indolylmethyl		Glucobrassicin
N-Metoxy-3-indolylmethyl		Neoglucobrassicin

Figure 14.4 Formation of isothiocyanates and nitriles from glucosinolate in *Cruciferae* plants.

(*Brassica napus* L.), Chinese cabbage (*Brassica campestris* var. *amplexicaulis*), chingensai (*Brassica campestris* var. *chinesis* L. Schulz), komatsuna (*Brassica campestris* var. *perviridis*), nozawana (*Brassica campestris* var. *rapifera*), leaf mustard (*Brassica juncea* L. Czern.et Coss.), white or yellow mustard (*Brassica hirta* Moench), brown or oriental mustard (*Brassica juncea* Cosson), black mustard (*Brassica nigra* Koch), wasabi (*Wasabia japonica* Matsum.), horseradish (*Armoracia rusticana* Lam. Gaertn., Mey. et Scherb.), radish (*Raphanus sativus* L.), and watercress (*Nasturtium officinale* R. Br.).

Cabbage (*Brassica oleracea* L. var. *capitata* L.)

The flavor components in raw cabbage mainly consist of C6 compounds having a "green" note such as (*Z*)-3-hexenol and hexanol, and also of compounds derived from glucosinolates such as allyl isothiocyanate and methylthiocyanate (Kurobayashi 1997). During cooking, the amount of the C6 compounds decreases and that of sulfides increases, while isothiocyanates show flexible changes in quantity (MacLeod and MacLeod 1970). Glucoiberin with a 3-methylsulfinylpropyl group and sinigrin with an allyl group are principal glucosinolates in cabbage (Fenwick et al. 1983). Allyl isothiocyanate derived from sinigrin notably contributes to fresh cabbage flavors (Chin et al. 1996). Dimethyl sulfides such as dimethyl monosulfide, dimethyl disulfide, and dimethyl trisulfide are produced from *S*-methyl-L-cysteine sulfoxide in the same way as in the case of the *Allium* plants (Chin and Lindsay 1994) and they also contribute to the flavor of cooked cabbage (Buttery et al. 1976, Takeoka 1999).

Broccoli (*Brassica oleracea* L. var. *italica* Plenck)

4-Methylthiobutyl isothiocyanate and 4-methylthiobutyl cyanide are the principal flavor components in broccoli (Buttery et al. 1976). 4-Methylsulfinylbutyl isothiocyanate (sulforaphane) and 4-methylsulfinylbutyl cyanide (sulforaphane nitrile), both produced from glucoraphanin, are also ubiquitous (Chiang et al. 1998). Sulforaphane is currently attracting attention because it has an anticarcinogenic activity by inducing phase 2 enzymes such as glutathione-*S*-transferase (Zhang et al. 1994, Fahey et al. 1997). Glucoraphanin exists in larger quantities in broccoli sprouts than in mature broccoli, comprising about 80–90% of the total glucosinolates (Fahey et al. 1997, Baik et al. 2003).

Mustard (*Brassica hirta* Moench, *Brassica juncea* Cosson, *Brassica nigra* Koch)

There are three main types of mustard: white or yellow mustard (*Brassica hirta* Moench), brown or oriental mustard (*Brassica juncea* Cosson), and black mustard (*Brassica nigra* Koch). Their seeds are used as spices. Leaf mustard (*Brassica juncea* L. Czern.et Coss.) is also eaten in some countries. The pungent flavor of mustard is due to allyl isothiocyanate derived from sinigrin. The sinigrin content of brown mustard is about 100 times higher than that of white mustard (Tsao et al. 2002), and the former has a much hotter flavor note. 3-Butenyl isothiocyanate, phenylacetonitrile, and 3-phenylpropiononitrile also exist in large quantities (Kameoka and Hashimoto 1980).

Wasabi (*Wasabia japonica* Matsum.)

Wasabi, Japanese horseradish, has a strong pungent flavor to which allyl isothiocyanate is mainly attributed. Wasabi is commonly cultivated in water and its rhizome is used as a spice. The leaves of upland wasabi cultivated in soil are also used as pickled foods. The amount of isothiocyanates in the rhizome is greater for wasabi cultivated in water than for that cultivated in soil (Sultana et al. 2003). In addition to the allyl isothiocyanate that comprises about 80% of the total flavor components, ω-alkenyl isothiocyanates such as 3-butenyl isothiocyanate and 4-pentenyl isothiocyanate, ω-methylthioalkyl isothiocyanate such as 6-methylthiohexyl isothiocyanate and 7-methylthioheptyl isothiocyanate, and ω-methylsulfinylalkyl isothiocyanates such as 6-methylsulfinylpentyl isothiocyanate and 7-methylsulfinylheptyl isothiocyanate also contribute to the wasabi flavor (Ina et al. 1989, Etoh et al. 1990). ω-Methylsulfinylhexyl isothiocyanate has an anticancer effect (Fuke et al. 2003) and ω-methylthioalkyl isothiocyanate has an antiplatelet effect (Kumagai et al. 1994).

THE *SOLANACEAE* PLANTS

The abundant volatile compounds in the *Solanaceae* (Nightshade family) plants are C6 compounds such as (*Z*)-3-hexenal and hexanal derived from fatty acids

probably by lipoxygenase (Luning et al. 1995) and some other enzymes (Hatanaka et al. 1977, Gatfield 1999, Yilmaz et al. 2002) (Figure 14.5) although the key flavor component is different in some plants (Table 14.1). The common species of the *Solanaceae* plants include tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.), bell pepper (*Capsicum annuum* L. var. *grossum* Bailey), and red pepper (*Capsicum annuum* var. *annuum*).

Tomato (*Lycopersicon esculentum* Mill.)

More than 400 compounds have been identified as tomato flavors. The principal flavor compounds are (*Z*)-3-hexenal and hexanal that account for about 50% and 30% of total flavor compounds, respectively (Buttery et al. 1988) (Table 14.1). Both of them have a green note and significantly contribute to the tomato flavor. Their flavor dilution (FD) factors can reach up to about 4,000 and 2,000, respectively (Krumbein and Auerswald 1998).

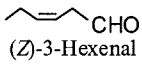
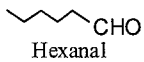
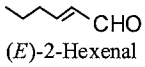
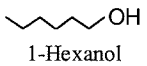
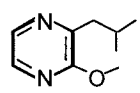
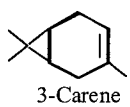
Bell pepper (*Capsicum annuum* L. var. *grossum* Bailey)

About 100 compounds have been identified as bell pepper flavor. C6 compounds, such as 1-hexanol and (*E*)-2-hexenal, are abundant (Wu and Liou 1986) (Table 14.1). However, the compounds that characterize the bell pepper are 2-methoxy-3-isobutylpyrazine and 3,7,7-trimethyl-bicyclo[4.1.0]hept-3-ene (3-carene) but are less than 1% of the total flavor compounds (van Ruth et al. 2003, Luning et al. 1994). The threshold of the 2-methoxy-3-isobutylpyrazine is 0.002 parts per billion (ppb) (Buttery et al. 1969) and its FD factor is about 250 (Zimmermann and Schieberle 2000).

THE *UMBELLIFERAE* PLANTS

The major volatile compounds in the *Umbelliferae* (parsley family) plants are terpenes, which have the isoprene unit (CH_2=CHC[CH_3]=CH_2) in their structure. However, the flavor that characterizes the plant is sometimes different from the terpenes (Table 14.2). The common species of the *Umbelliferae* plants include celery (*Apium graveolens* L. var. dulce), parsley (*Petroselinum crispum* [Mill.] Nyman), coriander (*Coriandrum sativum* L.), carrot (*Daucus carota* L. var. *sativa* DC.), lovage (*Ligusticum levisticum* L.), water dropwort (*Oenanthe javanica* DC.), and Japanese hornwort (*Cryptotaenia japonica* Hassk.).

Table 14.1 Major and Key flavor compounds in the *Solanaceae* plants.

	Major compound	Key compound
Tomato	 (*Z*)-3-Hexenal  Hexanal	
Bell pepper	 (*E*)-2-Hexenal  1-Hexanol	 2-Methoxy-3-isobutylpyrazine  3-Carene

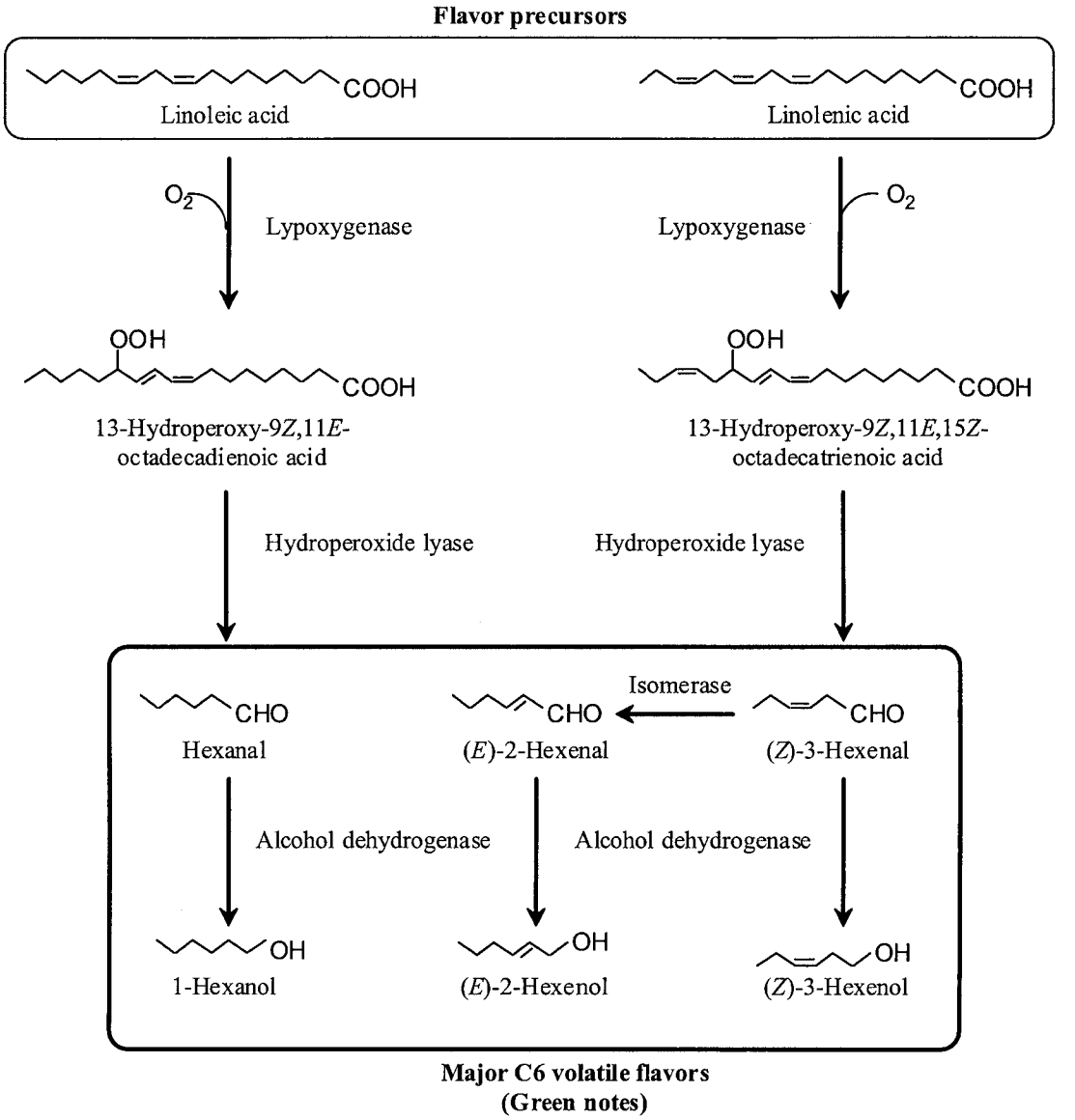
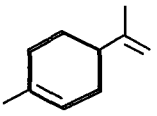
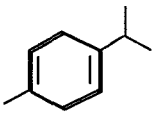
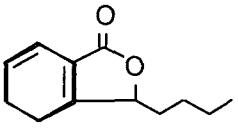
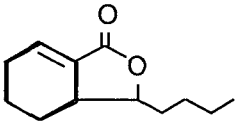
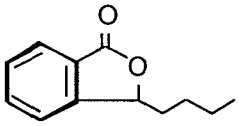
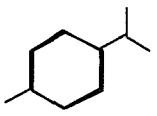
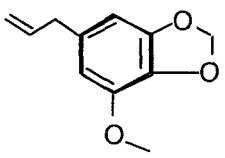
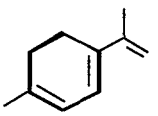
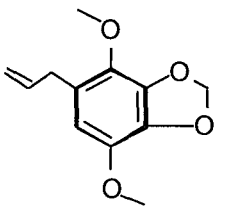


Figure 14.5 Formation of C6 volatile flavor compounds from fatty acids.

CELERY (*APIUM GRAVEOLENS* L. VAR. DULCE)

Volatile flavor compounds of celery mainly consist of terpenes such as *p*-mentha-1,8-diene (limonene) and *p*-mentha-1,4-diene (γ-terpinene), and phthalides such as 3-*n*-butylphthalide, 3-*n*-butyl-4,5-dihydrophthalide (sedanenolide or senkyunolide A), *cis*-3-*n*-butyl-3a,4,5,6-tetrahydrophthalide (*cis*-sedanolide or isocnidilide), and *trans*-3-*n*-butyl-3a,4,5,6-tetrahydrophthalide (*trans*-sedanolide or neocnidilide) (Van Wassenhove et al. 1990) (Table 14.2). The phthalides are the key compounds of celery flavor (Takeoka 1999, MacLeod and Ames 1989,

Table 14.2 Major and Key flavor compounds in the *Umbelliferae* plants.

	Major compound	Major key compound	Minor key compound
Celery	 Limonene [*p*-Mentha-1,8-diene]  *γ*-Terpinene [*p*-Mentha-1,4-diene]	 Sedanenolide [3-*n*-Butyl-4,5-dihydrophthalide]	 Sedanolide [3-*n*-Butyl-3a,4,5,6-tetrahydrophthalide]  3-*n*-Butylphthalide
Parsley	 *β*-Phellandrene [*p*-Menthadiene]  Myristicin [4-Methoxy-6-(2-propenyl)-1,3-benzodioxole]	 *p*-Mentha-1,3,8-triene  Apiole [4,7-Dimethoxy-5-(2-propenyl)-1,3-benzodioxole]	

Wilson 1970). Sedanenolide are 3 to 100 times more abundant than sedanolide and 3-*n*-butylphthalide. In quantity, sedanenolide accounts for about 5–65% of the total flavor compounds (Uhlig et al. 1987, MacLeod and Ames 1989, Tang et al. 1990, Van Wassenhove et al. 1990).

Parsley (*Petroselinum crispum* [Mill.] Nyman)

The major flavor compounds in parsley are *p*-mentha-1,3,8-triene and *p*-menthadiene (β-phellandrene), amounting to 9–56% and 12–59% of the total flavor

compounds, respectively (MacLeod et al. 1985, Masanetz and Grosch 1998, Broda et al. 2001, Diaz-Maroto et al. 2002) (Table 14.2). 4-Methoxy-6-(2-propenyl)-1,3-benzodioxole (myristicin) and 4,7-dimethoxy-5-(2-propenyl)-1,3-benzodioxole (apiole) are also found to be abundant, the amounts of which are 8–39% and 18–34%, respectively (MacLeod et al. 1985, Diaz-Maroto et al. 2002). *p*-Mentha-1,3,8-triene is the key compound of parsley that has a parsley-like flavor and its odor activity value (ratio of concentration to odor threshold) is about 26,000 (Masanetz and Grosch 1998). β-Phellandrene has a terpenic flavor and its odor activity value is about 5,000. Apiole is sometimes called "parsley camphor" and has been reported to have a parsley-like flavor (MacLeod et al. 1985), although it was not detected in some studies (Masanetz and Grosch 1998, Broda et al. 2001).

THE *LABIATAE* PLANTS

Most of the *Labiatae* (mint family) plants have a strong characteristic flavor and they are often used as food spices. Their major volatile compounds are terpenes and some of them also play roles as key compounds (Table 14.3). The common species of the *Labiatae* plants include thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), lemon balm (*Melissa officinalis* L.), perilla (*Perilla frutescent* Britt. W. var. *crispa* W. Decne), peppermint (*Mentha pipertia* L.), spearmint (*Mentha spicata* L.), horsemint (*Mentha longifolia* L.), and Japanese mint (*Mentha arvensis* L.).

THYME (*THYMUS VULGARIS* L.)

Thyme is often used as a spice for meat, fish, dressings, and sauces. The major flavor compounds are 5-methyl-2-(1-methylethyl)-phenol (thymol), 1-methyl-4-(1-methylethyl)-benzene (*p*-cymene), and *p*-mentha-1,4-diene (γ-terpinene) that account for 15–40%, 19–30%, and 12% of the total volatile compounds, respectively (Piccaglia and Marotti 1991, Mookherjee et al. 1989) (Table 14.3). Thymol is an alcohol having an herbal phenolic flavor (Benn and Boake 1998), while *p*-cymene and γ-terpinene are hydrocarbons, both having a citrus flavor (Moyler and Moss 1998). Carvacrol is assumed to be biosynthesized from γ-terpinene via p-cymene, but its content is only 1–2% (Piccaglia and Marotti 1991, Mookherjee et al. 1989).

ROSEMARY (*ROSMARINUS OFFICINALIS* L.)

The principal flavor compounds in rosemary are 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene(α-pinene), 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (1,8-cineole), and 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (camphor) (Table 14.3). Their quantities, however, vary depending on the variety, harvesting time, and extraction conditions. The amount of α-pinene, 1,8-cineole, and camphor ranges from 1–47%, 1–64%, and 2–54%, respectively (Mookherjee et al. 1989, Guillen et al. 1996, Domokos et al. 1997, Tuberoso et al. 1998, Elamrani et al. 2000). α-Pinene belongs to a hydrocarbon, 1,8-cineole to an ether, and camphor to a ketone. α-Pinene has a light piney flavor (Benn and Boake 1998), while 1,8-cineole has a fresh eucalyptus flavor (Moyler and Moss 1998).

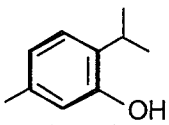
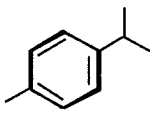
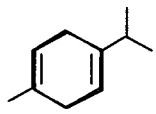
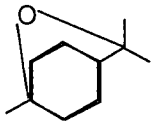
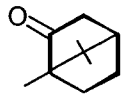
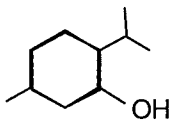
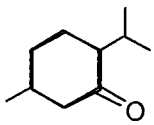
PEPPERMINT (*MENTHA PIPERTIA* L.)

More than 200 compounds have been identified in peppermint oil (Court et al. 1993a). The refreshing flavor of mint is attributed to the major compounds such as 5-methyl-2-(1-methylethyl)-cyclohexanol (menthol) and 5-methyl-2-(1-methylethyl)-cyclohexanone (menthone) (Table 14.3), but their respective amount varies depending on the cultivating conditions, harvesting time, and extraction conditions. The amount of menthol and menthone ranges from 17–57% and 2–55%, respectively (Mookherjee et al. 1989; Court et al. 1993a, 1993b; Piccaglia and Marotti 1993; Marotti et al. 1994; Chalchat et al. 1997; Chalchat and Michet 1997; Moyler and Moss 1998; Güntert et al. 2001). The major flavor contributors obtained by Aroma Extract Dilution Analysis (AEDA) are menthofuran and 1,8-cineole, and their AEDA values are 12 and 10, while those for menthol and menthone are 8 and 7, respectively (Benn and Boake 1998). Menthofuran has a rubbery plastic flavor, 1,8-cineole has a cooling eucalyptus flavor, and both menthol and menthone have a cooling/clean minty flavor.

FRUITS

Fruits are botanically defined as the ripened ovaries of seed-bearing plants. Public recognition would be, however, sweet ripened ovaries consumed as desserts, snacks, or juice. This section follows this common public recognition. In addition to the sweet

Table 14.3 Major and Key flavor compounds in the *Labiatae* plants.

	Major key compound		
Thyme	 Thymol [5-Methyl-2-(1-methylethyl)-phenol]	 *p*-Cymene [1-Methyl-4-(1-methylethyl)-benzene]	 *γ*-Terpinene [*p*-Mentha-1,4-diene]
Rosemary	 *α*-Pinene [2,6,6-Trimethyl-bicyclo[3.1.1] hept-2-ene]	 1,8-Cineole [1,3,3-Trimethyl-2-oxabicyclo[2.2.2] octane]	 Camphor [1,7,7-Trimethyl-bicyclo[2.2.1] heptan-2-one]
Peppermint	 Menthol [5-Methyl-2-(1-methylethyl)-cyclohexanol]	 Menthone [5-Methyl-2-(1-methylethyl)-cyclohexanone]	

taste, the sweet fruity flavor exists in most fruits and is attributable to esters as their impact-aromas (Table 14.4). Orange, grapefruit, lemon, and lime belong to the *Rutaceae* family. Apple, strawberry, raspberry, peach, apricot, and pear belong to the *Rosaceae* family, while blueberry, cranberry, and cowberry belong to the *Ericaceae* family, and black currant and gooseberry to the *Saxifragaceae* family. Grape belongs to the *Vitaceae* family, watermelon and melon to the *Cucurbitaceae* family, and banana to the *Musaceae* family. Guava, feijoa, and spiceberry belong to the *Myrtaceae* family, mango to the *Anacardiaceae* family, and mangosteen to the *Guttiferae* family.

Orange

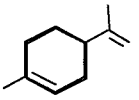
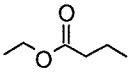
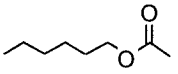
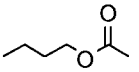
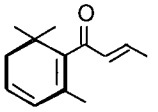
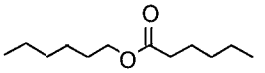
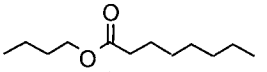
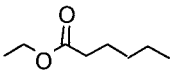
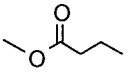
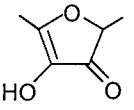
Orange belongs to the *Rutaceae* family (citrus family), and there are many varieties of oranges such as Valencia (*Citrus sinensis* Osbeck), Navel (*Citrus sinensis* Osbeck), Blood (*Citrus sinensis* Osbeck), Mandarin (*Citrus deliciosa* Tenore), Satsuma (*Citrus unshiu* Marcov.), Sour (*Citrus aurantium* L.), Hassaku (*Citrus hassaku* Hort. Es Tanaka), Iyo (*Citrus iyo* Hort.ex Tanaka), and Porcupine (*Citrus hystrix* DC.). Limonene is the principal compound, amounting to about 70–96% in the total volatile compounds in peel (Sugisawa et al. 1989, Boelens 1991, Dugo et al. 1994, Moshonas and Shaw 1994,

Tirado et al. 1995), and about 50–80% in the total volatile compounds in juice (Yajima et al. 1979, Araki and Sakakibara 1991) (Table 14.4). The key compound of orange is ethyl butanoate. The odor threshold of ethyl butanoate is 0.13 ppb, while that of limonene is 60–210 ppb, the concentration of ethyl butanoate in orange juice being about 20–1,000 part of limonene (Ahmed et al. 1978, Moshonas and Shaw 1994, Shaw and Wilson 1980). The odor activity value of ethyl butanoate is about 12,000 and its flavor dilution (FD) factor is about 1,000 (Schieberle and Buettner 2000).

Apple (*Malus pumila* Mill)

Apple belongs to the *Rosaceae* family (Rose family), and about 250 compounds have been identified in its flavor extract (Willaert et al. 1983). The major flavor compounds are esters such as hexyl acetate and butyl acetate (Song et al. 1997, De Pooter et al.

Table 14.4 Major and Key flavor compounds in fruits.

	Major compound	Key compound
Orange	 Limonene [*p*-Mentha-1,8-diene]	 Ethyl butanoate
Apple	 Hexyl acetate  Butyl acetate	 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one [*β*-Damascenone]  Hexyl hexanoate  Butyl octanoate
Strawberry	 Ethyl hexanoate  Methyl butanoate	 DMHF [2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone]

1983, Leahy 1999) that account for 19–31% and 3–44%, respectively (Willaert et al. 1983) (Table 14.4). Hexyl acetate has a sweet, fruity, slightly floral flavor, and butyl acetate has a very diffusive, ethereal-fruity, pungent, pear flavor (Dimick and Hoskin 1983). The compounds that mainly contribute to the apple flavor include 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone), hexyl hexanoate, butyl octanoate, ethyl butanoate, butyl hexanoate, and hexyl butanoate, and their odor-activities detected by charm analysis are 12%, 9%, 9%, 8%, 7%, 7% charm, respectively (Cunningham et al. 1986). The amount of β-damascenone in apple juice is about 50–180 nanograms per milliliter (ng/ml), but its contribution to the total aroma potency increases from 2 to 32% by heating (Zhou et al. 1993). This indicates that β-damascenone is especially important for heated apple juice.

Strawberry (*Fragaria x ananassa*)

Strawberry also belongs to the *Rosaceae* family (rose family), and about 360 compounds are involved in its flavor (Schieberle and Hofmann 1997, Brunerie et al. 1998). Esters such as ethyl hexanoate, ethyl butanoate, methyl hexanoate, methyl butanoate, and hexyl acetate are the major flavor compounds accounting for 11–40%, 3–22%, 1–18%, 2–21%, 0.2–22% of the total flavor compounds, respectively (Hirvi 1983, Kuchii 2002, Hakala et al. 2002) (Table 14.4). Some of these esters such as ethyl butanoate and methyl butanoate, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) are the key compounds of strawberry (Zabetakis and Holden 1997), the former having a fruity flavor and the latter having a caramel-like flavor (Schieberle and Hofmann 1997). The FD factors of DMHF, ethyl butanoate, and methyl butanoate are about 4,000, 500, and 250, respectively, and their odor activity values are about 1,600, 400, 1,000, respectively (Schieberle and Hofmann 1997).

SUMMARY

This chapter overviews the natural volatile flavors in the *Allium* plants, mushrooms, the *Cruciferae* plants, the *Solanaceae* plants, the *Umbelliferae* plants, the *Labiatae* plants, and fruits. The major volatile compounds in the *Allium* plants, mushrooms, the *Cruciferae* plants, and the *Solanaceae* plants are enzymatically produced when the plant tissues are damaged. Therefore, the production of volatile compounds would vary depending on the processing conditions. Sulfides are mainly produced in the *Allium* plants, C8 compounds in mushrooms, isothiocyanates in the *Cruciferae* plants, and C6 compounds in the *Solanaceae* plants. The major volatile compounds in the *Umbelliferae* plants, the *Labiatae* plants, and fruits are biosynthesized during ripening. Terpenes are the major volatile compounds in the *Umbelliferae* plants and the *Labiatae* plants, and esters in most fruits. However, the key compounds are sometimes different from those major compounds, which indicates that the sensation perceived by smell is highly specific to a certain compound or a few impact aromas. Further investigation is necessary to clarify this phenomenon.

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15
Wood Smoke Flavor

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INTRODUCTION

Wood smoke has been used for tens of thousands of years to impart flavor, aroma, and color to foods, as well as to preserve them (Baltes et al. 1981, Pszczola 1995). The traditional method of direct smoking (i.e., direct contact with hardwood smoke in a smokehouse) is still used today in the production of various smoked meats, such as ham, bacon, sausage, etc., fish, cheese, and a wide variety of other food products. However, in the past few decades, commercial, natural wood smoke flavorings, or liquid smokes have become more popular in the United States and throughout the world as a convenient and consistent way to add smoke flavor and color to foods without having to use a smokehouse.

The original primary objective behind smoking of foods was to preserve them through the combined effects of dehydration and reduction of surface bacteria by the antimicrobial action of certain smoke components (Boyle et al. 1988, Suñen 1998). As a consequence of smoking, desirable and characteristic smoked color and flavor also were imparted to the food. Currently, traditional direct smoking is primarily used to enhance the sensory properties of foods. Natural liquid smoke extracts, on the other hand, have found numerous other food uses in addition to their use as flavoring agents. Depending upon their method of production and composition, these products may be used to impart or modify color as well as flavor, and they can provide antimicrobial, antioxidative, and protein denaturing properties when added to foods.

The following discussion will focus on the production and chemistry of smoke flavorings, with emphasis given to the characterization of important aroma constituents of smoke flavorings. For additional information, the reader is encouraged to consult several literature reviews and overviews on the subject of smoke flavor (Baltes et al. 1981; van Chuyen and Kato 1983a; Maga 1987, 1988; Rozum 1998; Underwood and Shoop 1998).

GENERATION OF WOOD SMOKE

Wood smoke flavor is generated by the controlled pyrolysis of the major components of wood, namely cellulose, hemicellulose, and lignin (Figure 15.1) (Wittkowski et al. 1992, Fisher and Scott 1997). During pyrolysis, hemicellulose is most readily degraded followed by cellulose and finally lignin (Browning 1967). Hemicellulose degradation yields primarily lactones and furans, while pyrolysis of cellulose produces anhydro-sugars that participate in further classical carbohydrate thermal-degradation reactions. The thermal degradation of lignin gives rise to the most important class of smoke flavor compounds, the phenols. These include guaiacol, phenol, 4-methylguaiacol, and syringol among

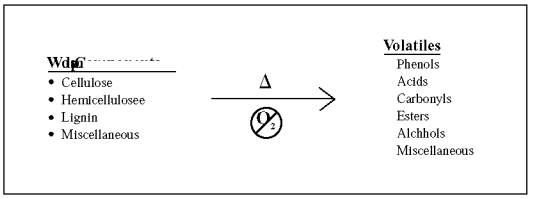

Figure 15.1. Controlled pyrolysis of wood components.

many others. The unique composition of the various types of woods is an important factor affecting the volatile composition of the smoke flavoring, e.g., hickory versus mesquite smoke flavors (Table 15.1). The pyrolysis of the three main wood constituents is discussed below.

Pyrolysis of Cellulose

Cellulose is a linear polymer of β-D-glucopyranosyl units and represents the major component of most woods. Therefore, its pyrolysis products are significant both in number and in abundance in the smoke. Tremendous variation in volatile degradation products is known to occur due to type and abundance of celluloses present, as well as the conditions of pyrolysis, especially temperature. Pyrolysis of cellulose commences at 350°C; evolution of volatile organic compounds occurs at 450°C (Tzamtzis et al. 1997). Degradation occurs through dehydration, hydrolysis, oxidation, decarboxylation, and transglucosylation (LeVan 1989). The primary reaction at high temperature is depolymerization through cleavage of the glycosidic linkage to produce glucose, which undergoes dehydration to form levoglucosan (1,6-anhydro-β-D-glucopyronose) and oligosaccharides.

Table 15.1. Percent composition of hickory and mesquite sawdust.

	Hickory	Mesquite
Moisture	4.0	5.1
Nitrogen	0.13	0.18
Cellulose	53.6	8.0
Lignin	17.7	64.0
Hemicellulose	7.1	8.1

Data from Maga 1986.

Aliphatic acids and aldehydes are the major volatile products of cellulose pyrolysis (Shafizadeh 1984).

Pyrolysis of Hemicellulose

Unlike cellulose, hemicellulose is a heterogeneous group of polysaccharides composed of subunits of hexoses (glucose, mannose, and galactose), pentoses (xylose and arabinose), uronic acids, and other constituents. The pyrolysis chemistry of hemicellulose is very complex. Hemicelluloses are more thermally labile than cellulose (Baltes et al. 1981, LeVan 1989). Much of the methanol, formaldehyde, and acetic acid, which are major volatile components of liquid smoke flavorings, are pyrolysis products of hemicelluloses (Baltes et al. 1981).

Pyrolysis of Lignin

Lignin is a high molecular weight, randomly cross-linked polymer consisting of an irregular array of hydroxyl- and methoxy-substituted phenylpropane units. Pyrolysis of lignin yields phenols, methoxyphenols (guaiacols), and syringols from the cleavage of ether and carbon-carbon linkages (LeVan 1989, Wittkowski et al. 1992, Izumi and Kuroda 1997). The guaiacol and syringol derivatives (Figure 15.2) are of particular importance in smoke flavor. Syringol and 4-substituted syringol derivatives are formed at elevated levels in hardwoods that contain a higher amount of methoxy-substituted lignin, which is characteristic of hardwoods such as oak and hickory. In softwood the main lignin subunit is guaiacylpropane, which yields primarily guaiacol derivatives upon pyrolysis (Alén et al. 1996). Therefore, it is possible to determine from the proportion of syringyl to guaiacyl derivatives the type of wood (hard versus soft) used in smoke generation process (Baltes et al. 1981). Wittkowski et al. (1992) demonstrated that at temperatures exceeding 400°C, guaiacols can undergo further transformation into pyrocatecols and alkyl- and dialkylphenols. Another important lignin degradation reaction is initiated by fission of the heterocyclic furan/pyran rings and ether linkages that produce ferulic acid that further degrades to yield 4-vinylguaiacol, which can undergo further degradation to give rise to phenol and cresols. In addition to the lignin monomers, such as the phenols and methoxyphenols, lignin dimers and trimers have also been reported as components of

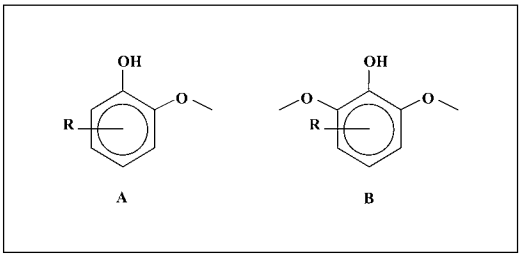

Figure 15.2. Structures of guaiacol (A, R = H) and syringol (B, R = H) and their derivatives.

liquid smoke flavors (Guillén and Ibargoitia 1998, 1999a).

SMOKE FLAVORINGS

In the late 1880s, the Kansas City, Missouri pharmacist Wright developed and patented a crude distillation process for condensation of wood smoke to yield the first commercial liquid smoke flavoring (Pszczola 1995). The product was intended for home curing of meats and for flavoring of certain foods such as baked beans, barbeque sauce, etc. However, liquid smoke did not catch on as a mainstay flavoring until the early 1970s after the development of more advanced production methods. Commercial liquid smoke is defined as a colloidal suspension of the aqueous condensate produced by smoldering wood chips or sawdust under controlled conditions, in particular in the absence of air. The product is further refined to remove tars and particulate matter and is later standardized according to flavoring or coloring strength. Today, liquid smoke and flavorings derived from it are used extensively in the food industry, especially by meat processors where an estimated 7 out of 10 use the product. Applications for liquid smoke include meat and poultry, fish (salmon), cheese and processed cheese products, nuts, sauces, soups, seasonings, and miscellaneous snack foods. Use of liquid smoke flavorings has several advantages over traditional smokehouse processes, including consistent and uniform flavoring and coloring strength, shorter processing cycles, easier smokehouse cleanup, and reduced smokehouse-related pollution. Liquid smoke flavorings can also be applied in a number of ways including incorporation into curing brine, by injection, or by electrostatic spraying. Liquid smoke is considered a natural flavoring and has been granted Generally Recognized As Safe (GRAS) status by the Flavor and Extract Manufacturers Association.

PRODUCTION

The general commercial process for production of smoke flavorings has been described by Pszczola (1995) and is based on a process originally patented in the early 1960s. Wood chips and sawdust from hickory and other hardwoods, or in some cases "soft" woods such as pine, are smoldered in specially designed rotary or multiple-hearth furnaces that allow for control of oxygen, moisture, temperature, and throughput time. Any combustible gases generated in the process, such as methane, are recycled and used in the combustion furnace, resulting in minimal emissions.

The smoke produced in the combustion process is mixed with cold water in a condensing tower to produce crude liquid smoke. The condensate is held for at least 10 days to allow for the bulk insoluble materials to settle out, especially polycyclic aromatic hydrocarbons (PAH). The liquid smoke then undergoes a multistage filtration process.

The refined liquid smoke is further processed into three main products, namely aqueous, vegetable oil-based, and dry (powder) smoke flavorings. Important quality control indices include titratable acidity, phenol and carbonyl content, staining index, specific gravity, moisture, and particle size. The particular type of wood used in the generation of the smoke strongly influences the characteristics, especially flavor, of the final smoke flavoring.

VO C

Wood smoke contains numerous volatile compounds, and its composition varies widely depending upon species of tree, age and growing conditions, and on parameters used during pyrolysis. The "active" components of smoke include volatile acids that can affect flavor and pH as well as stability of the product; carbonyl compounds that can react with proteins and other nitrogen compounds to develop stain or color; and phenols that are considered the main flavor compounds and are also primarily responsible for both antimicrobial and antioxidant activities. Numerous

other chemical classes also contribute to smoke flavor. These include aliphatic and aromatic hydrocarbons as well as alcohols, organic acids, carbonyl compounds, and various oxygen- and nitrogen-containing heterocyclic compounds. Prior to 1988, around 400 volatile compounds had been identified in smoke (Maga 1987, 1988). Table 15.2 contains a listing, subdivided by chemical class, of 149 volatile compounds commonly reported in aqueous smoke condensates and liquid smoke flavorings. This list is taken primarily from reports on the volatile compositions of wood smoke intended for food flavoring use and of liquid smoke flavor preparations. The following discussion briefly reviews the literature on smoke flavor from the early 1960s to the current state of knowledge. A separate section is later devoted

Table 15.2. Volatile compounds commonly identified in wood smoke flavorings.

Acids
Formic acid
Acetic acid
Propanoic acid
Butanoic acid
2-Butenoic acid (crotonic) acid (*cis*- and *trans*-)
2-Methylpropanoic (isobutyric) acid
3-Methylbutanoic (isovaleric) acid
Pentanoic (valeric) acid
2-Methyl-2-butenoic (tiglic) acid (*cis*- and *trans*-)
Levulinic acid
Hexanoic (caproic) acid
Isocaproic acid
Heptanoic acid
Octanoic (caprylic) acid
Nonanoic acid
Decanoic (capric) acid
Dodecanoic (lauric) acid
Tetradecanoic (myristic) acid
Hexadecanoic (palmitic) acid
Benzoic acid

Alcohols
Methanol
Ethanol
2-Propanol
2-Propen-1-ol

Aldehydes
Formaldehyde
Acetaldehyde
Propanal
2-Butenal
3-Methylbutanal
2-Methyl-2-butenal
Pentanal
4-Hydroxybenzaldehyde (anisaldehyde)
4-Hydroxy-3-methoxybenzaldehyde (vanillin)
4-Hydroxy-3,5-dimethoxybenzaldehyde (syringealdehyde)

Aldehydes (continued)
3-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-2-propenal (sinapaldehyde)

Esters
Methylformate
Methyl acetate
Hydroxy-2-butanone acetate

Furans
Furan
2-Methylfuran
2-Acetylfuran
2-Methylbenzofuran
Furfuryl alcohol
2-Furfural
5-Methylfurfural
5-Hydroxy-2-furfural
2-Furfuryl methyl ketone

Ketones
Acetone
1-Hydroxypropanone
3-Methyl-2-butanone
2,3-Butanedione (diacetyl)
2-Pentanone
2,3-Pentanedione
2,5-Hexanedione
Methyl ethyl ketone
Acetophenone
Cyclopentanone
2-Cyclopentenone
Cyclohexanone
2-Methylcyclopentenone
2,3-Dimethyl-2-cyclopentanone
2-Hydroxy-3-methylcyclopent-2-ene-1-one (cyclotene)
4′-Hydroxy-3′5′-dimethoxyacetophenone (acetosyringone)

Table 15.2. Continued

Ketones (continued)
2-Hydroxy-3-methylcyclopent-2-ene-1-one (cyclotene)
4′-Hydroxy-3′5′-dimethoxyacetophenone (acetosyringone)

Miscellaneous
γ-Butyrolactone
3-Hydroxy-2-methylpyrone (maltol)
1,2-Dimethoxybenzene
1-Indanone

Phenols
Phenol
2-Methylphenol (*o*-cresol)
3-Methylphenol (*m*-cresol)
4-Methylphenol (*p*-cresol)
2,3-Dimethylphenol
2,4-Dimethylphenol
2,5-Dmethylphenol
2,6-Dimethylphenol
3,4-Dimethylphenol
3,5-Dimethylphenol
2,3,5-Trimethylphenol
2,4,6-Trimethylphenol
Diethylphenol
4-Isopropylphenol
4-Vinylphenol
2,6-Dimethoxyphenol (syringol)
4-Methylsyringol
4-Ethylsyringol
4-Propylsyringol
4-Isopropylsyringol
4-Propenylsyringol (*cis*- and *trans*-)
4-Allylsyringol
4′-Hydroxy-3′-methoxyacetophenone (2-Acetovanillone)
2-Methoxyphenol (guaiacol)

Phenols (continued)
4-Methylguaiacol
4-Ethylguaiacol
4-Propylguaiacol
4-Vinylguaiacol
2-Methoxy-4-allylphenol (eugenol)
2-Methoxy-4-propenylphenol (isoeugenol) (*cis*- and *trans*-)
1,2-Benzenediol (pyrocatecol)
3-Methylpyrocatecol
4-Methylpyrocatecol
3-Methoxypyrocatecol

Hydrocarbons and polycyclic aromatic hydrocarbons (PAHs)
Benzo[*a*]pyrene
Benzo[e]pyrene
Benz[*a*]anthracene
Phenanthrene
Benzo[*ghi*]perylene
Chrysene
Pyrene
Fluoranthene
Triphenylene
Naphthalene
1-Methylnaphthalene
2-Methylnaphthalene
1-Methylphenanthrene
2-Methylphenanthrene
2-Methylanthracene
Benzo[*a*]fluorene
Benzo[*b*]fluoranthene
Benzo[*k*]fluoranthene
Perylene

to PAHs, which do not actually contribute to smoke flavor per se, but are relevant with respect to the potential safety of smoke flavorings.

Pettet and Lane (1940) first reported the presence of aldehydes, alcohols, ketones, acids, and phenols in steam-distilled fractions from wood smoke. Sometime later, Husaini and Cooper (1957) reported mainly acids in the steam volatiles of smoke. Hollenbeck and Marinelli (1963) used paper chromatography to determine the presence of formic, acetic, propanoic, vanillic, and syringic acids, acetaldehyde, ethanol, acetone, glyoxal, methyl glyoxal, furfural, guaiacol (2-methoxyphenol), and syringol (2,6-dimethoxyphenol) in an aqueous liquid smoke preparation. Since that time and after gas chromatography became commonplace in most flavor research laboratories, there has been extensive research on the composition of wood smoke and smoke flavorings.

Prior to the 1990s, mainly phenolics, acids, carbonyls, and furfuryl compounds were reported in whole smoke and smoke condensates (Baltes and Söchtig 1979; Baltes et al. 1981; Doerr et al. 1966; Fiddler et al. 1966; Fiddler et al. 1970a, 1970b; Fujimaki et al. 1974; Hamid and Saffle 1965; Porter et al. 1965; Knowles et al. 1975; Radecki et al. 1976, 1977; Radecki and Grzybowski 1981; van Chuyen and Kato 1983a, 1983b; Wittkowski et al. 1981). Fiddler et al. (1970b) fractionated an ether-soluble extract of liquid smoke and demonstrated that the essential "smoky" flavor fraction contained primarily phenols and carbonyls. This finding supported the earlier observations of Bratzler et al. (1969) who suggested that phenols are, perhaps, the most important class of flavor compounds in smoke flavor. Wasserman (1966) mentioned that 4-methylguaiacol may be the single most important aroma component of smoke due to its relatively high abundance and low threshold. However, by use of recombination experiments, he later demonstrated that the phenols alone could not duplicate smoke flavor exactly and that smoke flavor must be more complex consisting of many compounds of different chemical classes. The complexity of smoke flavor was later supported by the results of Spanyar et al. (1966), who could not duplicate smoke flavor even when using simulated smoke solutions of different chemical classes based on the results of gas chromatographic analysis of smoke preparations.

Since the publication of Maga's (1987, 1988) reviews on smoke flavor, there have been a considerable number of reports on the volatile composition of smoke extracts and flavorings. The vast majority of these have been conducted by Guillén and coworkers (Guillén et al. 1995, 2000a, 2000b, 2001; Guillén and Ibargoitia 1996a, 1996b, 1998, 1999a, 1999b; Guillén and Manzanos 1996a, 1996b, 1997, 1999a, 1999b, 2002; Ojeda et al. 2002), with only a few additional studies conducted elsewhere (Maga and Chen 1985, Potthast and Eigner 1988, Wittkowski et al. 1990, Edye and Richards 1991, Chen and Maga 1995). Influence of vegetal source (i.e., wood substrate for pyrolysis) on volatile composition has been the subject of many of these studies (Guillén et al. 2001; Guillén and Ibargoitia 1996b; Guillén and Manzanos 1999a, 1999b). Recently, numerous studies have been conducted on commercial smoke flavorings. Alkyl and carbonyl derivatives of guaiacol and syringol have been reported as the major volatile constituents of dichloromethane extracts prepared from a commercial liquid smoke flavoring, with syringol derivatives predominating over guaiacol derivatives (Guillén et al. 1995, 2001; Guillén and Manzanos 1996b, 1997). Total guaiacol and syringol derivatives were in nearly equal abundance in a liquid smoke prepared from French oak (*Quercus* sp.) (Guillén and Manzanos 2002). Lignin dimers and trimers were reported in commercial liquid smoke flavorings (Guillén and Ibargoitia 1998, 1999a). These dimers and trimers have very little flavor impact but possess potent antioxidative activity. Guillén et al. (2001) analyzed polar constituents (methanol extracts) of commercial liquid smokes and found the cellulose pyrolysis product levoglucosan was the main constituent. Some previously unidentified furan, pyran, pyridine, and phenol derivatives also were reported.

Polycyclic Aromatic Hydrocarbons

PAHs are compounds consisting of two or more condensed aromatic rings, either *cata*-annellated (linearly or angularly) or *peri*-condensed (Šimko 2002). PAHs are noteworthy since they are prevalent in a number of wood smokes and comprise the largest class of carcinogenic compounds. For this reason, the determination of PAHs in smoked foods and in smoke flavorings has been the subject of numerous studies (Guillén et al. 2000a, 2000b; Šimko 2002). Temperature during smoking plays a very important role in the generation of PAHs, and the amount of PAHs is linearly related to the smoking temperature in the range 400–1000°C (Tóth and Blaas 1972). Wood composition, especially lignin content, also influences the levels of PAHs produced (Maga 1986). Products undergoing direct exposure to smoke, especially heavy smoking during processing, contain higher PAHs concentrations than products treated with liquid smoke flavorings, where the PAHs have been nearly eliminated by condensation with tars and by further processing (Roda et al. 1999). Legal limits of 10 μg/kg have been established for benzo[*a*]pyrene, a marker compound for the presence of PAHs, in smoked foods, and for foods treated with liquid smoke flavorings (FAO/WHO 1987). Evaluation of commercial liquid smoke flavorings by Guillén et al. (2000a) revealed that benzo[*a*]pyrene concentrations did not exceed the 10 μg/kg limit.

FLAVOR-IMPACT COMPONENTS OF SMOKE FLAVORINGS

Although considerable progress has been made on the identification of the numerous volatile constituents of smoke condensates, very little is known about the aroma and taste components of smoke flavorings. The compounds most readily associated with the "smokiness" character of smoke flavorings are the guaiacyl and syringyl derivatives (Figure 15.2). These include guaiacol, 4-methylguaiacol, syringol, *trans-iso*eugenol, and 4-methylsyringol, in particular, which comprise approximately 50% of the total abundance among 22 phenolic compounds identified in liquid smoke condensates (Knowles et al. 1975).

Using the sensory-instrumental analysis methods of gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA) described by Grosch (1993), Wittkowski et al. (1990) found that guaiacol, 4-, 5-, and 6-methylguaiacols and 2,6-dimethylphenol were the predominant aroma components of headspace extracts of smoked ham, regardless of whether the ham was produced by softwood or hardwood smoking. Semivolatile components such as syringol and its derivatives were found to play only minor roles in the headspace aroma of smoked ham.

Cadwallader (1996) compared aroma extracts prepared from hickory and mesquite liquid smokes by GCO and AEDA. In that study, aroma extracts were prepared by simultaneous steam distillation-solvent extraction with dichloromethane from two types of liquid smokes obtained from two commercial sources. Sixteen high-impact odorants, including 10 phenols, were commonly identified in both the hickory and mesquite liquid smokes (Table 15.3). Compounds exhibiting the highest odor activity values (calculated by dividing the concentration of an odorant by its odor detection threshold) included guaiacol, diacetyl, 3-(methylthio)propanal, 4-ethylguaiacol, eugenol, 4-methylguaiacol, and butanoic acid. The results of this study indicated that while phenolic compounds are indeed key components of smoke flavor, other constituents such as carbonyls and acids are important contributors to smoke flavor. Additional instrumental-sensory directed studies that combine GCO and descriptive sensory analysis of model flavor systems are needed to more fully understand the complex nature of smoke flavorings.

Table 15.3. High impact odorants identified in commercial hickory and mesquite liquid smokes[a].

Compound	Odor Description
2,3-Butanedione (diacetyl)	Buttery
1-Penten-3-one	Plastic
2,3-Pentanedione	Buttery
3-(Methylthio)propanal	Potato
Butanoic acid	Spoiled milk
3-Methylbutanoic acid	Dried fruit
2-Methoxyphenol (guaiacol)	Smoky
4-Methylguaiacol	Smoky, vanilla
2-Methylphenol (o-cresol)	Ink, phenol
4-Ethylguaiacol	Cloves, smoky
4-Methylphenol (p-cresol)	Stable, fecal
Eugenol	Cloves, smoky
4-Propylguaiacol	Cloves, smoky
4-Vinylguaiacol	Cloves, spicy
2,6-Dimethoxyphenol (syringol)	Smoky
Isoeugenol	Cloves

Data from Cadwallader 1996.

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16
Blended Flavors

Sheng X. Ma, Zuo Bin Xiao, and Feng Chen

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INTRODUCTION

Flavor creation is an art of mixing various flavoring materials in specific ratios through special processing procedures (Lin 2001). The mixture bears a unique flavor profile with harmonious and balanced flavor notes. Most importantly, all the raw materials used in the flavor formula must be safe for human consumption, if they are applied as food additives.

Flavor has always been an essential part of people's life. From the foods we eat, the beverages we drink, to the condiments we use for cooking, most, if not all, of them contain desirable and unique flavors. Besides their inherent applications in foods, flavors are also widely used in medical, tobacco, and chemical products (Sun et al. 2000). The common applications of flavor include, but are not limited to, the following:

1. Developing new products with novel flavors and/or taste profiles.
2. Maintaining and/or improving product quality through:
    A. Compensating flavor losses due to multiple processing procedures and supplementing flavors already present in the products.
    B. Enhancing, extending, rounding off, or increasing the potency of flavors already present in the products. For example, we could enhance the vanilla flavor in the formulated products using vanilla flavor extract or vanilla beans.
    C. Assuring the stability of the total flavor profile and right specifications of the end product.
    D. Masking less desirable and/or undesirable flavors naturally present or developed during processing, such as those volatile active ingredients in ethnic products and the original flavors from the raw materials that are commonly practiced in pharmaceutical and energy drink products.
3. Simulating other more expensive flavors or replacing unavailable flavors or aromatizing the original tasteless ingredients for economical reasons.

A typical flavor normally consists of three major parts: top note, body note, and base note, which can be provided respectively by individual type of flavor materials as classified based on the volatilities (Table 16.1). Top note is the foremost smelled flavor characters that are brisk, fresh, exciting, and elegant. Body note is the main aroma that lasts quite a long

Table 16.1. Flavor materials with different volatilities.

Volatility	MW	Lasting	Contributions
Top Note	<120	Minutes	Impact, lift compounds
Body Note	120–200	Hours	Fullness of flavor
Base Note	≥200	Weeks/Days	Taste and rounding off

time with stable and unique aroma characteristics. The base note is the residual fragrance that can last an even longer time for flavor reminiscence.

The aroma characteristics of the three flavor parts are quite different. Therefore, it is very difficult to develop a highly satisfied product by using only one flavoring chemical since normally no single flavoring chemical can encompass all three flavor notes perfectly. This reality makes it necessary to use more than one flavoring ingredient with different aroma profiles in a product to achieve the most desired result.

BLENDED FLAVOR IS BECOMING A TREND

Definition of Blended Flavor

From the name itself, blended flavor could be defined as two or more different flavorings used or blended together on a specific ratio to give the finished product a unique flavor profile that is different from any one of the individual flavors used alone. The flavorings used in blending can be natural, natural identical, or artificial. They can be applied in different forms such as liquid, emulsion, or solid. Therefore, blended flavor is not a classical type of flavor according to any traditional flavor classification. Instead, blended flavor is an art more related to the blending technique that flavorists can use to express the whole developmental process of a flavor profile, rather than a specific flavor stage in the finished product.

Advantages of Using Blended Flavors

Flavor blending is a flavor creation technique that has been widely used in our common life practice but often ignored in literature. It is originated from and has been greatly improved by flavorists in their flavor creative practice, which made it possible to achieve the required high degree of harmony between the smell and taste of foods. Moreover, it provides a useful channel to further improve and enhance product quality. Therefore, using blended flavor in food products is a trend and fashion, especially to the younger generations. The major advantages of using blended flavors include, but are not limited to, the following:

A. To make up some flavor limitations: As previously described, a typical flavor consists of top note, body note, and base note. Each of them has a different releasing process for flavor development. Since it is nearly impossible for one flavor to have all three perfect releasing processes reflecting the flavor characteristics, it is very difficult to make a perfect product using only a single flavor. However, it is possible to select and blend several flavors with different flavor characteristics to achieve the best flavor profile for a finished product.
B. To satisfy a customer's sensory requirement in different regions: In different countries or different regions of a country, consumer's flavor preferences are quite different. For example, regarding the milk flavors used in ice creams, people in some regions may prefer the flavors more toward vanilla note while in other regions, consumers may want fresh milk note or sometimes need more bakery note or even with a little caramel note, all of which could be resolved by the flavor blending technique now commonly used by ice cream makers.
C. To meet product development needs: The products using blended flavors often have certain specialities and uniqueness in their flavor profiles, which can prevent use as adulterated foods or reduce the possibility of the products being simulated by competitors. On

the other hand, blended flavors can specifically create a product with unique flavor profiles that will attract some consumers with a certain flavor preference.

D. To enhance and modify low cost flavor formula: Blended flavors can be used to simulate, maintain, and even improve product image and quality when less expensive ingredients have to be used to achieve a cost-effective formulation.

E. Others:

   a. To modify flavor profiles that are tailor-made for customers
   b. To meet various consumer targets
   c. To stir consumer's interest with unique product profile
   d. To make product more interesting and not boring
   e. To provide synergistic effect, for example, blended cooling flavor can maximize cooling and mint intensity and minimize irritation
   f. To strengthen the stabilization effect, i.e., addition of one flavor will make another flavor more stable

THE BASIC PRINCIPLE OF FLAVOR BLENDING

1. Flavor Note is the Basic Element of Flavor Blending Flavor: Notes are the basic units of a flavor structure. They are also the building blocks of a blended flavor. Moreover, it must be pointed out that flavor notes respectively describe certain parts of aromas in a flavor profile, rather than a description of the whole flavor profile. In recognition of the above facts, flavor creation to a flavorist is actually an art of formulating different flavor notes in terms of what and how flavor materials can be used and the ratio of flavorings to express a special flavor note. In this creative artwork, a smooth transition between the flavor notes is especially important and necessary during the process of the whole flavor creation and in the final products. Therefore, flavorists are required to master the skills of re-creation and connection of flavor notes to keep the consistency of the process and to enable the replication of the end result with the specified flavor.

2. The Basic Principles of Flavor Blending: There are several basic principles that can be followed in developing a successful blended flavor.

   A. Blended flavor with duplicated flavor notes: In this case, a blended flavor can be prepared from the same flavor series and/or the same flavor category, such as blended strawberry flavor (fresh strawberry with ripe strawberry) and blended peach flavor (sweet peach with yellow peach). This technique is especially useful in the simulation of fruity flavor profiles since the fruity aromas not only possess the special flavor characters, but also reflect the trace of flavor evolution at different ripening stages. Therefore, the prerequisite for a successful fruity flavor creation relies on the actual representation of the product under its natural environment because all fruits have a maturing process with a full flavor development. However, for a flavorist who always tries to duplicate the best and the most typical part of the flavor, the most difficult thing is to show the whole flavor development process. This dilemma can be in a high degree resolved by the flavor blending technique. For example, blending fresh strawberry with ripe strawberry can not only substantially simulate a characteristic strawberry flavor, but also reflect the smooth transition from fresh, green, and sour flavor notes to a sweet, fruity, and berry flavor notes, which represents the whole flavor development process of strawberry. Therefore, in most cases of simulation of the flavor development in products, blended flavor is the best choice for duplicating the process. Likewise, this technique can be applied to a processed food that usually has a flavor-changing process.

   B. Blended flavor with complementary flavor notes: Blended flavors can have complementary flavor notes when they are prepared from the same flavor category but different flavor types, e.g., blended orange and ripe pineapple flavor, blended mango and sweet peach flavor, etc. Blended flavor with complementary flavor notes are essential to flavor industries because single flavor often lacks the third dimension in expressing the actual flavor profile and mouthfeel. Therefore, blended

flavors have wide and successful applications. For example, the sweet taste could be enhanced by adding a little sour flavor note; foods can become more tasty and refreshing by adding a little green flavor note. Regarding the blended flavor of great delicacy, it is important for a flavorist to master the accuracy and the completeness of a flavor theme during food flavor creation. In addition, when the expected flavor theme seems to lack the flavor saturation and/or flavor imagination, blended flavor with complementary flavor notes is a good resolution. For example, although ripe pineapple's three major flavor notes (i.e., tangerine, fruity, and green) also exist in the orange flavor, their focuses are different so that they are complementary in many cases. Fresh orange juice is generally required not only to express its typical flavor character, but also to encompass the ripeness of fresh orange pulps. To strengthen the mouthfeel of orange ripeness, the ripe pineapple flavor is often used to help make the juice taste more natural. Similarly, blending mango flavor, sweet peach flavor, passion fruit flavor, guava flavor, pineapple flavor, and tangerine flavor can give an optimum result of expressing a tropical fruit theme in a harmonious and complete aroma and mouthfeel.

C. Blended flavor with traditional combinations: Flavors that have been traditionally used in blending in many cases belong to different categories, e.g., strawberry and milk flavors; red bean and sweet-scented osmanthus flavors, but they are closely associated with related flavor notes. For example, both milk flavor and strawberry flavor have the same flavor note with different directions of expression. Regardless of their differences, the blending of the two flavoring ingredients has resulted in a desirable combination. In this case, the aroma of strawberry will not be altered by the addition of milk since the aroma of milk is quite flat. On the contrary, it can extend and enhance the impact of strawberry flavor. Therefore, this is the reason people consume strawberry ice cream or drink strawberry-flavored yoghurt. In striking similarity, vanilla flavor is often blended with dairy flavors to make the finished product more complex and tasty. Nevertheless, no matter whether the blended flavors belong to the same or different categories, the consistency among the flavor notes is the base for flavor creation.

COMMON PROBLEMS ASSOCIATED WITH BLENDED FLAVOR

Flavor itself is a very complex system within which inherent components sometimes can interact with each other resulting in undesirable off flavors. Blended flavors bear a risk of increasing the complexity of the system by several folds depending on the number of flavors blended. The common problems associated with blended flavors mainly result from flavor profile incompatibility and undesired physical phenomena such as discoloration, separation, and sedimentations.

Incompatibility of Flavor Profiles

The incompatibility of flavor profiles has to be first considered for blended flavors. The incompatibility of flavor profiles could be reflected in the following situations:

A. Disappearance of a clear flavor theme: Individual food flavor normally has a clear flavor theme which is the base to simulate the natural mouthfeel. However, unsuccessful flavor blending could result in the blended flavor lacking a clear flavor theme.
B. Lack of harmony among flavor notes: Good harmony among flavor notes is the base of flavor blending. The closer the flavor notes, the greater the possibility of the successful rate of the business; furthermore, the more complete the transition between the flavor notes, the better the flavor harmony. However, not all of the flavor notes are compatible, so those should be cautiously avoided in flavor blending.
C. Lack of good mouthfeel: The ultimate goal of flavor blending is to provide desirable products that have a perfect combination of flavor and mouthfeel. Pleasing mouthfeel with an impressive characteristic food aroma is an

index of a successful flavor blending in food application. However, sometimes it is a very challenging task for flavorists.

D. Unfavorable interactions between the flavors: In light of the complexity of flavors, flavoring ingredients will nearly inevitably interact with each other. For example, flavors containing phenolics (e.g., salicylic acid) will interact with aldehydes so as to weaken the desirable flavor. Therefore, some unfavorable interactions should be avoided as much as possible.

Discoloration, Separation, and Sedimentation

Besides the potential interactions among different flavor molecules, physical phenomena such as discoloration, separation, and sedimentations are other common problems often associated with blended flavors. Among those physical phenomena, discoloration is especially a more serious problem that flavorists need to pay special attention to when coloring flavor is added to white products. One of the most significant examples is the mixture of orthovanillin with phenylmethyl anthranilate. These two flavoring chemicals, if mixed, will produce a fresh red color. The sensitivity is achieved in such a high degree that one's existence can be used as the qualitative measurement for the existence of its counterpart. Blending N-containing compounds with aldehyde-type flavors together also often generate colored products, though the reaction is slower or the discoloration is not so obvious. Phenolic type chemicals often cause the discoloration problem when used with other flavorings, especially in the case of the existence of trace metal ions or metal compounds such as iron. For example, the color change in toothpaste is commonly caused by the interactions between the flavors containing trace amounts of metal irons and the chalk base. All of those have to be carefully avoided during the flavor blending. Separation and sedimentation are ready to happen in the presence of terpene chemicals (especially limonene), which widely exist in natural lemon oil, orange oil, tangerine oil, lime oil, and grapefruit oil. When using those flavoring materials to prepare products with fruity flavor, cloudiness and separation and even sedimentation often happen. Although adding large amounts of phenylethyl alcohol or terpineol can increase the limonene solubility, a better common solution is to use terpeneless oil or folded oil in flavor application.

APPLICATION OF BLENDED FLAVOR IN FOOD FLAVOR CREATION

The following list contains a few examples of using blended flavor in cold drinks (Bao 2004, Wang 2004):

1. Low End Milk Flavored Products

A. Prototype formula:

Sugar	25.0
Stabilizer	0.6
Milk powder	1.0
Starch	8.5
Flavor	appropriate amount
Add water to	100.0

B. Blended flavor recommendation:

a. Fresh milk flavor 0.06% + condensed milk flavor 0.04% + butter flavor 0.02%: The blended flavor has fresh milk aroma, pure taste, and good aftertaste.

b. Fresh milk flavor 0.04% + condensed milk flavor 0.06% + butter flavor 0.03%: The blended flavor has natural enzymatic hydrolyzed buttermilk flavor, and is dense and thick.

c. Vanilla flavor 0.04% + pure milk flavor 0.02% + butter flavor 0.02% + condensed milk flavor: The blended flavor has milk aroma with vanilla's special sweet note, and is very pleasant.

Middle Level Milk Flavored Products

A. Prototype formula:

Sugar	20.0
Stabilizer	0.6
Milk powder	4.0
Starch	5.0
Flavor	appropriate amount
Add water to	100.0

B. Blended flavor recommendation:

a. Fresh milk flavor 0.05% + condensed milk 0.04% + melon tea flavor 0.03%:

The blended flavor has milk aroma with caramel aroma.

b. Ice cream yolk flavor 0.05% + ice cream condensed milk flavor 0.03% + fresh milk flavor 0.03%: The blended flavor has a nice combination of bakery aroma, cake aroma, milk aroma, and sweet aroma with very good mouthfeel.
c. Fresh milk 0.08% + melon flavor 0.008%: The blended flavor has a refreshing, clean milk aroma.
d. Pineapple flavor 0.07% + pineapple flavor emulsion 0.05%: These same types of flavor complement each other. Water-soluble flavor has elegant and strong top notes while flavor emulsion gives a long lasting and good aftertaste. The combination of the two can give a nice effect.

FUTURE DEVELOPMENT OF BLENDED FLAVOR

Blended flavor is evolving from simple blending between flavors toward higher level blending with flavor modules. The two major development trends follow:

1. Breaking a complete flavor into various flavor modules, which is normally a challenge for flavorists. Developing a series of flavor modules for a class of flavors as the top note, body note, and base note so that technologists can blend those flavor modules during their applications to meet customers' needs.
2. Bringing modules together to recreate a unique flavor, which is also a challenge for application technologists, who should, based on food properties and processing conditions, selectively combine individual modules to form a novel flavor that can better meet customer's needs in terms of the cost, product properties, shelf life, etc.

From developing flavor modules to flavor applications is a systematic process, which requires higher qualifications for both flavorists and application technologists. First of all, it requires our flavorists to have a more sensitive business vision and a feel to catch up natural products' aroma, fragrances, and their changing process. Second, it requires our flavorists to consider more thoroughly the interactions between flavor modules within foods and the harmony between flavor modules. These two qualifications will guarantee the success of the new product development when launched into the market. Moreover, a food technologist is no more an operator of adding flavor to foods, but a flavor re-creator who uses the flavor modules provided by the flavorist to remake a complete flavor based on customers' needs and special processes. This is a more professional and more systematic job, which is the challenge for the future.

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17
Off Flavors and Rancidity in Foods

Ronald B. Pegg and Fereidoon Shahidi

Introduction
Absorption/Contamination
Hydolytic and Enzymatic Rancidity
Oxidative Rancidity
Photooxidation
Examination of WOF: An Off Flavor in Meat Products
Strategies to Avoid WOF Development
- Meat Quality
- Primary Antioxidants: Synthetic and Natural
- Dietary Antioxidants
- Secondary Antioxidants
- Nitrites and Nitrates
- Natural and Liquid Smoke
- Packaging
- Flavor Masking

Summary
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INTRODUCTION

Off flavor is the term used to describe unpleasant odors or tastes resulting from the natural deterioration of a food, with extensive deterioration giving rancidity. A taint, on the other hand, is generally regarded as unpleasant odors or tastes arising from contamination of a food by some foreign chemical with which it has accidentally come into contact (Baigrie 2003). Detecting any of these "conditions" involves organoleptic appraisal of the off flavor quality of a food. This is subjective in nature, as the ability to perceive an off flavor varies from person to person. To illustrate this point, Baigrie (2003) noted that there are approximately 1,000 olfactory receptor genes influencing an individual's perception of odors, making it possible for the human nose to detect very low concentrations of some of these compounds (i.e., below 0.01 parts per billion). Consequently, identifying an off flavor or taint, both qualitatively and quantitatively, can be a challenging process.

Although microbial spoilage of food can generate an unpleasant odor in a product, this chapter will focus solely on the chemical and enzymatic processes resulting in off flavor and rancidity development. For example, when considering off flavors in lipid-rich foods, there are at least four types of mechanisms that need to be taken into consideration, namely hydrolytic, enzymatic, oxidative, and photooxidative rancidity (Palm 2002). Of these mechanisms, the oxidation of unsaturated lipids has been extensively studied because it relates to the deterioration of lipid-containing foods, production of both desirable and undesirable scission products, and numerous reactions with other food constituents (Wong 1989). The impact of lipid oxidation on muscle foods and edible oils has been the subject of numerous investigations. In fact, it has been over 45 years since the term "warmed-over flavor" was first coined in reference to a marked deterioration in the quality of cooked meat products that have been chill-stored (Tims and Watts 1958). Warmed-over flavor or "WOF" is a common phenomenon that originally referred to the rapid development of lipid-derived oxidized flavor in refrigerated cooked-meat products. In meats, this distinctive off flavor can become readily apparent within a few hours of thermal processing, and is most noticeable when refrigerated cooked-meat products are reheated. Products develop a flavor (i.e., odor and taste) that consumers do not like, and at the same time desirable meaty flavor notes are lost. The stale off flavors so formed have often been described as "cardboard-like," "painty," and "rancid" (Love 1988, Vega and Brewer 1994).

Although WOF development was first recognized as being a dynamic process of flavor change in cooked meat, principally because of a cascade of oxidation events, the original definition has been expanded to include fresh meat that has been stored in a freezer. While freezing can delay the onset of WOF development, it does not prevent it. Stale or off flavor notes such as "ice box," "rancid," and "freezer-burn" have been used to describe the phenomenon. Although the aroma profile related to stored fresh meat is somewhat different from the characteristic WOF of reheated meats, the flavor compounds involved are qualitatively the same but present at different concentrations. When the specter of WOF is raised, it often is in relation to comminuted products such as meat loaves, chicken nuggets, and precooked burgers. Thus, it is generally accepted that any process involving disruption of the integrity of muscle tissue, such as cooking, grinding, mechanical deboning, massaging, restructuring, or freezing will enhance the development of WOF. Warmed-over flavor is not solely a result of lipid oxidation. Scientists in the 1980s suggested that protein degradation reactions contributed toward WOF development (St. Angelo and Bailey 1987). In an effort to better describe the complex series of chemical reactions that contribute to an overall increase in off flavor notes and a loss in desirable meaty ones, the term "meat flavor deterioration" was proposed.

ABSORPTION/CONTAMINATION

Foods can develop taints simply by coming in contact with an off flavor constituent during processing, packaging, or storage. Even the development of a slightly unpleasant aroma or taste may be sufficient for a consumer to reject a food. The lipid component of a food or an edible oil acts as a good reservoir or sink into which volatile off flavor compounds can dissolve. The amount of volatile material absorbed by a food product is likely to be minute; nevertheless, this can have a significant impact on the product by causing a taint in the flavor. Whether a chemical can cause an unpleasant taste or odor is a function of the food product in question: if the taint imparts a taste or odor in keeping with the food, a deleterious effect may not be perceived (Lord 2003). As an example, an acidic taint in a fermented meat product will not impact its flavor, whereas the same taint in a cooked chicken product will have a deleterious impact on its flavor.

The contribution of a single organic volatile compound to an overall smell is based on its odor quality and odor intensity. The odor intensity is dependent on both the concentration of the component in question and the odor threshold in the food matrix. Odor quality and odor threshold data are, therefore, useful tools to gain insight into the influence of a volatile compound on a given food flavor (Rychlik et al. 1998). In various resources, flavor threshold data of chemicals in a specified medium are available, but in most cases the threshold values are quoted simply against water. As a result, detection thresholds of off flavors or taints in foods may be lower; therefore, they have been defined in sensory studies as the minimum concentration which a pure compound can be perceived by 50% of the members of a trained panel. Some general flavor thresholds for volatile classes of compounds found in foods are presented in Table 17.1. A more specific listing of odor thresholds of key food odorants has been compiled by Rychlik and others (1998).

HYDOLYTIC AND ENZYMATIC RANCIDITY

Most off flavors in food develop as a result of hydrolytic, enzymatic, and oxidative rancidity. Hydrolytic rancidity is associated with the presence of water. At typical food production temperatures, water can dissolve in oil from 0.1 to 0.5%. If a suitable catalyst such as a lipase from bacterial contamination is present and the temperature is sufficient, hydrolytic rancidity can take place. The process involves the hydrolysis of triacyglycerol molecules to first diacylglycerols, then monoacylglycerols and finally free fatty acids. The release of short-chain fatty acids has a direct effect on food aroma. These fatty acids have their own flavor threshold values and can impart specific flavors to food. In some instances, release of free fatty acids can combine with sodium ions in food to form soaps, thereby giving a soapy flavor to some food products. Lipoxy-genases and cyclooxygenases are enzymes found in many plants. They catalyze the oxidation of unsaturated fatty acids containing a 1-*cis*,4-*cis*-pentadiene system to their corresponding monohydroperoxides and endoperoxides, respectively. Cyclooxygenase incorporates two molecules of oxygen into the fatty acid

Table 17.1. Threshold values of different volatile compound classes[a].

Compound Class	Threshold Values in Water (mg/kg)
hydrocarbons	90–2,150
substituted furans	2–27
vinyl alcohols	0.5–3
1-alkenes	0.02–9
2-alkenals	0.04–2.5
alkanals	0.04–1.0
2-*t*,4-*t*-alkadienals	0.04–0.3
isolated alkadienals	0.002–0.3
isolated *c*-alkenals	0.0003–0.01
2-*t*,4-*c*-alkadienals	0.002–0.006
vinyl ketones	0.00002–0.007

[a]Adapted from Palm (2002). *t* = *trans* and *c* = *ci.*

and can be considered as a special case of lipoxygenase that incorporates only one molecule of oxygen. The preferred substrates are linoleic and linolenic acids for the enzyme in plants and arachidonic acid in animals (Belitz and Grosch 1987). In fruits and vegetables, the hydroperoxides formed from lipoxygenase breakdown into volatile secondary products with a variety of flavors. These volatiles are responsible for the taste of bananas, grapes, apples, cucumbers, and mushrooms (Palm 2002). In general, lipoxygenase produces similar flavor volatiles to those formed during autoxidation, although the relative proportions of the products may vary widely depending on the specificity of the enzyme conditions (Hamilton 2003). Apart from these wanted flavors, however, oxidation continues and many of the resultant volatiles serve as signal substances warning us from eating rancid food.

OXIDATIVE RANCIDITY

The major reaction in the formation of off flavors is a free-radical chain reaction referred to as autoxidation. Autoxidation of lipid molecules (LH) can be catalyzed by the presence of heat, light, ionizing radiation, metal ions, and metalloporphyrins (Wong 1989). There are many factors related to the propensity of lipids to oxidize, but an important difference between autoxidation in muscle foods and in refined vegetable oils is the presence of several catalytic systems for oxygen activation in meat. Although formation of the first lipid free radical may occur by several different means, one possibility is via metal ion (e.g., M^{3+}) catalysis. Autoxidation then follows initiation, propagation, and termination steps, as outlined below.

$$LH \rightarrow L\cdot + H\cdot$$
$$(\text{e.g., } M^{3+} + LH \rightarrow L\cdot + H^{+} + M^{2+})$$

$$L\cdot + {}^{3}O_2 \rightarrow LOO\cdot$$
$$LOO\cdot + LH \rightarrow LOOH + L\cdot$$

$$L\cdot + L\cdot \rightarrow L\text{-}L$$
$$L\cdot + LOO\cdot \rightarrow LOOL$$
$$LOO\cdot + LOO\cdot \rightarrow LOOL + O_2$$

Hydroperoxides are the primary products of lipid autoxidation. Even though they are odorless and tasteless, their degradation to alkoxy radicals and then additional scission products leads to the formation of complex mixtures of low molecular-weight compounds with distinctive aromas. These secondary products of lipid oxidation principally include alkanes, alkenes, aldehydes, ketones, alcohols, esters, furans, lactones, epoxy compounds, polymers, and acids (Shahidi et al. 1986, Shahidi 1989). It should be stressed, however, that not all flavors derived from oxidation give rise to unpleasant off odors. Short-chain aldehydes with unsaturation at the 2-position are described as sweet and pungent while longer chain analogues have been described as sweet, fatty, and green. Some characteristic flavors and associated compounds isolated and identified in oxidized fats of various foodstuffs are presented in Table 17.2.

In the case of cooked meat, aldehydic breakdown products play a significant role in the flavor, as they

Table 17.2. Characteristic flavors and associated compounds isolated and identified in oxidized fats from various foodstuffs[a].

Flavor Note	Compounds Identified
Cardboard, tallowy	octanal; alkanals (C_9–C_{11}); alk-2-enal (C_8,C_9); 2,4-dienals (C_7, C_{10}); nona2-*t*, 6-*t*-dienal
Fatty/oily	alkanals (C_5–C_7); hex-2-enal; 2,4-dienals (C_5–C_{10}); 2-*t*-pentenylfuran
Painty	alkanals (C_5–C_{10}); alk-2-enals (C_5–C_{10}); hepta-2-*t*,4-*t*-dienal;
Oxidized	2-heptanone; pent-2-*t*-enal; but-2-*t*-enal
Fishy	oct-1-ene-3-one; octanal; hept-2-enal; 2,4-heptadienal; alkanols (C_2–C_9)
Grassy	alkanals (C_5–C_{10}); alk-2-enals (C_5–C_{10}); hepta-2-*t*,4-*t*-dienal; 2-alkanones (C_3–C_{11}); oct-1-en-3-one; deca-2-*t*,4-*c*,7-*t*-trienal; pent-1-en-3-one
Mild, pine-like	2-*t*-hexenal; nona-2,6-dienal; 2-*c*-pentenylfuran; hexanal; heptanal
Green-beany	3-*t*-hexenal
Beany	3-*c*-hexenal
Deep-fried fat	alkanals, non-2-enal
Sweet aldehydic	2-*t*,4-*t*-decadienal
Mushroom, moldy	2-*t*,4-*c*-decadienal
Metallic	oct-1-en-3-one; oct-1-en-3-ol
Rancid	pent-1-en-3-one; oct-1-en-3-one; 2-*t*-pentenylfuran; 1-*c*,5-octadien-3-one
Nutty	2-*t*-nonenal; volatile fatty acids (C_4–C_{10}) 2-*t*,4-*t*-octadienal; 2-*t*,2-*c*-octenal

[a]Adapted from Kochhar (1996). *t* = *trans* and *c* = *cis*.

possess low odor threshold values and are responsible for the development of WOF and rancidity. Meat lipids are made up of adipose and intramuscular tissues, and they contain both saturated and unsaturated fatty acids. However, it is the membrane lipids (i.e., the phospholipids) that tend to possess the lion's share of the polyunsaturated fatty acids (PUFA), and these are most prone to oxidation. When meat is ground, chopped, or cooked, membranes are disrupted releasing cell contents; PUFAs are then exposed to oxidative stress. Consequently, the process of WOF development can begin within several hours of cooking the meat product compared to regular lipid oxidation, which can take several days to develop. Research has shown that subcutaneous fat from meat can produce about 50 volatile compounds during WOF development, whereas intramuscular lipids can generate more than 200. There seems to be no question that oxidation of phospholipids is the primary source of off flavor notes generated during WOF development.

Oxidation of the unsaturated C_{18} fatty acids of meat, namely oleic (18:1*n*-9), linoleic (18:2*n*-6), and -linolenic (18:3*n*-3) acids, has been reported to produce low molecular-weight aldehydes (C_3–C_{12}) such as pentanal, hexanal, and *trans,trans*-2,4-decadienal as well as oxocompounds like the very potent *trans*-4,5-epoxy(E)-2-decenal (Konopka and Grosch 1991); these are believed to be partially responsible for WOF and rancidity development of cooked meats during storage. The impact of dominant aldehydic scission products from the autoxidation of methyl linoleate and their odor threshold values are shown in Table 17.3. It can be reasonably hypothesized that meats containing higher levels of PUFAs are more susceptible to oxidation. This is indeed so, because certain muscle tissue exhibits more of a propensity toward the development of WOF. Tichivangana and Morrissey (1985) have shown that the autoxidation of muscle foods occurs in the following order:

fish → poultry (i.e., chicken and turkey) → pork → beef → lamb

PHOTOOXIDATION

An alternate mechanism resulting in oxidation can occur in the presence of light; thus, the term photo-

Table 17.3. Dominant volatile aldehydes derived from the autoxidation of methyl linoleate[a].

Aldehyde	Quantity[b] μg/g	Odor Threshold Value (μg/kg)	
		in Water	in Oil
pentanal	55	10	100
hexanal	5,100	4.5	150
heptanal	50	30	45
trans-2-heptenal	450	50	14,000
octanal	45	40	50
cis-2-octenal	990	-	-
trans-2-octenal	420	4	7,000
cis-3-nonenal	30	-	-
trans-3-nonenal	30	-	-
cis-2-decenal	20	-	-
trans-2, *trans*-4-nonadienal	30	90	460
trans-2, *cis*-4-decadienal	250	-	20
trans-2, *trans*-4-decadienal	150	0.1	200

[a]Adapted from Belitz and Grosch 1987.
[b]One gram of linoleate was autoxidatized at 20°C by an uptake of 0.5 mole of oxygen per mole linoleate.

oxidation has been given to it. Photooxidation involves the interaction of singlet oxygen with lipid constituents. Singlet oxygen can react directly with unsaturated lipid moieties in contrast to autoxidation. The presence of sensitizers such as myoglobin, riboflavin, and chlorophyll is necessary, as this allows triplet oxygen to interact with light to produce singlet oxygen. In turn, the singlet oxygen reacts with unsaturated lipid constituents and forms hydroperoxides. These build up quickly as soon as oxidation begins because there is little or no inductive period. The photosensitized oxidation process is illustrated as follows, where Sens, ^{1}Sens*, and ^{3}Sens* represent the sensitizer, excited singlet state, and excited triplet state, respectively (Kochhar 1996).

$$\text{Sens} \xrightarrow{\text{light, hv}} {}^1\text{Sens}^* \rightarrow {}^3\text{Sens}^*$$
$${}^3\text{Sens}^* + {}^3\text{O}_2 \rightarrow {}^1\text{Sens} + {}^1\text{O}_2{}^*$$
$${}^1\text{O}_2{}^* + \text{LH} \rightarrow \text{LOOH}$$

There are two types of photooxidation: types I and II. In type I, the typical hydroperoxides are generated as in autoxidation, but for type II, the intermediates formed are different. In type II photooxidation, an ene addition reaction occurs whereby a molecule of singlet oxygen adds across the lipid double bond. This has the effect of generating hydroperoxides that are different from those formed by autoxidation (Hamilton 2003). This in turn leads to different volatile breakdown products and off flavors than those generated by autoxidation. Photooxidation cannot be curbed by synthetic antioxidants such as BHA, which is a great concern for the edible oil industry. Carotenoids, such as b-carotene, are naturally present in many crude oils and some foods, and can quench singlet oxygen and protect lipids against light-induced oxidation, as the carotenoid reacts more quickly with singlet oxygen than triacylglycerol molecules. Refining and bleaching of edible oils remove, at least in part, the endogenous photosensitizers. This along with using suitable packaging that is absorbent to the light energy necessary for photosensitization or storage in the dark will protect oils against deterioration by singlet oxygen.

EXAMINATION OF WOF: AN OFF FLAVOR IN MEAT PRODUCTS

The contribution of sensory analysis toward the development of descriptors, definitions, and references to describe the phenomenon of WOF in cooked meat products has come a long way since the time of Tims and Watts. Today, sensory descriptive vocabularies for WOF in meats of different species have been prepared. An example lexicon of flavor descriptors employed for pork is presented in Table 17.4. Such a vocabulary can be used by panelists to describe perceived sensory characteristics in a sample set; the

Table 17.4. Sensory descriptive terms with definitions developed for the evaluation of warmed-over flavor in cooked, chill-stored, and reheated pork meat.

Term	Definition with Reference Material[a]
Odor	***Odor associated with***
Cardboard	Shredded wet cardboard
Linseed oil	Warmed linseed oil/linseed oil-based paint
Rubber/Sulphur	Warmed rubber or the white of a boiled egg
Nut	Crushed fresh hazelnuts
Green	Fresh French green beans
Fatty	Pig back fat (fresh, nonoxidized)
Taste	***Taste associated with***
Sweet	Sucrose, 1 g/L aqueous solution
Salt	Sodium chloride, 0.5 g/L aqueous solution
Sour	Citric acid monohydrate, 0.3 g/L aqueous solution
Bitter	Quinine chloride, 0.05 g/L aqueous solution
Umami/MSG	Monosodium glutamate, 0.5 g/L aqueous solution
Flavor	***Aromatic taste sensation associated with***
Metallic/Bloody	Ferrous sulphate, 0.1 g/L aqueous solution
Fresh cooked pork	Oven cooked pork without browning
Rancid	Oxidized vegetable oil
Lactic acid/Fresh sour	Natural yoghurt
Vegetable oil	Fresh vegetable oil
Piggy/Animal	Skatole, 0.06 μg/mL refined vegetable oil
Fishy	Fish stock in boiling water
Tinny	Stainless steel strip
Livery	Cooked beef liver
Aftertaste	***Feeling factor on skin surfaces of the oral cavity associated with***
Astringent	Aluminum sulphate, 0.02 g/L aqueous solution

[a]Definitions of sensory terms, as derived during vocabulary development by O'Sullivan et al. (2002).

resultant profile is a perceptual map of the variations in a sample type that can be employed alone or in combination with chemical/instrumental data to help explain and elucidate underlying sensory and chemical relationships. It is important, however, that any analytical technique employed to assess causes and to find solutions for the problem of WOF must be conducted in combination with sensory analysis. The descriptors need to be well defined to allow trained panelists to accurately track the development of WOF with time. Data from the mid-1980s indicate that the sensory perception of WOF was similar across meat patties of beef, pork, chicken, and turkey, but the intensity of samples varied. Some specific data on beef indicated that when it is just cooked, the intensity of fresh cooked beef notes is strong. Over time, however, cardboard notes develop and then disappear; this generally happens before oxidized notes become apparent. At about the same time, there is a marked reduction in fresh beefy notes. For samples that had been stored for 3–7 days, oxidized/rancid/painty notes were dominant (Johnson and Civille 1986). From recent studies on WOF development in pork, sensory profile data clearly demonstrated the specific association and distinct relationship of at least two groups of odor and flavor terms (see Table 17.4) with increasing days of chill-storage and WOF development (i.e., rancid-like flavor, linseed oil-like odor and fish-like flavor, rubber-like odor, and cardboard-like odor) (Byrne et al. 2001).

Various chemical methods to semiquantify WOF development in meat and meat products include

measuring changes in conjugated dienes and carbonyl values as well as the more recent employment of electronic noses. Malonaldehyde is a relatively minor production of autoxidation of polyunsaturated fatty acids in muscle tissue; yet, its presence and concentration in meat products is monitored as an indicator of lipid oxidation by the classical 2-thiobarbituric acid (TBA) test. This assay involves the reaction of malonaldehyde in oxidized foods with the TBA reagent under acidic conditions; a pink adduct forms with a distinctive absorption maximum at 532 nanometer (nm) (Tarladgis et al. 1960, Siu and Draper 1978). The TBA test was once believed to be specific for malonaldehyde, but this is not so. In fact, the TBA method has been criticized as lacking specificity and adequate sensitivity toward the dialdehyde. Due to the ambiguity concerning the identity of compounds that can react with the TBA reagent, the term "thiobarbituric acid-reactive substances" (TBARS) is now employed in lieu of TBA number or value (Ke et al. 1984, Gray and Pearson 1987). Nevertheless, determining the content of TBARS (i.e., often reported as mg malonaldehyde equivalents/kg meat) appears to be a useful indicator of meat quality deterioration. Recent studies have shown that TBARS are highly correlated with many of the sensory terms related to WOF from pork (Byrne et al. 2001). On the other hand, the importance of hexanal, a dominant volatile oxidation product of linoleic acid, as an indicator of WOF development by sensory perception has come into question. Hexanal has a characteristic "tallowy" or "green leafy" aroma, but this odor term was not strongly perceived as being associated with WOF odor terms (e.g., linseed oil-like and cardboard-like) during vocabulary development for pork and chicken meat (Byrne et al. 1999a, 1999b). Results from gas chromatography/mass spectrometry (GC/MS) analyses have indicated that oxidation compounds such as pentanal, 2-pentylfuran, octanal, nonanal, 1-octen-3-ol, and hexanal covaried with the sensory descriptor green.

STRATEGIES TO AVOID WOF DEVELOPMENT

Important questions facing the food scientist and technologist include how does one control or limit WOF development in meat and meat products and what arsenal of countermeasures is available to address WOF. By examining the causes of off flavor development, strategies can be designed to improve the situation.

Meat Quality

The choice of meat is an important factor with regard to the problems of oxidation. Fish and other muscle tissues containing high levels of PUFAs exhibit more of a propensity toward off flavor development. Chicken has less tendency to develop oxidized flavors than turkey, because the higher level of vitamin E in chicken fat retards oxidation; however, the problem will be worse in chicken thigh meat than in white meat, as the darker meat contains more lipid and heme iron. Fresh meat used in product formulations shows less of a tendency to develop WOF than older meat. If the meat is fresh then little enzymatic oxidation has taken place, which can generate autocatalytic compounds that propagate oxidation even after enzymes have been inactivated by thermal processing. Antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, continue to function postmortem at curbing lipid oxidation in uncooked muscle foods; however, their efficacy diminishes with increasing age of the meat. Ensuring that high quality meat is used in product formulations is critical. Incorporating antioxidants into the product and reducing the time from cooking to plate are other means by which food service operators can minimize WOF.

The actual cooking method employed for precooked products can also influence the extent of WOF. One might assume that grilling of meat would exaggerate the potential for WOF on account of the high temperatures to which lipid and protein constituents are subjected, but this is not so. In fact, thermal processes that employ very high temperatures like grilling seem to inhibit WOF development through the formation of Maillard-browning intermediates (Bailey et al. 1987, van Ruth et al. 1998). Similarly, conditions that favor browning, such as addition of glucose or smoke intermediates, may help to retard or inhibit WOF development.

Primary Antioxidants: Synthetic and Natural

Food technologists attempt to reduce the problem of WOF in meat products by adding food-grade antioxidants, when permitted, or ingredients that impart

antioxidant properties. Antioxidants extend the induction period and delay the onset of fatty acid oxidation by acting as free radical acceptors or hydrogen atom donors. Such antioxidants (AH) trap free radicals directly and delay the free-radical chain reaction in a concentration-dependent manner. The antioxidant radical (A·) formed from the reactions shown below is much more stable than the alkyl (L·), alkoxy (LO·) or hydroperoxy (LOO·) free radicals and does not partake in the propagation reactions.

$$L\cdot + AH \rightarrow LH + A\cdot$$
$$LO\cdot + AH \rightarrow LOH + A\cdot$$
$$LOO\cdot + AH \rightarrow LOOH + A\cdot$$

Synthetic compounds such as tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are commonly used antioxidants by the food industry. Their usage levels are strictly regulated, and in the past few years, concerns have been raised about the safety of these compounds (Valentão et al. 2002). As "chemical soup" is not customer-friendly, a wide variety of natural compounds can offer protection against oxidation in the form of food ingredients. The most common natural antioxidants utilized to fight WOF are vitamin E, rosemary and sage extracts, and carotenoids such as β-carotene and lycopene. In addition, many other spices, fruits, and vegetables contain constituents with antioxidant properties, and these might provide benefits to a meat formulation. Reducing exposure of these antioxidants to oxygen and light will enhance their effectiveness in minimizing WOF development.

Addition of spice and herb extracts to meat products is a popular means of incorporating natural antioxidants. Rosemary, oregano, and sage extracts as well as their combinations with tocopherols and/or ascorbate have been utilized. Rosemary in particular appears to be the most effective (Barbut et al. 1985, Lai et al. 1991); it contains a number of antioxidant compounds called diterpenes, with carnosic acid being the most prevalent and also possessing the greatest antioxidant potency. Because carnosic acid and rosmanol, another antioxidant constituent of rosemary, are odorless, manufacturers can develop spice-based ingredients with reduced flavor impact and increased protection against oxidation, and the subsequent WOF development. Nevertheless, spice companies have been working to develop odorless extracts possessing antioxidant activity for addition to meat systems.

Dietary Antioxidants

Typically, antioxidants are added to a meat product during its formulation, but scientists are experimenting with means to increase the antioxidant capacity of muscle tissue before the animal is harvested. One such approach is by supplementing the feed of domesticated species with dietary antioxidants. Vitamin E's supplementation in the form of α-tocopherol acetate to the feed of animals prior to their slaughter can minimize the potential for WOF development (Buckley et al. 1989, Asghar et al. 1991). This effect has been seen in a number of studies where the basal diets of hogs have been supplemented with increasing levels of vitamin E (O'Sullivan et al. 1997). A progressive increase in the concentration of α-tocopherol has been found in the muscle tissue, mitochondria, and microsomes of hogs. α-Tocopherol migrates into muscle cell membranes where it lies adjacent to highly oxidizable phospholipids; this localization makes α-tocopherol a particularly effective antioxidant. Sensory studies of meat products have shown that vitamin E supplementation can prolong flavor freshness, inhibit WOF development, and positively influence tenderness and juiciness.

Secondary Antioxidants

Another method of reducing oxidation is by the incorporation of chelators into meat products. A chelator or sequestrant is a food additive, which reacts with trace metal ions in foods and forms tightly bound complexes, thereby preventing the metal ion's interaction and subsequent catalytic action on lipid constituents. Typical chelators added to processed meat products are alkaline phosphates. Not only do alkaline phosphates improve the functionality of the meat product in question (e.g., via water-binding capacity, chewiness, and other textural attributes), but they also have the ability to complex with or "chelate" free iron ions in the meat matrix. Furthermore, sodium tripolyphosphate, tetrasodium pyrophosphate and sodium hexametaphosphate have the ability to complex with iron ions that are released from the heme moiety of myoglobin during thermal processing and the subsequent denaturation of meat proteins. Besides these phosphates, citric, ascorbic, and ethylenediaminetetraacetic acids are common food-grade additives, which help to stabilize metal ions by reducing their activity and

ability to act as oxidants. Ascorbic acid, its sodium salt, and isomer (erythorbate) also function synergistically with other antioxidants and added polyphosphates to give protection to meats against oxidative degradation.

Nitrites and Nitrates

Nitrites and nitrates are unique additives to meat products, in that they perform a multifunctional role in the meat matrix. One role is to retard lipid oxidation and WOF development (Pegg and Shahidi 2000). The mechanism by which nitrite prevents or retards the oxidation of meat lipids, and consequently WOF development is still a matter of discussion. From the literature, however, four different mechanisms have been proposed for the antioxidative effect of nitrite in meats: (1) formation of a stable complex between heme pigments and nitrite, thereby preventing the release of iron ions from the porphyrin molecule; (2) stabilization of unsaturated lipids within tissue membranes against oxidation; (3) interaction of nitrite as a metal ion chelator so that it ties up trace metals in meat as well as any liberated nonheme iron from denatured heme pigments; and (4) formation of nitroso and nitrosyl compounds in meat which possesses antioxidative properties by acting as radical scavengers.

Natural and Liquid Smoke

Smoking is another popular meat-preservation technique. Like nitrites, constituents of smoke impart antioxidant properties to meat and meat products. Additionally, incomplete combustion of gases during natural smoke generation from the pyrolysis of hardwood can result in the formation of various nitrogen oxides; some of these function as nitrite does in the curing process. Research has shown that certain smoke flavorings can reduce the occurrence of WOF and extend shelf life when added to fresh, precooked and processed meats. Formulators can add liquid smoke to their products directly by atomizing, dipping, drenching, spraying, or injecting. Smoke ingredients with strong flavors can help mask WOF, whereas flavorless smoke fractions can be employed at low levels in marination systems to reduce the extent of WOF. In some cases, the incorporation of smoke flavors has reduced lipid oxidation by 20 to 30%. It is the phenols present in smoke that act as antioxidants. The flavors also contain certain carbonyls that react with amino groups of meat proteins to inhibit WOF production.

Packaging

Packaging is a physical means to reduce off flavor development in meat and meat products. Because light and the presence of oxygen can accelerate oxidation, eliminating their exposure through packaging technologies will help. Vacuum packaging controls oxygen interactions at the meat surface, and thereby minimizes oxidation. This technique, coupled with nitrogen flushing or modified atmosphere packaging (MAP; e.g., 70% N_2 and 30% CO_2) techniques, can give substantial shelf life to finished products. Other packaging technologies, such as oxygen scavengers, can be added to the package either as stand-alone or be incorporated into the packaging film. An approach used by the packaging industry has been the development of films that act as oxygen barriers; this can help decrease WOF development. In fact, packages of the future may become part of the product itself. Edible films containing natural antioxidants have been examined as a means to control WOF development in cooked meat products. The films were composed of modified cornstarch, soy protein, wheat gluten, corn zein, and natural antioxidants. Some problems encountered, however, had to do with solubility issues; that being, the natural antioxidant was fat-soluble (i.e., hydrophobic) whereas the film itself was hydrophilic in nature. Therefore, the release and delivery of the active components need to be clarified. In some cases, it is more effective to mix the antioxidant directly into the meat formulation, but this does not work for whole-muscle and some restructured meat products.

Flavor Masking

Flavor masking or other methods using flavors that modify the perception of off notes can be effective tools in the fight against oxidative off flavors in meat products. An example of which has already been eluded to is that of smoking. Addition of complementary (i.e., savory) flavors from mild herbs and spices or their extracts can potentiate meat flavors. These inhibit lipid oxidation and help stabilize desirable meaty flavors, which fade during processing and

refrigerated storage. Beer flavoring is another ingredient that helps eliminate flavor problems that occur during the reheating of meats. At low levels, it functions as an enhancer, but at higher concentrations it imparts strong beer flavor notes. Its anti-WOF effect may be a function of the typical yeast notes found in the brew, because yeast-based flavors can improve and enhance flavors, mask bitterness, increase aroma, and provide some protection against oxidation. Still, masking agents that have been developed to cover off flavors, such as metallic notes in high-intensity sweeteners or beany notes in soybean products, have also been proven effective in masking WOF. A good masking agent will not have much flavor on its own when employed at low levels. However, co-processing with other flavors, especially desirable meaty notes, can tailor the mask to specific applications, such as masking WOF. In vacuum-packaged refrigerated products where one is looking for a 60-day shelf life, it holds up well and is stable in retort products. Because the flavor can also mask certain desirable meaty notes if used at too high of a concentration, product testing is necessary to develop the best application level. Merely disguising the oxidative notes is another option. That is, highly flavored systems such as those found in spicy Mexican or East Indian seasonings can distract the consumer from any off notes. The sensations of heat and tanginess dominate consumers' palates. Heavy spicing of meat products is common practice in countries where proper refrigerated storage is an issue.

In developing a flavor system designed to combat WOF, it is important to consider the overall flavor profile of the finished product, along with the prevention of off flavor development. Both temperature and hold time during the cooking process will influence the overall taste. Therefore, to achieve an optimal flavor, spices and flavoring agents should be incorporated at a slightly higher level than would be needed for immediate consumption. Once an appropriate flavor system is developed, it is incorporated into the product by means of a mix-in seasoning, rub, or marinade, depending on the type of product formulated. In a whole-muscle product, marination is recommended to enhance protection and minimize the possibility of off notes developing within the internal area of the product. Because the product's surface is more prone to oxidation than the interior of the meat, topical application would certainly help to minimize the formation of WOF notes.

SUMMARY

Off flavors have always been a problem in lipid-rich foods. In particular, WOF has often been a known adversary in prepared food service entrées. As far as oxidation is concerned, it is not possible to prevent the decomposition of hydroperoxides once they are formed; therefore, the goal is to prevent or retard their formation as long as possible. This may be achieved to some degree by the incorporation of synthetic or natural antioxidants and chelators to foods (i.e., extended shelf life and reduced off flavor development). From the point of view of taints, using proper packaging to prevent the absorption of chemical and microbial contaminants from the environment will be advantageous.

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18
Land Animal Products

Terri D. Boylston

Contributors to Flavor
- Volatile Flavor Compounds
  - Maillard Reaction
  - Lipid Oxidation
  - Flavors Formed Through the Interaction Between Lipid Oxidation and Maillard Reactions
- Taste Compounds

Sensory Evaluation of Meat Flavor
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  - Sex
  - Diet
- Postslaughter Processing
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  - Cooking
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  - Irradiation
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Challenges in Meat Flavor Research
Acknowledgment
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Extensive research over the past years has identified over 1,000 flavor compounds in meats. However, a single compound or group of compounds responsible for "meaty flavor" has not and perhaps never will be identified due to the overall complexity of meat flavor. Meat flavor is dependent on the pool of flavor precursors in the meat tissue and the chemical reactions that occur during processing. Processing and subsequent storage contribute to the development of the characteristic flavors of meats. Because the precise flavor precursors vary between and within species, beef, pork, lamb, and poultry each have distinctive flavor characteristics. In this chapter, the major contributors to meat flavor, from an instrumental and sensory point of view, and factors that affect the final flavor quality of the meat when it is consumed will be discussed.

CONTRIBUTORS TO FLAVOR

Flavor is composed of aroma or volatile flavor compounds, which are detected by specialized cells of the olfactory epithelium of the nasal cavity and nonvolatile taste compounds, which are detected by the taste buds on the tongue and back of the oral cavity. The integration of aroma and taste is critical to the overall perception of meat flavor.

VOLATILE FLAVOR COMPOUNDS

Numerous complex reactions occur during the cooking of meat to contribute to flavor development. The meaty flavor of muscle foods is attributed to the lean portion of the muscle, which is similar between the different species (Wasserman 1979). The unique flavor characteristics that distinguish the different species of land animals from each other is attributed to differences in the fatty acid composition of these muscles (Hornstein and Crowe 1960, Sink 1979, Wasserman 1979) and the lipid oxidation reactions that occur during cooking (Shahidi and others 1986, Mottram 1998). The volatile flavor compounds that contribute to the desirable meaty and browned flavors are present at parts per billion levels, while grassy, cardboardy, and painty flavors, which contribute to undesirable flavors are present at parts per million levels (Vercelloti and others 1987). The differences in

the concentrations, thresholds, and aroma qualities of these compounds have a significant impact on the flavor characteristics and desirability of meat flavor.

Maillard Reaction

The Maillard reaction is a nonenzymatic browning reaction involving carbonyl and amine compounds. Through a series of complex reactions, aromas with roasted, toasted, and meaty flavor characteristics and polymerized brown pigments are formed to contribute to the characteristic aroma of cooked meat. Chapter 20 discusses the chemistry of the Maillard reaction and its contribution to flavor in foods in more detail.

Heterocyclic and sulfur-containing compounds formed through the Maillard reaction are important contributors to desirable meaty flavors (Figure 18.1). The flavor descriptions of the volatile flavor compounds that are formed through the Maillard reaction are presented in Table 18.1. Although these compounds are present at parts per billion levels, they are

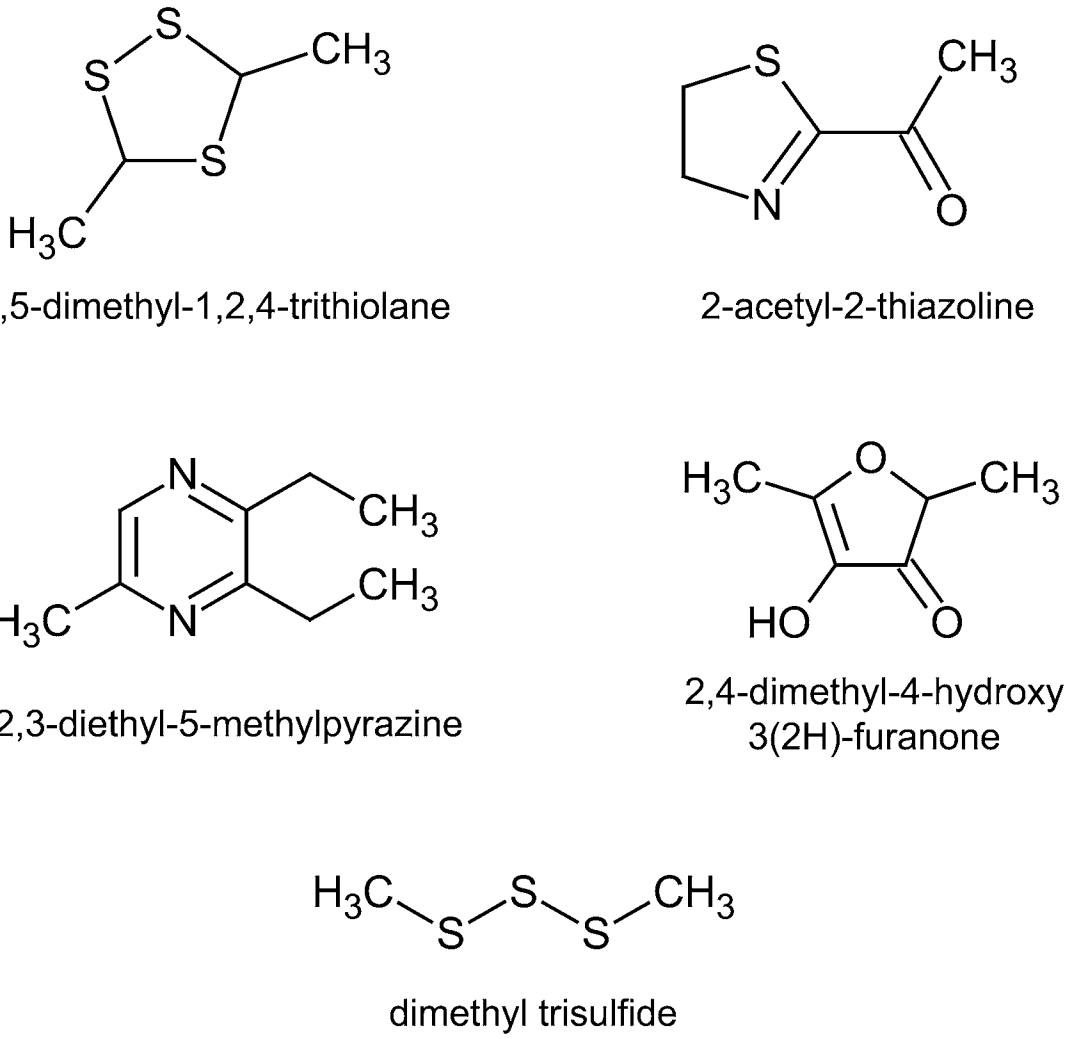

Figure 18.1. Examples of heterocyclic and sulfur-containing compounds found in meats.

Table 18.1. Flavor characteristics of volatile flavor compounds formed through Maillard reactions.

Volatile compound	Flavor description	Reference
Aliphatic sulfur compounds		
Hydrogen sulfide	boiled or rotten eggs	E
Methanethiol	rotten eggs, meat or fish, cheesy, cooked cabbage	A E
Dimethyldisulfide	strong onion, garlic-like, pungent	B
Dimethyl trisulfide	roasty, rotting green vegetables, cabbage	A B
Methional	cooked potatoes, meat broth	B C
3-(Methylthio) propanal	cooked potato	D
3,5-Dimethyl-1,2,4-trithiolane	putrid	D
Heterocyclic sulfur compounds		
4,5-Dimethylthiazole	smoky, roasty, nutty	B
2-Acetyl-2-thiazoline	roasty, floral	A C
2,4,6-Trimethyltetrahydro-1,3, 5-thiadiazine	popcorn, burnt	D
5,6-Dihydro-2,4,6-trimethyl-4H, 1,3,5-dithiazine	roasty, musty	D
2-Methyl-3-(methylthio)furan	meaty (not roasted)	A
2-Methyl-3-furanthiol	meaty, sweet	D
2-Furfuryl thiol	roasty, coffee	D
Heterocyclic nitrogen compounds		
Methylpyrazine	green, fragrant	A
2,3-Dimethylpyrazine	meaty	A
2,5 (and/or 2,6)-Dimethylpyraine	meaty roast, green	A
Trimethylpyrazine	roasty, earthy, caramel	A C
Tetramethylpyrazine	walnuts, green	A
2-Ethyl-3-methylpyrazine	green, floral, fresh nuts	A
2-Ethyl-5-methylpyrazine	fruity, sweet, toffee, pungent	A B
2-Ethyl-3,5-dimethylpyrazine	roasty, earthy, nutty	C D
2-Ethyl-3,6-dimethylpyrazine	burnt, pungent, roasty, nutty	A B
2,3-Diethyl-5-methylpyrazine	meaty, roasty, fragrant, sweet, earthy, crushed leaves	A B C
2,5-Diethyl-3-methylpyrazine	sweet, caramel	A
6,7-Dihydro-(5H)cyclopentapyrazine	green, fatty	A
2-acetyl-1-pyrroline	roasty, overheated meat-like, cured ham-like	C E
Heterocyclic oxygen compounds		
2-Furanmethanol	meaty roast	A
4-Hydroxy-5-methyl-3(2H)-furanone	caramel, fried chicken	D
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	roasted almonds, sweet, caramel	B C D
3-Hydroxy-4,5-dimethyl-2(5H)-furanone	seasoning-like	C
4,5-Dihydro-5-propyl-2(3H)-furanone	fruity, fatty, sweet, roasty	B
4,5-Dihydro-5-butyl-2(3H)-furanone	caramel-like, pungent, roasty, fruity	B

[a]References for aroma characterization: A: MacLeod and Ames 1986; B: Specht and Baltes 1994; C: Kerler and Grosch 1996; D: Farkaš and others 1997; E: Carrapiso and others 2002.

critical to the desirable flavors of meat because of their low aroma thresholds (Min and others 1979, MacLeod and Seyyedain-Ardebili 1981, Hsu and others 1982, Hartman and others 1983, Galt and MacLeod 1984, MacLeod and Ames 1986, St. Angelo and others 1987, Shahidi 1989, Ramarathnam and others 1993, Mottram 1998). The importance of sulfur-containing volatile flavor compounds to the roasted meaty flavor characteristics has been demonstrated in model systems consisting of cysteine, cystine, thiamin, reducing sugars, and other meat flavor precursors (Güntert and others 1990, Werkhoff and others 1990, Mottram and Nobrega 2002). The flavor quality of the sulfur-containing compounds is dependent on the concentration, with pleasant meaty aromas exhibited at low concentrations and objectionable aromas exhibited at high concentrations (Shahidi 1989).

The addition of Maillard reaction products, formed through reactions between sugars and amino acids, have been shown to function as a meat flavor enhancer and as an antioxidant when added to meat products (Lingnert and Eriksson 1981, Bailey and Um 1992). The basic amino acids have been shown to form the most effective antioxidants and the choice of amino acid rather than sugar is most critical in the formation of Maillard reaction products with antioxidant activity (Lingnert and Eriksson 1981).

Lipid Oxidation

Lipid oxidation is the major cause of loss of desirable meat flavor. Tims and Watts, in 1958, first described the development of off flavors in cooked meats following refrigerated storage and coined the term "warmed-over flavor" (WOF). Research since then has shown that WOF can develop whenever the muscle tissue is disrupted by processes such as grinding, restructuring, or cooking of the muscle (Greene 1969, Sato and Hegarty 1971). A detailed discussion of the mechanism of WOF formation and processing techniques, including use of antioxidants, to minimize WOF formation, are discussed in Chapter 17.

The unsaturated fatty acids associated with the phospholipids in the muscle tissue are the major precursors of the flavor compounds associated with WOF. The oxidation of these lipids, which is catalyzed by iron, results in the formation of a complex mixture of aldehyes, ketones, and alcohols (Younathan and Watts 1960, Sato and Hegarty 1971, Igene and others 1979). These lipid oxidation compounds are present at parts per million concentrations and are the predominant peaks in the gas chromatogram of meat samples (St. Angelo and others 1987). Pentanal, hexanal, heptanal, 2,3-octanedione, 1-octen-3-ol, nonanal, and 2-pentylfuran are among the major lipid oxidation products identified in meats as contributors to WOF (Figure 18.2). The carbonyl compounds have the greatest impact on flavor due to their high rate of formation during lipid oxidation and low flavor threshold in comparison to the other oxidation products (St. Angelo and others 1987, Drumm and Spanier 1991, Decker and Hultin 1992). Table 18.2 summarizes the aroma character-

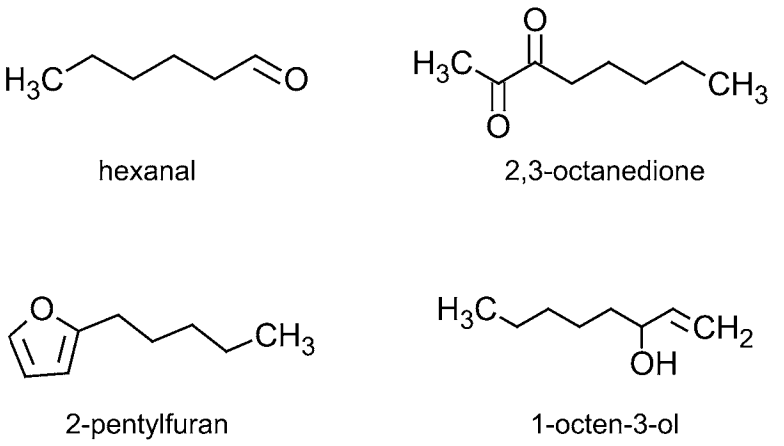

Figure 18.2. Examples of lipid oxidation products found in meats.

Table 18.2. Flavor characteristics of volatile flavor compounds formed through lipid oxidation reactions.

Volatile compound	Flavor description	Reference[a]
Aldehydes		
2-Methylpropanal	toasted, fruity, pungent	E
3-Methylbutanal	green, burnt, caramel, malty, pungent, fruity, toasted	A B C E
2-Methylbutanal	sweet, floral, fruity, roasty, malty	B C
Hexanal	green, grassy, fatty	A B C
Heptanal	fruity, fatty, sweet, caramel, buttery	A B
Octanal	soapy, sweet, fruity, nutty	A C
Nonanal	fragrant, sweet, fatty, green, tallowy	B C
trans-2-Nonenal	pungent, fragrant, fruity, roasty, tallowy, fatty	B C
cis-3-Nonenal	fatty	C
cis-2-Nonenal	soapy	C
trans-2, *cis*-6-Nonadienal	cucumber-like	C
trans, trans-2,4-Nonadienal	fatty, soapy	C
Decanal	sweet, fruity, roasty	B
trans-2-Decenal	pungent, green, fragrant, fruity	B
trans-2, *trans*-4-Decadienal	sweet, fatty, pungent, fried potato, chickeny	B C D
trans-2-Undecenal	sweet, fruity, fatty	B
trans-2-Dodecenal	sweet, fruity, roasty, pungent	B
Benzaldehyde	nutty, almonds	A
Phenylacetaldehyde	sweet, fruity, flowery, honey-like	B C
Ketones		
2,3-Butanedione	sweet, buttery, caramel	A B
2,3-Pentanedione	buttery, lemon-like, sweet fruity, caramel	A B
1-Penten-3-one	rotten, fruity	E
Octan-2-one	caramel	A
1-Octen-3-one	mushroom-like	B C D
1-Octadien-3-one	green, geranium-like	C
Nonan-2-one	boiled milk, slightly burnt	A
3-Methylcyclopentanone	meaty, fatty, fruity, caramel	A
Alcohols		
Hexanol	fragrant	A
Heptanol	sweet, fruity, butterscotch	A
1-Octen-3-ol	green	A
Acids		
Butanoic acid	cheese-like, rancid	A

[a]References for aroma characterization: A: MacLeod and Ames 1986; B: Specht and Baltes 1994; C: Kerler and Grosch 1996; D: Farkaš and others 1997; E: Carrapiso and others 2002.

istics of volatile flavor compounds formed through lipid oxidation reactions.

Thiobarbituric acid reactive substances (TBARS) and hexanal contents are frequently used as indicators of lipid oxidation in meats. Thiobarbituric acid reacts with carbonyl compounds formed through lipid oxidation to form a colored product. Although this assay lacks specificity, it is widely used to monitor lipid oxidation reactions, especially in meats. Hexanal, which is the major product of linoleic acid oxidation, is present in the highest intensity and is formed at significantly higher rates than the other

oxidation products (St. Angelo and others 1987, Drumm and Spanier 1991, St. Angelo and others 1992, Decker and Hultin 1992, Kerler and Grosch 1996). These chemical indicators have been shown to be positively correlated with the intensity of sensory descriptors of undesirable flavor notes, such as cardboard, painty, warmed-over, oxidized, sour, and bitter and negatively correlated with desirable flavor notes, such as cooked beef/brothy, meaty, browned/caramel, serum, and sweet (Hwang and others 1990; St. Angelo and others 1987, 1992). However, because the rates of formation for the individual oxidation products are different and independent of the initial concentration, the use of a single compound, such as hexanal, does not adequately characterize the overall flavor (Ullrich and Grosch 1987, Drumm and Spanier 1991).

Flavors Formed Through the Interaction Between Lipid Oxidation and Maillard Reactions

The interaction of carbonyl compounds generated through lipid oxidation reactions and Maillard reaction products also contributes to the formation of heterocyclic compounds with meaty flavor characteristics. Alkylthiazoles, alkylthiophenes, alkylpyridines, alkylpyrazines, and trithiolanes have been proposed to form through reactions between aliphatic aldehydes and hydroxyketones formed from lipid oxidation products and ammonia and hydrogen sulfide from the decomposition of amino acids (Farmer and others 1989, Farmer and Mottram 1990, Mottram 1998, Elmore and others 1999). Phospholipids are critical to the formation of a pronounced "cooked meat, beefy" aroma, as shown in comparing model systems consisting of cysteine + ribose with and without phospholipids (Farmer and others 1989, Farmer and Mottram 1990). In beef, increases in polyunsaturated fatty acid content of the muscle were correlated with increases in content of alkylthiophenes and ethylthiapyran (Elmore and others 1999). The greater contribution of phospholipids, rather than triglycerides, in the formation of meaty aromas was attributed to the higher degree of unsaturation and higher water solubility of the phospholipids (Mottram and Edwards 1983, Farmer and Mottram 1990).

Taste Compounds

Meat constituents that contribute to the basic tastes include inorganic salts (salty), hypoxanthine (bitter), sugars (sweet), and organic acids (sour). In addition, the amino acids contribute sweet, sour, bitter, and salty tastes to meat. Hydrophilic amino acids, such as hydroxyproline, glycine, serine, and threonine, contribute sweet tastes. Hydrophobic amino acids, such as histidine, arginine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan, contribute bitter tastes. Aspartic acid, glutamic acid, and asparagine are noted for their sour taste characteristics, and the sodium salts of glutamic and aspartic acids have salty taste characteristics (MacLeod and Seyyedain-Ardebili 1981; Spanier and others 1988, 1990). Sour taste characteristics in meat have also been attributed to lactic acid formed from glycogen through anaerobic glycolysis (Larick and Turner 1990). Proteolytic activity during postmortem aging produces peptides that can increase the overall taste intensity and palatability of the meat (MacLeod and Seyyedain-Ardebili 1981). Degradation products of ribonucleotides, including inosine 5′-monophosphate and guanosine 5′-monophosphate also function as flavor potentiators to enhance meaty flavor and contribute a umami taste characteristic (Nagodawithana 1995).

SENSORY EVALUATION OF MEAT FLAVOR

Sensory evaluation provides the human perception aspect of meat flavors. During the past 20 years, there has been a substantial effort to develop lexicons to describe beef (Johnson and Civille 1986, Lynch and others 1986), lamb (St. Angelo and others 1991), pork (Jeremiah and others 1990, Byrne and others 1999a), cured ham (Flores and others 1997), and poultry (Lyon 1987, Chambers and others 1992, Byrne and others 1999b) flavor. The basic tastes, sweet, sour, salty, and bitter, as well as universal flavor descriptors, such as meaty, serum, browned, painty, and cardboard are common across all species. In addition to these universal flavor descriptors, descriptors and definitions that are unique to each species, such as beefy, gamey/muttony, porky, and chickeny/poultry provide species-specific descriptors (Bett 1993, St. Angelo and others 1991, Imafidon and Spanier 1994).

Principal factor analysis of sensory and instrumental measures of flavor in beef patties has shown the sensory attributes sweet, cooked beef/brothy, and browned/caramel to be associated with the desirable freshly cooked patties. On the other hand, the

sensory attributes, painty, sour, bitter, and cardboard, were associated with cooked and stored patties that did not receive treatments to reduce lipid oxidation and had undesirable flavor characteristics. High contents of the volatile flavor compounds, hexanal, nonanal, 2,3-octanedione, propanol, pentanal, decanol, and butanol, and high TBARS values were also associated with those patties with undesirable, oxidized flavor characteristics (Spanier and others 1992a, 1992b).

Gas chromatography-olfactometry (GC-O) is a useful tool to establish the relationship between flavor compounds detected by instrumental techniques and sensory evaluation. A panelist sniffs the volatile compounds using a sniffer apparatus as these compounds are eluted from the gas chromatograph and provides descriptive terms and intensities for the individual flavor compounds. Due to differences in the thresholds of volatile flavor compounds, the use of GC-O allows food scientists to determine the impact of individual compounds on the flavor of the food. Several researchers have used GC-O and other techniques to describe the flavor characteristics of the individual compounds that contribute to meat flavor, as shown in Tables 18.1 and 18.2 (MacLeod and Ames 1986, Specht and Baltes 1994, Kerler and Grosch 1996, Farkaš and others 1997, Carrapiso and others 2002).

FACTORS INFLUENCING FLAVOR CHARACTERISTICS OF MEATS

Species, breed, sex, and diet have a significant impact on the fatty acid composition of the meat. Following slaughter, processing and storage conditions contribute to the development of desirable and undesirable flavors through reactions involving the lipids and other constituents of the muscle tissue.

Preslaughter Factors

Species

The basic precursors for meat flavor, that is the proteins, sugars, and lipids are similar across all land animal species. Carbonyl compounds and hydrocarbons are most often recognized for flavor differences between species (Ramarathnam and others 1993). The degree of unsaturation of the lipids in muscle tissues varies greatly from species to species. Because the susceptibility of fatty acids to oxidation increases with the degree of unsaturation, the rate of lipid oxidation reactions and the flavors that are formed through these reactions are species dependent.

Shahidi and others (1986) reviewed meat flavor and identified the species from which specific volatile flavor compounds had been isolated. Many of these compounds were identified in several different species. However, failure to identify a specific compound may be attributed to differences in processing treatments and methods for isolation of the volatile flavor compounds and may not be necessarily due to species differences.

Breed

A majority of the research focused on the effects of breed on flavor characteristics has been conducted in beef animals. Lean muscle from bison has a higher polyunsaturated fatty acid content than lean muscle from either Hereford or Brahman cattle. The more intense off flavors and aftertastes, described as ammonia, bitter, gamey, liverish, and sour, detected in bison was attributed to differences in the fatty acid composition between these three beef species (Larick and others 1989). A comparison of flavor characteristics of Pirenaica (beef type) and Friesian (dairy type) breeds of beef from the Mediterranean area resulted in the beef from the Friesian breed as having a higher intensity of fatty flavor and aftertaste, and a higher content of aliphatic hydrocarbons, but lower content of butanal and dimethylsulfide. These breeds are characterized as having low-fat carcasses, although the Friesian breed has a higher content of saturated intramuscular fat (Gorraiz and others 2002). Higher contents of hexanal and other lipid oxidation products in Japanese Wagyu and American Wagyu beef cooked and stored for 3 days, in comparison to beef from Angus or Longhorn breeds, was attributed to significantly higher total lipid and unsaturated fatty acid contents in the Wagyu breeds (Boylston and others 1996). Genetic control of animal lipid metabolism affects the fatty acid composition of the muscle tissue, and thus, contributes to the observed differences in flavor characteristics between breeds (Larick and others 1989).

Sex

Beef from bulls is characterized as having a stronger livery and bloody flavor, while beef from heifers has

a more common meaty flavor. Contents of 2-propanone and dimethylsulfide were significantly higher in the beef from the bulls (Gorraiz and others 2002). Heifers typically have higher triglyceride and lower phospholipids percentages in the intramuscular fat than in the fat of bulls, which may be attributed to the role of sex hormones and the genetic control of animal development on lipid composition (Sink 1979).

Diet

Nutritional awareness of the importance of decreases in saturated fatty acids and increases in polyunsaturated fatty acids in the diet has led to an interest in manipulating the fatty acid composition of meat tissue through the animals' diet. In addition to changing the nutritional value, changes in the diet of land animals have an impact on the flavor of the cooked meat. The fatty acid content of the diet has a more direct impact on the fatty acid content of the muscle tissue in pork and poultry than in beef and lamb due to the biohydrogenation reactions in the rumen (Wood and others 2004).

The supplementation of pig diets with polyunsaturated fatty acids, such as safflower oil (Larick and others 1992), canola or flaxseed (Shackelford and others 1990), and rapeseed oil and fish oil (Leskanich and others 1997) resulted in increases in the content of hexanal and other lipid oxidation products. The formation of off flavors is further increased if the meat undergoes oxidative stress during processing (Shackelford and others 1990). Similarly, in broilers, the fatty acid composition of dietary oils is reflected in the fatty acid composition of the neutral lipid and phospholipid fractions (Lin and others 1989). The higher content of polyunsaturated fatty acids in the tissues of pork and poultry contributes to greater susceptibility of oxidation reactions and off flavor formation during refrigerated storage following cooking.

Beef from grain-fed cattle is described as having a higher intensity of roasted beef, beefy, blood-like, tallowy, cooked beef fat, and sour flavors and a lower intensity of sweet, grassy, and gamey flavors than beef from forage-fed cattle (Larick and Turner 1990, McMillin and others 1991, Maruri and Larick 1992). Grain-fed beef have higher percentages of linoleic acid and lower percentages of oleic and linolenic acids than pasture-fed beef (Yang and others 2002). The intensity of the grassy and gamey flavors is correlated with the content of 2,3-octanedione and several diterpenoid compounds in beef steaks (Larick and others 1987, Maruri and Larick 1992). Lactones have been shown to be present in higher concentrations in grain-fed cattle and are positively correlated with roasted and blood-like flavor characteristics (Maruri and Larick 1992). The higher glycogen content of corn-fed cattle could contribute to higher lactic acid contents and the increased sour taste (Larick and Turner 1990). Steaks with increased polyunsaturated fatty acid contents, due to the feeding of different supplemental fats, result in higher contents of aldehydes and alcohols formed through lipid oxidation reactions (Elmore and others 1999).

The supplementation of diets with α-tocopherol results in an increased content of the antioxidant in the membranes and has been shown to be effective in protecting unsaturated phospholipids from oxidation. The presence of tocopherols in the tissues stabilizes the membrane lipids and minimizes lipid oxidation and off flavor development that occurs during refrigerated and frozen storage of beef, pork, and poultry (Lin and others 1989; Asghar and others 1990, 1991; Yang and others 2002).

Postslaughter Processing

Aging

Aging of beef contributes to the development of desirable flavor characteristics through proteolytic, lipolytic, and other enzyme reactions, resulting in increases in the sugars, peptides and free amino acids, and nucleotides (Spanier and others 1990). Proteolytic activity has a significant effect on subsequent meat quality. Top round beef aged for up to 4 days was judged to have the optimal flavor by a sensory evaluation panel (Spanier and others 1997). The concentration of peptides in the 3–10 kilodalton (kDalton) molecular weight range increased with aging time and was shown to be correlated with taste intensity in beef and pork (Claeys and others 2004). Amino acids generated through the proteolytic reactions can directly function as flavor compounds or react with sugars and other carbonyl compounds in the meat through Maillard reactions to produce roasted and meaty flavors. Lipid oxidation, however, has only a minor impact on flavor during the aging process (Spanier and others 1997).

Cooking

Raw meat has very little flavor, but contains a number of nonvolatile flavor precursors, including amino acids, peptides, reducing sugars, and vitamins. It is only through the cooking process that the cooked, roasted, and meaty flavors associated with meats are developed through the Maillard reaction and other heat-dependent reactions. Cooking methods, such as boiling, roasting, frying, and pressure-cooking, vary in their temperature of heating and moisture and thus affect the interactions between carbonyl and amine compounds to form Maillard reaction products and influence overall meat flavor.

Sensory evaluation has shown an increased intensity of browned and species-specific flavor notes and a decreased intensity of bloody-serumy, metallic, and sour flavor notes as the final internal temperature of the meat increased (Bowers and others 1987, Heymann and others 1990). Higher cooking temperatures, longer cooking times, or cooking treatments that increase surface browning increase the formation of Maillard reaction products. These products contribute to browned and meaty flavors. In addition, the Maillard reaction products (MRP) have been shown to have antioxidative properties and thus, provide additional benefits in minimizing off flavor formation during subsequent storage (Huang and Greene 1978, Gros and others 1986, Specht and Baltes 1994). However, lipid autoxidation reactions are also enhanced at elevated temperatures due to enhanced myoglobin degradation and release of free iron (Spanier and others 1990, 1992b). Above approximately 70°C, the Maillard reaction predominates, with MRP antioxidants contributing to a reduction in lipid oxidation and off flavor formation (Spanier and others 1990).

Treatments to Minimize Lipid Oxidation Reactions

Processing of meats through mechanical deboning, grinding, restructuring, or cooking disrupts the tissue membranes and allows the catalysts of lipid oxidation to react with the unsaturated fatty acids to initiate lipid oxidation reactions. The effects of these processing treatments on lipid oxidation reactions have been shown as increases in the contents of TBARS, hexanal, and other lipid oxidation products and increases in the intensity of cardboard and painty flavors assessed by sensory evaluation panels (Gray and others 1996). The addition of synthetic and natural antioxidants during the processing of meat products has been shown to be effective in reducing the lipid oxidation reactions and maintaining desirable meat quality. Antioxidants may function as free radical terminators or free radical preventers. Phenolic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butyl hydroquinone (TBHQ), and tocopherol, are widely used in meat products to inhibit oxidation by donating hydrogens to the free radicals formed in lipid oxidations. Other antioxidants, such as ethylenediamine tetraacetic acid (EDTA), citric acid, and phosphates bind metal cations to inhibit the initiation of the lipid oxidation reaction (Shahidi and others 1987, Ladikos and Lougovois 1990).

Consumer interest in increasing the use of natural rather than synthetic additives in foods has led to interest in the identification of natural antioxidants. The antioxidant activity of numerous plant extracts, including rosemary, sage, tea catechins, and rice hull extracts (Nissen and others 2000; McCarthy and others 2001a, 2001b; Kim and others 2003) have been studied in meat products. The antioxidant activity of these plant extracts is attributed to the ability of the polyphenolic compounds present in these plant extracts to quench free radicals (Foti and others 1996). Carnosine, a naturally occurring skeletal muscle dipeptide, functions as a chelator, free radical scavenger, and hydrogen donor in the aqueous phase of the muscle tissue to inhibit lipid oxidation (Decker and Crum 1991, 1993; Decker and Faraji 1990). Honey has also been shown to be an effective antioxidant in processed meats. The antioxidant activity of honey is attributed to the presence of reducing sugars, which can participate in Maillard reactions, phenolic compounds, and other natural antioxidants (McKibben and Engeseth 2002).

Because oxygen is critical to the lipid oxidation reactions and the development of warmed-over flavor, packaging treatments that exclude oxygen from the environment have a significant impact on the flavor quality of meat during storage. Vacuum packaging has been shown to effectively reduce warmed-over flavor formation and maintain desirable meaty flavor characteristics in raw ground beef (Lynch and others 1986), cooked beef (McDaniel and others 1984; Hwang and others 1990; Spanier and others 1992a, 1992b), and cooked turkey and pork (Nolan

and others 1989) products. Hot vacuum packaging following cooking effectively excludes oxygen from the packaging system so that prooxidants, fat content, and fatty acid composition have no effect on lipid oxidation during storage (Ahn and others 1992).

Curing

Curing of meats with sodium nitrite was originally developed as a means of preservation. However, the nitrite also has a significant impact on the meat flavor. The addition of nitrite (50–500 mg/kg) to meat contributed to an increase in ham aroma and flavor and a decrease in off odors and off flavors, as detected by a sensory evaluation panel (MacDonald and others 1980a). However, in a comparison of the volatile flavor profiles of cured and uncured meats, the curing process contributed to a significant decrease in the concentration of hexanal and other carbonyl compounds, but did not result in the formation of new flavor compounds that would contribute to the cured meat flavor. Nitrite stabilizes the microsomal lipids and heme pigments to reduce lipid oxidation reactions and the resulting off flavor formation (MacDonald and others 1980b, Shahidi and others 1987, Ramarathnam and others 1991). Shahidi (1989) suggested that the flavor of nitrite-cured meats reflects the natural flavor of the meat without the flavor compounds contributed by the lipid oxidation reactions.

Irradiation

Irradiation is an effective processing method to improve the safety of meats through decreasing the load of spoilage and pathogenic microorganisms (Lefebvre and others 1992, Fu and others 1995). Irradiation doses of 1 to 10 kilogray (kGy) have been approved by the United States Department of Agriculture for raw meat and poultry products (USDA 1999). However, the irradiation process produces reactive hydroxyl radicals that react with proteins and lipids in the meat to produce off flavors. The formation of dimethyl disulfide, dimethyl sulfide, and other volatile sulfur compounds from the radiolysis of sulfur-containing amino acids in irradiated meats contributes to an undesirable irradiation off odor, described as rotten egg, sweet, bloody, cooked meat, or barbecued corn, burnt, sulfur, metallic, alcohol, or acidic (Ahn and others 2000, Lee and Ahn 2003, Zhu and others 2004). The presence of oxygen during irradiation accelerates lipid oxidation reactions through the formation of free radicals and hydroperoxides, breakdown of hydroperoxides, and destruction of antioxidants (Nawar 1996). These degradative oxidation reactions and the subsequent off flavor formation are minimized when meats are vacuum packaged prior to irradiation (Luchsinger and others 1996, Ahn and others 1998). The addition of antioxidants to the meats has also been shown to be effective in reducing the formation of sulfur compounds and lipid oxidation products through the ability of the antioxidants to scavenge and quench free radicals (Nam and others 2002, Nam and Ahn 2003, Lee and Ahn 2003).

Storage

Storage has an adverse effect on the flavor quality of cooked meat products. In cooked beef patties, following 4 days of refrigerated storage, the intensity of beefy and brothy sensory attributes decreased, while the intensity of painty, sour, and bitter sensory attributes increased. The increased intensity of the painty attribute is attributed to lipid oxidation reactions and correlates with increases in hexanal and TBARS contents. During storage of cooked meat, the content of the hydrophilic peptide residues decreases and the content of the hydrophobic peptide residues remain unchanged. These changes in the distribution of peptides in the cooked and stored meat resulted in an increase in sour and bitter sensory attributes and a decrease in cooked beef and brothy flavor attributes (Spanier and others 1990). Antioxidant and vacuum treatments designed to reduce lipid oxidation and off flavor formation also minimize losses in the desirable meaty attributes (Spanier and others 1992b). During the storage of beef patties, contents of heterocyclic compounds, which contribute to desirable meaty characteristics, were stable, while contents of lipid oxidation products and aliphatic and cyclic sulfur compounds increased. The increases in the lipid oxidation and sulfur-containing compounds were attributed to free-radical degradation reactions (Drumm and Spanier 1991). Because the flavor characteristics of the sulfur-compounds are dependent on concentration (Shahidi 1989), these sulfur compounds may contribute undesirable flavor characteristics following extended refrigerated storage. The decrease in intensity of desirable flavor notes during refrigerated storage may be attributed to masking of the desirable flavor notes by

the increased content of undesirable flavor compounds, rather than a degradation of desirable flavor compounds (Drumm and Spanier 1991).

CHALLENGES IN MEAT FLAVOR RESEARCH

Flavor has an important impact on the consumer acceptability of all foods and meat from land animals is no exception. Food scientists will continue their research to identify and characterize those compounds that impact meat flavor and further develop production and processing methods to enhance the desirable flavor characteristics of meats.

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Marine Animal and Plant Products

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INTRODUCTION

The marine environment is an extremely large geographical area, comprising about 71% of the surface of this planet. This gigantic proportion incorporates an immense diversity of plants and animals, which thrive in this environment and constitute an enormous amount of the food chain, catering to the human population with a great variety of seafood, nutrients, pharmaceuticals, and other important chemical compounds. The entire system of both marine plants and animals is quite intricate in its nature and is subjected to a very complex survival under a habitat full of variations and limitations when compared with those from the land resources. However, marine plants are absolutely essential for the survival and existence of animals living in this environment. Both produce organic compounds that regulate and control the activity of potential predators, pathogens and other stresses caused by the lack of solar radiation, oxygen and other inherent deficiencies of physicochemical parameters.

Marine animals are generally classified as invertebrates and vertebrates. Invertebrates possess a shell or a hard exoskeleton and lack a backbone, which is a basic characteristic of vertebrates. Some of the invertebrates are sponges, jellyfish, corals, hydroids, clams, snails, comb jellies, crabs, shrimps, lobsters, copepods, squids, octopi, starfish, brittle stars, and marine worms. Vertebrates possess a backbone and represent thousands of fish species such as bony fish, snappers, hagfish, angelfish, sharks, dolphins, sea turtles, alligators, whales, etc. Moreover, among each of these animals, there may be hundreds of species varying in their flavor and nutritional characteristics. It has been estimated that the total number of fish species lies between 15,000 to 40,000, most of which are captured at least to some extent (de Groot 1995). Some fish are considered dangerous and should not to be touched (jellyfish, corals, and hydroids) as these are known to contain toxic poisonous substances.

Marine plants have evolved unique biochemical processes and structures in adapting to their chemical, physical, and biological environments. Plants usually grow only in the depths to which light penetrates the water. Some coral produces certain amino acids, which absorb solar radiation and serve as a

sunscreen that consequently protects algae and their hosts from sunburn. The most common marine plants are algae, which form the basis of the marine food chain serving as potential sources of pharmaceuticals, agricultural chemicals, foods and food ingredients, industrial chemical feedstock, and other useful products.

Several reviews on flavor generation in seafood have been published (Josephson and Lindsay 1985, Heath and Reineccius 1986, Lindsay 1990, Josephson 1991, Arganosa and Flick Jr. 1992, Hsieh 1994, Lindsay 1994, Kawai 1996). Initially, Josephson and Lindsay (1985) presented a mechanism for enzymatic degradation of eicopentaenoic acid (EPA) leading to the biosynthesis of volatile compounds such as 1-penten-3-ol, (*E*)2-hexenal, (*Z*)3-hexenal, various nonadienals, (*Z*)1,5-octadien-3-one and alcohols such as (*Z*)1,5-octadien-3-ol and (*Z*)3-hexen-1-ol. Later, an excellent review was published by Josephson (1991) who described precursor-based detailed mechanisms for the formation of volatile compounds such as lipid-derived aroma compounds, which included enzyme-catalyzed conversion and autoxidative deterioration of polyunsaturated fatty acids, carotenoids, and other chemical constituents (sulfur- and nitrogen-containing compounds) serving as precursors for various chemical reactions or microbial degradation leading to the formation of volatiles (dimethyl sulfide and trimethylamine), which contributed to aroma or off flavor in seafood. Several volatile carbonyl compounds, enzymatically derived from the lipid moiety, contributed to fresh, plant-like aromas in freshly harvested fish. Furthermore, these reviews also detailed the change in volatile profiles during processing such as salting, pickling, drying, frying, boiling, baking, and smoking of seafood. The more abundant volatiles generated during lipid autoxidation were hexanal and various isomers of 2,4-heptadienals, 3,5-octadien-2-ones, 2,4-decadienals, and 2,4,7-decatrienals. Some character-impact compounds formed during thermal processing of seafood were identified such as isomers (*Z,Z,Z* and *E,Z,Z*) of 5,8,11-tetradecatrien-2-one and dimethyl-2-phenylethylamine in boiled shrimps, dimethyl sulfide in stewed clams and oysters, and 2-methyl-3-furanthiol in canned tuna fish. It was also reported that thermally processed flavors could be generated from nonvolatile constituents such as amino acids, nucleotides, sugars, oxygenated lipids, and partly due to the Maillard reactions between sugars chiefly glucose and ribose and free amino acids such as serine, alanine, and lysine, commonly found in the flesh of many fish species.

The reviews or texts cited above were published before 1997, and since then, in spite of the vast amount of literature published over the last decade, there has been very little data assembled in the form of reviews dealing specifically on marine flavors. Moreover, the topic of flavor resources from marine plants and animals is very broad in nature and hence it is beyond the scope of this chapter to delve exhaustively into details. It is therefore proposed to present initially a characterization of marine environment and aquaculture systems from the flavor standpoint, the potential it represents from its plants and animals, the state of knowledge generated on the flavor aspects of these organisms emphasizing on new data generated over the past decade, concentrating mainly on the identification of volatile aroma compounds, and finally, present the development of products based on potential marine resources.

MARINE ENVIRONMENT VERSUS AQUACULTURE SYSTEMS

The marine environment constitutes a basic medium of saltwater and is composed of, on average, 96.5% water and 3.5% dissolved substances mainly salts, which include ions of chloride (55.04%), sodium (30.61%), sulfate (7.68%), magnesium (3.69%), calcium (1.16%), and potassium (1.10%). Some minor ions such as bicarbonate (0.41%), bromide (0.19%), boric acid (0.07%), and strontium (0.04%) are also present (Nybakken 1993). Several metal ions such as sodium, potassium, magnesium, and calcium are essential to sustain marine biological life. The taste threshold for the calcium ion varies from 100–300 milligrams per liter (mg/L), depending on the associated anion, but higher concentrations are acceptable to consumers. The taste threshold of the chloride anion in water is also dependent on the associated cation, and concentrations above 250 mg/L lead to detectable taste in water. Taste thresholds for sodium chloride and calcium chloride in water vary between 200 and 300 mg/L (Zoeteman 1980). The guideline value of 0.5 mg/L for magnesium is considered adequate while sodium may affect the taste of drinking water at levels above 200 mg/L (WHO 1979).

Besides the taste attributes imparted in marine living organisms by the water medium, salt also serves

as a flavor enhancer and is used as a preservative for the improvement of texture and color of food products. Typically, salt increases the headspace concentration of nonpolar volatile compounds, which consequently affects the intensity of aroma attributes perceived by sniffing due to the change in concentration of these volatiles. In general, from the flavor point of view, sodium salts also characterize as suppressing agents for bitter-tasting compounds (Breslin 1996).

The habitat of marine species is determined both by the physical environment and by the organisms themselves. Traditionally, tidal migration and food selection by crustacean and fish species have been attributed to the innate ability of animals to sense, through taste and smell, specific flavors, nutrients, and toxins in plants. The survival of some of these species is being threatened due to unfavorable conditions in their habitat or because of excessive fishing. The overexploitation of the fishery resources with high commercial values also induces a leveling off of the total capture volume, resulting in a consecutive quantitative decline of certain species and size of fish landed. On the other hand, in the last two decades, there has been a great upsurge in the development of aquaculture systems all over the world. These systems constitute a cultivation of shrimp (Keys 2003) and some other high-priced commodity-specific species such as crawfish, salmon, tilapia, trout, and catfish. However, it is now well known that the ocean fish possesses its peculiar taste due to a diet rich in bromophenols, the chemical compounds that occur throughout the marine environment and are present in algae, sea mosses, sandworms, and sea salt. A distinct difference is still observed in iodine-like or marine-like flavors appreciated very much in fish and shrimp obtained from marine resources as opposed to those which are cultivated in aquaculture and in which there is lack of its characteristic flavor although both types of products look alike in appearance.

MARINE PLANTS

The most common marine plants belong to algae and sea grasses. There is a very wide difference between them. While algae are simple plants, sea grasses are known to be much more complex in nature. Algae are mainly green (chlorophyta), brown (phaeophyta), red (rhodophyta), blue-green (cyanophyta), and diatoms (chrysophyta). Seaweed are large marine algae that grow in coastal water. People of Asian cultures eat many kinds of seaweed including kelp, which are appreciated for their flavor characteristics and nutrients such as vitamins and iodine.

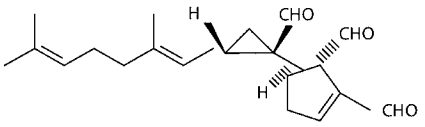
SEAWEED

Generally, seaweed does not possess any aroma at the time of harvest. However, some volatile compounds such as ammonia, methylamine, and trimethylamine are generated by enzymatic or chemical reactions or by microorganisms. Trimethylamine oxide, the precursor for the formation of trimethylamine, is also found in seaweed. On roasting dry green and purple seaweed, a stimulating aroma, much appreciated by Japanese, is generated by the formation of dimethyl sulfide from dimethyl-β-propiothetin (Ito and Hori 1989).

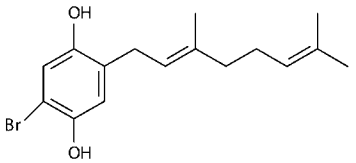
Some seaweed defends itself against herbivorous fishes and invertebrates by producing a diterpene known as halimedatrial (see [1] on Figure 19.1), a secondary metabolite that is very distasteful to these fish. Ito and Hori (1989) in their review on seaweed described the presence of several volatile compounds in different types of seaweed. Some important ones are discussed here. Green seaweed produces cymopole (see [2] on Figure 19.1), a condensation product of bromophenols and monoterpenes. Brown seaweed produces C_{11}-hydrocarbons, sulfur-containing compounds, and a series of diterpenes, the most authentic being (-)-dictyopterene C' [3] (see Figure 19.1), which serves as a sex pheromone and difucol [4] (see Figure 19.1).

Red seaweed is known to synthesize halogenated substances containing bromine and iodine. Several species including *Asparagopsis taxiformis* (commonly known as "Limu Kohu"), *Bonnemaisonia hamifera,* and *B. asparagoides* contain high concentrations of bromine and generate a series of polyhalogenated ketones (1,1,3,3-tetrabromo-2-heptanone and 1-iodo-3,3-dibromo-2-heptanone), halogenated sesquiterpenes and several bromophenols. Some of these halogenated compounds containing bromine impart a sweet flavor, especially in red seaweed when wet. The red seaweed *Palmaria palmata* is known to possess flavor characteristics peculiar to marine, crustacean, and green notes (Michel and others 1997).

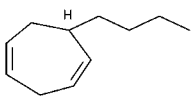
The volatile compounds (iodoethane and iodopentane) from the headspace of red algae were considered to be character-impact compounds (Pape



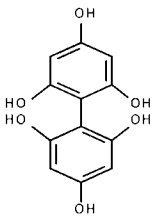
halimedatrial [1]



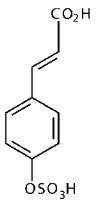
cymopole [2]



(-)-dictyopterene C' [3]



difucol [4]



zosteric acid [5]

Figure 19.1

and others 2004), however isoprene (2-methyl-1,3-butadiene) production has been reported in several species of red (*Chondrus crispus* and *Asparagopsis armata*), green (*Ulva intestinalis*), and brown (*Laminaria digitata, Ascophyllum nodosum, Pelvetia canaliculata, Fucus vesiculosus, Fucus serratus, Halidrys siliquosa,* and *Laminaria saccharina*) algae (Broadgate and others 2004).

Some volatile halogenated organic compounds (VHOC) such as dibromomethane, bromoform, dibromochloromethane, bromodichloromethane, diiodomethane, and chloroiodomethane were identified in 28 different species of Antarctic macroalgae (Laturnus and others 1996). Besides the contribution to the flavor characteristics of the algae, these compounds play an important role in chemical defense of Antarctic macroalgae against herbivores. The algae species *Desmarestia anceps* and *D. menziesii*, which release high quantities of VHOCs, are an important food for a variety of marine herbivores. In seaweed, an increased production of iodinated, brominated, or chlorinated low-molecular-weight VHOCs are associated with oxidative reactions (Mtolera and others 1996). In general, the compounds responsible for retention of the marine flavor of algae are better retained when stored in artificial seawater at 4°C for 15 days as compared to frozen algae, which develops a marked green flavor (Pape and others 2002).

Sea Grass

Very little data are available on flavor aspects of sea grasses. However, it is noteworthy that many marine organisms remain quite clean in spite of the abundance of algal spores and other larvae that could settle and grow on them. It is now known that one way some seaweed and invertebrates remain clean is by producing a potent antifouling compound, zosteric acid [5] (see Figure 19.1) that was isolated from young shoots of sea grass and that functions to deter or kill settling larvae and spores.

MARINE ANIMALS

In general, fresh saltwater fish are almost odorless because they contain a small quantity of volatiles while freshwater fish give off pyrrolidine and other earthy-odor compounds, which are responsible for their maturity and surrounding water pollution (Kawai 1996).

The compounds responsible for the development of flavor during seafood cooking can be classified in two groups. One, which represents the pleasant cucumber/green, almond/nutty, and potato aroma notes, consists of highly volatile, low-molecular-weight compounds belonging to various chemical classes such as aldehydes, ketones, alcohols, esters, nitrogen, phenols, and sulfur-containing compounds (Kubota and others 1996, Sekiwa and others 1997, Le Guen and others 2001, Lee and others 2001). The second is due to water soluble, low-molecular-weight free amino acids (taurine, glutamic acid, glycine), nucleotides (purine derivatives), organic acids (lactic acid), and inorganic salts (Na, K, Cl) (Konosu and Yamaguchi 1982).

Biogenic amines are nitrogen-containing compounds, which are present at very low levels in fresh fish. However, during storage and deterioration, biogenic amines can be produced by amino acid decarboxylation from bacterial enzymes. Among biogenic amines formed, putrescine and cadaverine have a putrid flavor while histamine and phenylethylamine have a pungent and fishy flavor, respectively. Biogenic amines are thermally stable and, therefore, have been used as indices to determine fish freshness (Glória and others 1999, Glória 2003). Volatile amines such as trimethylamine (TMA) or dimethylamine (DMA) are formed from trimethylamine oxide (TMAO), and these compounds also serve as a quality index for marine fish (Duflos and others 1999, Baixas-Nogueiras and others 2003).

Fish

Several studies have been undertaken to identify volatile compounds in different fish species, among them channel catfish (Grimm and others 2004), cod (Milo and Grosch 1995, 1996), hake (Baixas-Nogueras and others 2001), herring (Aro and others 2003), salmon (Milo and Grosch 1996, Whitfield and others 1996, Yoshiwa and others 1997), sardine (Yoshiwa and others 1997), squid (Koizumi and others 1990, Kubota and others 1996, Morita and others 2002), trout (Milo and Grosch 1995), and some Mediterranean fish (Simeonidou and others 1998). Some important features of these fishes are presented.

Catfish

Farm-raised channel catfish are known for their mild flavor and lack of the fishy odor that is peculiar to marine and wild freshwater fish. Flavor quality is the most important criterion for the acceptability of fish by the channel catfish processing plants. Catfish are primarily rejected for having woody, grassy, rotten, diesel, pesticide, pine, and earthy and muddy aromas. Approximately 80% of the rejected fish have the earthy, muddy aroma. Ploeg (1991) stated that the most common off flavor descriptors in catfish were earthy and musty/muddy, which are represented by the compounds geosmin (GSM) and 2-methylisoborneol (MIB), respectively. The threshold concentration of these compounds is 0.02 micrograms per kilogram (μg/kg). These compounds are produced by certain species of blue-green algae and Actinomycetes bacteria.

Grimm and others (2004) reported that the odor threshold of channel catfish varies from 0.1 to 0.2 μg/kg for 2-methylisoborneol and from 0.25 to 0.50 μg/kg for geosmin. The odor threshold values for both methylisoborneol and geosmin in pure water are around 15 nanograms per kilogram (ng/kg) while for catfish, the values for both these compounds range from 35 to 40 ng/kg.

Cod

The most prominent volatile compounds responsible for the flavor of boiled cod were characterized on their odor activity value (OAV). According to Milo and Grosch (1996), the OAVs of different flavor compounds in decreasing order are methional (OAV 275; concentration 11 μg/kg), (*Z*)-1,5-octadien-3-one (OAV 250, 0.1 μg/kg), 3-methylbutanal (OAV 204, 51 μg/kg), (*E,Z*)-2,6-nonadienal (OAV 175, 3.5 μg/kg), acetaldehyde (OAV 130, 1,300 μg/kg) and (*E,E*)-2,4-decadienal (OAV 117, 3.5 μg/kg). Methional, 1-octen-3-ol, and 2,6-nonadienal were identified as the most important compounds contributing to ripened cod odor. Spoilage flavors were mainly 3-methyl-1-butanol and 3-methylbutanal, which can be determined by an electronic nose and are suggested as quality indicators for objective assessing of cod ripening.

Hake

The main amines present in fresh hake (*Merluccius merluccius*) are the natural polyamines, spermine, and spermidine (Baixas-Nogueras and others 2001). During storage, the levels of volatile and nonvolatile

amines change as affected by temperature. In samples stored at −20°C, changes only occurred in dimethylamine and agmatine while at higher temperatures, major changes were observed in cadaverine, histamine, putrescine, and tyramine.

Salmon

Salmon contains carotenoids, astaxanthin (3,3′ -dihydroxy-β−β-carotene-4,4′dione) and canthaxanthin (β,β-carotene-4,4′ -dione), which are responsible for its attractive red color. Volatile compounds generated from carotenoid-related oxidation reactions played an important role in cooked salmon flavor (Josephson 1991). Differences in the overall eating quality occurred between river-caught and sea-caught salmon. River-caught salmon tended to score high on earthy flavor and aftertaste, and scored low for salmon-like odor and flavor (Farmer and others 2000).

Sardine

Prost and others (2004) monitored qualitative and quantitative changes in the volatile aroma compounds during storage of raw sardine (*Sardina pilchardus*). A sensory panel generated a list of 20 odorant descriptors for raw sardine. Thirty-four volatile components were identified as potent odorants using an olfactometric method. (*E,E*)-2,4-octadienal, (*E*)-2-penten-1-ol, and 2,3-butanedione were the most potent odorants of raw sardine. The odor-active compounds responsible for oxidized and rancid flavors increased during storage while sulfur-containing compounds associated with marine odors decreased. The decrease in marine odor could be attributed to the decrease in dimethyl sulfide during storage, while the increase in rancid odor was related to the increase in heptanal and (*Z*)-3-hexen-1-ol in the initial period of storage and in 2,3-pentanedione, hexanal, (Z)-4-heptenal, (*E*)-2-heptenal, methional, and 2-nonanol on longer storage.

Squid

More than 100 compounds have been identified in boiled squid (Koizumi and others 1990, Kubota and others 1996). Koizumi and others (1990) reported that the compounds in neutral and basic fractions contributed to cooked squid flavor. Furthermore, Kubota and others (1996) found that 10 compounds including pyrazines, thiazoles, thiazoline, and a hydroxy furanone were potent odorants. Based on 10 sensory attributes, Morita and others (2002) evaluated the effect of pH and different parts of squid on aroma characteristics of boiled squid. Aroma notes such as sweet, roasted shrimp, cooked fish, and cooked squid scored higher in both muscle and muscle with skin at pH 6.5.

Shrimp and Crustacean

Shrimp

Potent flavor-active compounds in cooked tail meat of freshwater prawn (*Macrobrachium rosenbergii*) were identified by flavor extract dilution analysis (Cadwallader and others 1996). The most intense compounds identified were 2-acetyl-1-pyrroline, 3-(methylthio)-propanal, and 2,3-butanedione representing popcorn-like, baked potato-like, and buttery flavors, respectively.

The aroma components of processed by-products (heads, shells, and tails) of Northern pink shrimp (*Pandalus borealis*) and spotted shrimp (*Trachypena curvirostris*) caught near Tongyeong, South Korea, were reported by Heu and others (2003). Volatile basic nitrogen of the by-products was lower than that of the edible parts. The total content of free amino acids of the processing by-products (2 g/100 g) was 15% higher than that of the edible parts (1.7 g/100 g). Major free amino acids were taurine, threonine, leucine, tryrosine, and phenylalanine. Based on taste threshold values in the by-products, the authors suggested that aspartic and glutamic acids most influenced the taste.

Bromophenols such as 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol are key flavor compounds in seafood (Whitfield and others 1988, 1997, 1998). In water, the strongest of these flavored compounds, 2,6-dibromophenol, 2-bromophenol, and 2,4,6-tribromophenol, have flavor threshold concentrations of 5×10^{-4}, 3×10^{-2}, and 0.6 ng/g, respectively (Whitfield and others 1996). At these concentrations, the flavors of 2,6-dibromophenol and 2,4,6-tribromophenol are iodoform-like and that of 2-bromophenol as phenolic/iodine like. However, at levels below the flavor threshold concentrations, these bromophenols contribute recognizable marine- or ocean-like flavors to seafood and enhance

the intensities of existing seafood flavors (Whitfield and others 1998). Whole wild ocean prawns had a total bromophenol content that ranged from 9.5 to 1,114 ng/g, depending on species, location, and harvest time. The concentration was higher in heads than in tail meats. Cultivated or farmed prawns contained a much lower tribromophenol content that ranged from 0.31 to 1.3 ng/g (Whitfield and others 1997). As a consequence, the flavor of cultivated prawns is frequently described as bland and lacking the natural flavors of wild crustaceans.

Volatile components of boiled prawns were analyzed and correlated with the sensory evaluation of its aroma by Morita and others (2001). Seafood-like odors were related with some unsaturated methyl ketones among more than 100 compounds identified, which included 40 sulfur- and/or nitrogen-containing compounds in cooked shrimp volatiles. Odor descriptions for 2-acetyl-1-pyrroline and 2-actylthiazole were nutty/popcorn-like, and these compounds were found to be aroma active in cooked shrimp (Baek and Cadwallader 1997). Ketones, alcohols, and pyrazines were selected for roasted shrimp, dimethyl trisulfide for green vegetable note, benzaldehyde for creamy and nutty (Tanchotikul and Hsieh 1989), and almond and fruity (Girard and Durance 2000) and 2,4-dimethylthiazole for meaty and cocoa-like. Every one of these compounds was considered important for boiled prawn flavor.

Live kuruma prawn (*Penaeus japonicusis*), which is rich in adenosine 5′-triphosphate (ATP), is considered to be delicious but not sweet. However, on boiling the prawn, a strong sweet taste develops due to the thermal degradation of ATP leading to the formation of adenosine 5′ monophosphate (AMP) (200 mg/100 g of muscle) inosine 5′-monophosphate (IMP) (4 mg/100 g of muscle). The two substances together impart the characteristic umami taste to the boiled prawns (Fuke and Ueda 1996).

Crustacea and Others

The flavor profile of several crustacean species have been reported, among them, clams (Tanchotikul and Hsieh 1991, Chung and Cadwallader 1994, Baek and Cadwallader 1996, Sekiwa and others 1997), lobsters (Lee and others 2001), mussels (Le Guen and others 2000a, 2000b), and oysters (Kim and others 2000). Chung and Cadwallader (1994) analyzed several volatile extracts from cooked blue crab (*Callinectes sapidus*) claw meat obtained by different extraction techniques and characterized four compounds with high flavor dilution factors: 2,3-butanedione (sour, creamy); (*Z*)4-heptenal (potato-like); 2-acetyl-1-pyrroline (nutty, popcorn-like); and 3-(methylthio)propanal (salty, soy sauce-like). Baek and Cadwallader (1997) reported 23 aroma active compounds in cooked crustaceans by using the aroma extract dilution analysis while Whitfield and others (1988) characterized an off flavor in Australian crustacean due to the presence of 2,6-dibromophenol.

Volatile generation in oysters (*Crassostrea gigas*) was related to compounds formed from fatty acid oxidation (86%), particularly to ω-3 polyunsaturated fatty acid oxidation (66%) while only one compound was related to amino acid degradation. The main flavors characterized were green/sulphur/crustacean, represented by dimethyl sulphide, 1-penten-3-one and hexanal, mushroom/citrus-like represented by (*E,E*)-2,4-heptadienal, 1-octen-3-one, 1-octen-3-ol, 6-methyl-5-hepten-3-one and octanal, and marine/cucumber notes attributed to (*E,Z*)-2,6-nonadienal and (*E*)-2-octenal, and decanal.

Three olfactometric methods (olfactometry global analysis, Osme, and aroma extract dilution analysis) were used to evaluate the main impact flavor compounds of cooked mussels (Le Guen and others 2000b). Six compounds seemed to contribute actively to the aroma of mussels: 2,3-butanedione (buttery, caramel-like odor), (*Z*)-4-heptenal (boiled potato-like odor), (*E*)-2-penten-1-ol (mushroom-like odor), 2-ethylpyrazine (nutty odor), methional (boiled potato-like odor), and (*E,E*)-2,4-octadienal (cucumber-like odor).

Wash water from clam processing plants is usually used to obtain a marketable natural clam flavoring agent. Kim and others (2000) concentrated oyster cooker effluent, which, on enzymatic hydrolyzes, led to an increase in free amino acids, with taurine comprising about 20% of the total. Inosine monophosphate was predominant (456 mg/100 g) among nucleotides and related compounds. Trimethylamine, trimethylamine oxide, and total creatinine levels were not affected by enzyme treatment. The predominant aroma-active compounds of enzyme-hydrolyzed oyster effluent were 2-acetyl-1-pyrroline and 3-(methylthio) propanal.

Off Flavors in Fish and Products

Off flavors in seafood may originate from environmental pollutants, the growth of microorganisms, oxidation of lipids, or endogenous enzymatic decomposition in the seafood or from the water assimilated by the fish. The off flavors most commonly detected are petroleum and blackberry-like flavors in salmon and cod; garlic-like flavors in prawns and sand lobsters; iodoform-like flavors in prawns, shrimp, and fish, and muddy and earthy flavors in brackish-water fish and shrimp (Wilkes and others 2000). Geosmin and 2-methylisoborneol are bicyclic tertiary alcohols produced by microorganisms, namely actinomycetes, cyanobacteria, and fungi (Nilsson and others 1999). Both compounds cause undesirable flavors (Young and others 1996). Even fresh, healthy pond-raised catfish may absorb off flavors from microbial metabolites in the water and sediments. Different catfish in the same pond may temporarily assimilate different amounts of geosmin and 2-methylisoborneol and then release them at different rates. Purge-and-trap GC has been used to determine 2-methylisoborneol in water, mud, and cooked channel catfish samples. The bioconcentration factor (concentration in water/concentration in fish) reported for 2-methylisoborneol was 28.1 ± 14.0 (Jackson and others 1997).

Other off flavors, especially in fish, result from the production of volatile microbial metabolites including volatile bases. Secondary products, such as propanal, can serve as a reliable indicator of flavor deterioration for fish products. Dimethyl sulfide and 2-pentanone can be used to monitor the flavor quality of cultured catfish and for their likelihood to occur in fish samples from contaminated environments, for distinctive odor characteristics that could be distinguished from inherent marine fresh fish flavor (Wilkes and others 2000).

In squid, spoilage was attributed to autolytic enzymes rather than microbial degradation (Gill 1990). Autolytic decomposition also occurs in shrimp that produces indole, a relatively nonvolatile amine, along with ammonia when stored at room temperature and only ammonia when stored at refrigerated temperature (Wilkes and others 2000).

Off flavors in ice-packed fish and seafood are produced during spoilage by several microorganisms, including *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., and *Shewanella putrefaciens* evolving several different unpleasant flavors (Jackson and others 1997). A strain of *Lactobacillus sake* used as an antibacterial agent in cold-smoked salmon was judged unsuitable because it produced sulfurous off flavors (Nilsson and others 1997).

Processed Products

Several products prepared from marine animal and plant resources have long been appreciated by people from Japan and other southeastern Asian countries for their flavor quality. Lately there has been a demand for the development of novel products derived from marine ingredients in the Western Hemisphere. A recent publication (Pszczola 2003) describes the current status on organoleptic, nutritional, therapeutic, and other health potential of several products elaborated from the use of marine or aquatic raw materials. Some of the important products related to the flavor aspects are presented here.

Surimi

In the preparation of high quality surimi from dark-fleshed fish mince, discoloration poses a problem. It is considered to be a critical operation that is controlled by optimizing washing conditions in order to remove undesirable materials such as lipids and trimethylamine oxide. Chen and others (1997) reported the beneficial effects of washing horse mackerel mince with ozonized water for 10–20 minutes. However, it also had limitations such as a marked decrease in pH and undesirable gel strength of the mince, accompanied by oxidation of the fish oil, which occurred during ozone treatment. A market test for surimi from Atlantic blue whiting (*Micromesistius poutassou*) was performed by evaluation of the main price-determining parameters such as gel-strength, whiteness, and taste. The results indicated that the respondents valued the samples at almost the same level as Alaska pollack surimi, at the same level as southern blue whiting surimi and better than Pacific hake surimi (Trondsen 1998).

Fish Sauce

Fish sauce is commonly used as a flavoring agent and is basically produced from a mixture of fish and salt (3:1) that has been allowed to ferment for a period of more than 6 months at 30–35°C. It is now

widely used in many prepared foods and sauces in Japanese and Western markets because of its favorable taste, resulting from an amino acid balance and high quantities of peptides. Based on flavor dilution studies on fish sauce, Fukami and others (2002) identified the presence of seven volatiles, which included 2-methylpropanal, 2-methylbutanal, 2-pentanone, 2-ethylpyridine, dimethyl trisulfide, 3-(methylthio)propanal, and 3-methylbutanoic acid. The first four compounds contributed distinctive sweet rancid flavor, and the last two compounds were characterized by a fishy fecal note in the fish sauce. The concentrations of 2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide in sauce were estimated to be 370.7, 38.5, 1.4, and 7.5 ng/mL, respectively. Besides these four compounds, the role of dimethyl disulfide and butanoic acid in the development of fish-sauce flavor was emphasized. Improvement in fish-sauce flavor was enhanced by treatment with bacteria (*Staphylococcus xylosus*) isolated from the fish-sauce mush (Moromi) made from frigate mackerel (Fukami and others 2004).

Deodorization of fish sauce is essential to improve its acceptability in areas outside Southeast Asia. Application of supercritical carbon dioxide for the extraction of lipophilic organic compounds and separation of highly unsaturated fatty acids from fish oil has been tried (Shimoda and others 2000). It was found that bringing microbubbles of supercritical carbon dioxide into contact with the aqueous solution enabled volatiles extraction with high efficiency. After the treatment at a CO_2 flow ratio of 0.29 and 10 mega Pascal (MPa) pressure, the remaining percentage (the concentration in treated sample/that in untreated one) was 5.2% trimethylamine, 8.0% S-methyl ethanethioate, 30% dimethyl disulfide, 55 to 61% aliphatic aldehydes, and 25 to 42% of carboxylic acids. Shimoda and others (1997) reported a column treatment using aromatic porous polymer beads to remove odorants in fish sauce.

Fish Oil

Fish is an important dietary source of polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA, 20:5-ω-3) and docosahexaenoic acid (docosahexaenoic acid [DHA], 22:6—ω-3) (Passi and others 2002). Their beneficial effects have been attributed to an increased ratio of -ω-3- to -ω-6-polyunsaturated acids in human blood lipids and cell membrane lipids, and fish having higher ratios of these acids are considered to result in greater health benefits (Yazawa and Kageyama 1991, Kitessa and others 2001). However, DHA and EPA in fish oil are more easily oxidized than the unsaturated fatty acids in vegetable oil. Lipid oxidation generally results in negative attributes of flavor quality and contributes greatly to the off flavors generated on improper use of stored foods (Durrington and others 2001).

The oxidative deterioration of fish oil involves the formation of hydroperoxides from PUFA in the triglycerides, which on further autoxidation lead to the formation of a complex mixture of secondary oxidation products. Although lipid hydroperoxides are known to be flavorless and odorless, the secondary oxidation products are responsible for the changes in the aroma and flavor properties of foods (Hsieh and Kinsella 1999; Venkateshwarlu and others 2004a, 2004b). The oxidative stability of DHA, eicosapentaenoic acid (EPA), and oxidized volatile compounds in two types of enriched fish oil—triacylglycerol and ethyl ester of DHA—was verified during storage at 80°C with aeration (Lee and others 2003). The rate of DHA autoxidation was higher in ethyl ester form, which was more susceptible to autoxidation than the triacylglycerol form. (*E*)-2-pentenal, 2-(1-pentenyl) furan, and (*E,E*)-2,4-heptadienal were commonly detected as oxidized volatile compounds.

Karahadian and Lindsay (1989) used the dynamic headspace purge-and-trap method to extract the volatile compounds generated during oxidation of fish oil. (*E,Z*)-2,6-nonadienal, (*E*)-2-hexenal, 1,5-octadien-3-one, (*Z*)-4-heptenal, (*E,Z,Z*), (*E,E,Z*)-2,4,7-decatrienal, hexanal, 2,4-heptadienal, and 2,4-decatrienal were the major volatile compounds associated with fishy flavor. In another study, using the dynamic headspace concentration procedure, (*Z*)-2-pentenal, (*E*)-2-hexenal, 2,4-heptadienal, 2,4-decadienal, and (*E,Z,Z*)- and (*E,E,Z*)-2,4,7-decatrienal were identified as the volatile compounds in menhaden fish oil produced from the oxidation of linolenic acid and other PUFAs (Hsieh and others 1989).

Several attempts have been made to develop functional foods by incorporating fish oil (Kolanowski and others 1999), among them, bread, mayonnaise (Hartvigsen and others 2000), and milk emulsion (Venkateshwarlu and others 2004ab). The develop-

ment of objectionable fishy off flavors was an obstacle in the development of fish oil-enriched foods. The volatile (*Z,Z*)-3,6-nonadienal, reported as a potent odorant in boiled cod by Milo and Grosch (1996), was detected neither in the milk emulsion enriched with cod liver oil nor in a commercial cod liver oil (Karahadian and Lindsay 1989).

Venkateshwarlu and others (2004ab) performed detailed studies on volatiles profiles of fish oil-enriched milk during cold storage (2°C) for 14 days. The potent odorants identified by gas chromatography-olfactometry (GC-O) were 1-penten-3-one, (*Z*)-4-heptenal, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, (*E,E*)-2,4-heptadienal, and (*E, Z*)-2,6-nonadienal. Despite their potency, none of the separated volatiles imparted a fishy or metallic odor. Two isomers, (*E,Z,Z*) and (*E,E,Z*) of 2,4,7-decatrienal, were identified in fish oil-enriched milk emulsions (Venkateshwarlu and others 2004a). Though decatrienals were not detected by GC-O, they had sensory significance at higher concentration, which may have impact in combination with other volatiles. The study showed further that the incorporation of fish oil with a very low initial peroxide value (0.1 milliequivalent/kilogram [meq/kg]) did not result in fishy off flavor development upon storage, while the development of distinct fishy off flavor was recorded when cod liver oil with initial peroxide value of 1.5 meq/kg was incorporated into milk. Based on the addition of the volatiles (*E,Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E,E*)-2,4-heptadienal to milk containing 1.5% fat, Venkateshwarlu and others (2004b) evaluated the individual and combinatory effects of these four potent volatiles on sensory properties. They suggested that (*E,Z*)-2,6-nonadienal and 1-penten-3-one could serve as useful marker for fishy and metallic off flavors in fish oil and fish oil enriched foods. In fish oil-enriched milk, Aidos and others (2002) had earlier reported 1-penten-3-one to contribute to the off flavor.

Encapsulation of fish oil by an enzymatic gelation process using transglutaminase cross-linked proteins was investigated by Cho and others (2003). They found that fish oil containing DHA could be encapsulated using double emulsification and subsequently an enzymatic gelation method, using microbial transglutaminase cross-linked proteins. Isolated soy protein was selected as a wall material because it showed better emulsion stability and higher reactivity with transglutaminase than other proteins. Microcapsules prepared by this method showed higher stability against oxygen and a low water solubility, which subsequently resulted in a sustained release of fish oil.

Other Processed Products

The physicochemical differences between pork and fish gelatin on the sensory characteristics of a gelatin-water gel were evaluated (Choi and Regenstein 2000). The flavored fish gelatin dessert gel product had less undesirable off flavor and off odor and a more desirable release of flavor and aroma than the same product made with an equal bloom but higher melting point, like pork gelatin.

The concentration of marine flavor is obtained using processes such as spray-drying, freeze-drying, drum-drying, and nanofiltration (Lin and Chiang 1993). Vandanjon and others (2002) used a membrane processing system consisting of various physical operations such as ultrafiltration followed by nanofiltration or reverse osmosis in order to reduce first the pollution load and then to concentrate flavor compounds of cooking juices from shrimp and tuna. Nanofiltration was not suitable for flavor recovery while reverse osmosis functioned very well for shrimp cooking juices. They showed that a good retention of marine flavors requires the use of reverse osmosis membranes. But these membranes presented a strong mass transfer limitation during concentration of cooking waters of shrimp. To maintain acceptable flows, it is necessary to combine a pretreatment by ultrafiltration followed by reverse osmosis.

Jayarajah and Lee (1999) evaluated a membrane concentration system consisting of tubular polysulphone ultrafiltration and polyamide reverse osmosis for concentrating key water-soluble flavor compounds from lobster extracts. Major flavor-giving compounds in the extract were glutamic acid, glycine, arginine, uridine 5-monophosphate, succinic acid, and glucose. The reverse osmosis system retained all dissolved flavor components at its optimized conditions (40°C, 2.8 MPa log mean transmembrane pressure and a flow rate of 15 L/min. A combination of ultrafiltration and reverse osmosis functioned well for the recovery of lobster flavor compounds. Ultrafiltration could be used to separate proteins and large molecular weight nonflavor compounds from the extract while the resulting perme-

ate enriched with low-molecular weight flavor components could be subsequently concentrated by reverse osmosis.

One of the procedures commonly employed to prevent the incidence of black spots in shrimps and lobsters tails is an immersion treatment in sodium metabisulfite solution prior to freezing. However, excessive use of this compound causes formation of high levels of sulfur dioxide (SO_2) in the muscle, which in turn contributes to an accelerated decomposition of trimethylamine oxide, which forms dimethylamine, trimethylamine, and formaldehyde. All of these compounds are highly undesirable in frozen shrimps or lobster tails.

Gennadios and others (1997) reported the advantages of using edible coatings with biopolymers such as gelatin, blood protein, feather keratin, fish myofibrillar protein, and the polysaccharide chitosan produced from crustacean shells for storage of frozen fish products. These coatings reduce moisture loss and lipid oxidation while maintaining better retention of color and flavor attributes. Silver salmon fillets coated by dipping in Myvacet 5-07 acetylated monoglyceride (60°C) lost less moisture and had lower peroxide values than uncoated control samples during frozen storage at -10°C. Carrageenan, a polysaccharide gum obtained from Irish moss (*Chondrus crispus*), and some other species of red seaweed could extend storage life of frozen fatty fish like mackerel.

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20
Maillard Reaction in Flavor Generation

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INTRODUCTION

Flavor is one of the major factors that determines consumer selection and acceptability of foods. It is even considered the single most important parameter that influences food choice (Glanz et al. 1998). A product with pleasing aroma and taste will draw a more favorable consumer response than another with impressive appearance or loaded with nutrients, but that has an unappetizing flavor. The perception of flavor is caused by a complex sensation from a combination of odor- and taste-active components.

The generation of flavor occurs through various means such as enzymatic reaction, microbiological action, lipid oxidation, and vitamin degradation. An important mechanism for thermally generated flavors is Maillard reaction, also known as nonenzymatic browning. This reaction was named after Louis-Camille Maillard, a French chemist who described the interaction between reducing sugars and amino acids (Billaud and Adrian 2003).

Maillard reaction plays a significant role in food science. It produces a wide variety of compounds that can cause either desirable or undesirable effects. Many books and articles have been devoted to this subject (Eriksson 1981, Waller and Feather 1983, Fujimaki et al. 1986, Baynes and Monnier 1989, Finot et al. 1990, Labuza et al. 1994, O'Brien et al. 1998, Horiuchi et al. 2002). The food industry also relies heavily on Maillard reaction (Ames 1998, Mlotkiewics 1998, Kerler and Winkel 2002). This chapter will focus specifically on the contribution of Maillard reaction on flavor generation.

MAILLARD CHEMISTRY

The Maillard reaction begins with the simple condensation of carbonyl and free amino groups. However, this is followed by the cascade of reactions that eventually give rise to a multitude of compounds that affect flavor, color, texture, and nutritional value of food. The complexity of Maillard chemistry is depicted in the so-called Hodge scheme (Hodge 1953). Hodge provided the basic foundation for understanding this complex reaction, and his scheme so far still serves as the framework for further elucidation of the mechanism.

Maillard reaction occurs in three stages (Nursten 1981). In the early stage, amino acid reacts with

reducing sugar to form a Schiff base that cyclizes to give the corresponding glycosylamine. This is followed by rearrangement reactions to produce Amadori (1-amino-1-deoxy-2-ketose) or Heyns (2-amino-2-deoxyaldose) products, which are important flavor precursors.

The intermediate stage involves the degradation of the rearrangement products through a series of reactions such as dehydration, deamination, cyclization, retroaldolization, isomerization, and fragmentation. This is where the key intermediates and products of flavor formation occur, as illustrated in Figure 20.1 (Vernin and Parkanyi 1982, Mlotkiewics 1998, Weenen 1998, Jousse et al. 2002). Essentially, flavor compounds can be categorized into two products: cyclization/condensation products and fragmentation products. Among the different pathways shown, Strecker degradation is recognized as a significant source of flavor precursors. In this process, carbonyl compounds react with amino acids to produce carbon dioxide and aldehydes with one less carbon atom. The flavor compounds formed include furans, pyranones, cyclopentenones, carbonyl compounds, sulfur compounds, pyrroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles, and others.

The final stage is involved in the formation of polymeric substances that leads to color development. The chemistry of the colored compounds is less studied than the flavor generation aspect of Maillard reaction. Ames and Nursten (1989) cited some of the relevant research conducted in this area. They classified the colored compounds into low-molecular weight products and the polymeric melanoidins. A recent work considered the thermal degradation of melanoidins (Tehrani et al. 2002). When water-insoluble melanoidins from a glucose/glycine mixture were heated (100 to 300°C), various kinds of compounds were generated. Maximum amount of furans, pyrroles, pyrazines, and carbonyl compounds were obtained at 200–220°C whereas pyridines and total oxazoles accumulated more at an even higher temperature.

CONTROLLING FACTORS

Maillard reaction is affected by several conditions such as time, temperature, pH, water activity, and high pressure. How some of the important processing conditions influence the outcome of the Maillard reaction is discussed by Ames (1998).

TIME AND TEMPERATURE

The formation of Maillard reaction products is in most cases positively correlated with reaction time and temperature. Magaletta and Ho (1996) studied the effects of roasting time and temperature on polyhydroxyalkylpyrazine formation and found an increase in the levels of these compounds with extended time and higher temperature. However, the reverse occurred for xylose-lysine solution over longer time but at lower temperature (Ames et al. 1996). A lower amount of compounds was observed for the sample that was further stored for 3 weeks. There was also a distinct peak that was present in another sample but not for the stored one. In addition, fewer resolved peaks were noticed for the chromatogram of the stored sample.

More volatile compounds were detected as xylose and glycine were reacted at increasing temperatures (Benzing-Purdie et al. 1985). The formed high-molecular weight products were characterized by different lengths of aliphatic carbons and fewer unsaturated carbons. A leucine and glucose system also depicted greater browning rate at 122°C than at 100°C (Renn and Sathe 1997). Similarly, a mixture of glucose and glycine generated more volatile compounds at elevated reaction temperatures from 100 to 300°C (Tehrani et al. 2002).

Other means of heat application show the same effects of time and temperature on the abundance of Maillard reaction products. In microwave heating, a longer irradiation time of 100 seconds produced more pyrrole derivatives (Zamora and Hidalgo 1995). In extrusion cooking, an overall increase in volatile compounds such as pyrazines, pyridines, pyrroles, furans, carbonyls, and oxazoles was observed at 180°C from lower temperatures (Ames et al. 2001). Likewise, a higher amount of pyrroles, thiophenes, thiophenones, thiapyrans, and thiazolines were generated at higher extrusion temperatures of wheat flour (Bredie et al. 2002).

pH

pH is an important parameter that greatly influences Maillard reaction. The reactivity of both the carbonyl and amino groups depends on the hydrogen ion concentration. Furthermore, different pH values lead to specific favored steps in the Maillard complex pathways, consequently giving rise to different

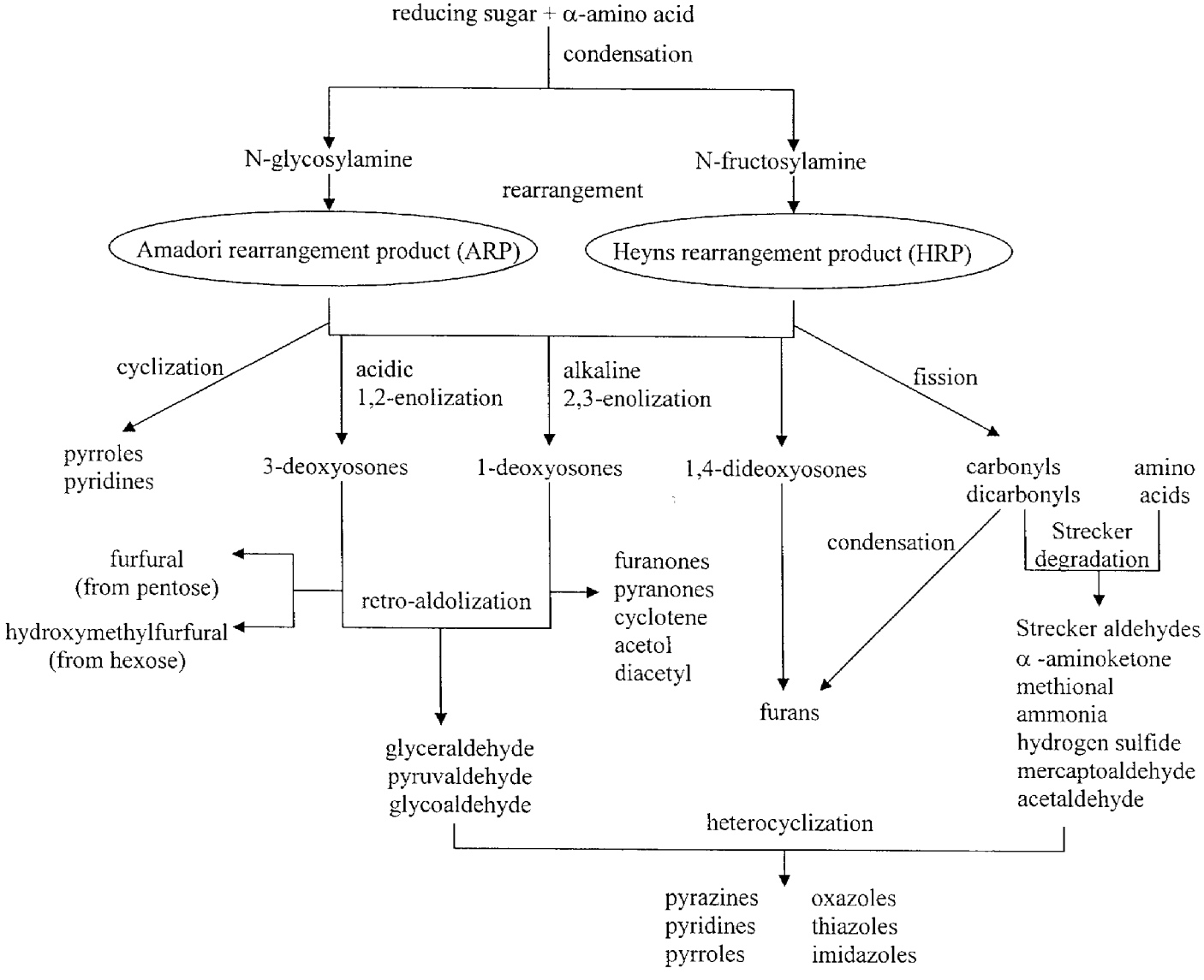


Figure 20.1. Formation of important flavor intermediates and products.

products. Higher pH favored the formation of pyrazines while lower pH tended to produce nonnitrogen compounds (Lu et al. 1997). Sulfur compounds were dominant at higher pH values whereas furan and derivatives were abundant at a lower pH range (Tai and Ho 1998).

The pH can alter the relative abundance of Maillard reaction products. The reaction between the meaty flavor precursor 4-hydroxy-5-methyl-3(2H)-furanone and cysteine at pH 4.5 (Whitfield and Mottram 1999) and pH 6.5 (Whitfield and Mottram 2001) yielded a similar number of compounds. However, sulfur-containing compounds dominated the reaction at pH 4.5 whereas more nitrogen compounds were found at pH 6.5 (Figure 20.2).

A higher pH condition/environment intensifies Maillard browning in most instances. More browning was observed in leucine-glucose (Renn and Sathe 1997), lysine-glucose (Ajandouz and Puigserver 1999), lysine-fructose (Ajandouz et al. 2001), and

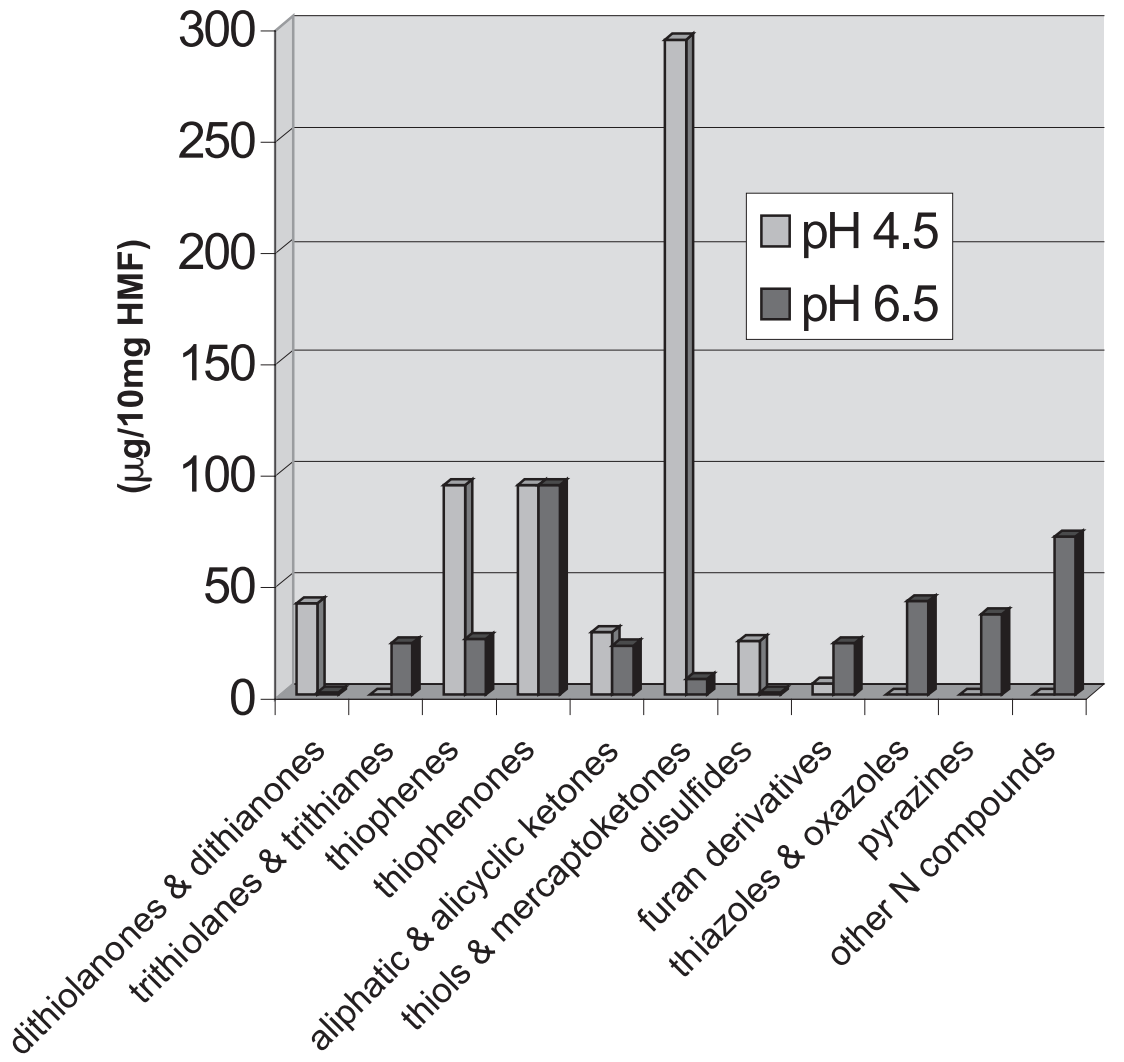


Figure 20.2. Flavor compounds formed at pH 4.5 and 6.5 (based on Whitfield and Mottram 2001).

proline-glucose (Blank et al. 2003) systems. Similar effects were observed for microwave-irradiated samples (Yeo and Shibamoto 1991b, Zamora and Hidalgo 1995). When extrusion cooking was used, the effects of pH were temperature dependent. Volatile compounds increased with decreasing pH at 180°C, but increased with rising pH at 120°C (Ames et al. 2001). Higher amounts of thiophenes, thiophenones, polythiacycloalkanes, thiazoles, thiazolines, pyrroles, and some pyrazines were attained at alkaline pH (Bredie et al. 2002).

Water Activity

Water plays an important role in Maillard reaction because it acts both as reactant and solvent. Therefore, water activity is often used to study the effect of moisture on the Maillard reaction. Labuza (1980) illustrated that the rate of browning was positively correlated to water activity, with maximum at a_w values 0.5 to 0.8, and then decreased at higher water activity. The initial increase in the rate of reaction was due to better solubility of the reactants resulting in more efficient interaction. However, additional water led to dilution of the reactants' concentration and subsequently, a decrease in browning rate (Mustapha et al. 1998).

Early investigations on the effect of water on Maillard reaction showed that both quantitative and qualitative changes occur in the volatile profile of a meat flavor model system with different water activity (Hartman et al. 1984a, 1984b). Maximum amount of volatiles was observed at a_w 0.72. The kind of major volatiles also differed: sulfur-containing compounds in the high water system and dehydration-type products in the low water system. As a result, boiled meatlike notes and roasted meatlike notes were observed for high and low water systems, respectively. In a glycine/glucose system, the presence of water drastically increased the amount of volatiles, with furans as the predominant product (Ames et al. 2001). Moisture content also affected Maillard reaction when microwave heating was used (Yeo and Shibamoto 1991b, Peterson et al. 1994).

High Pressure

High pressure processing has gained much attention because of its minimal effect on food flavor and nutrient qualities. However, in combination with other factors, high pressure may lead to some flavor variation. The influence therefore of high pressure on Maillard reaction is currently under investigation.

According to Hill et al. (1996), the effect of high pressure on Maillard reaction varied depending on the pH of the medium. In a glucose and lysine system with pH 7.0 to 7.5, high pressure had insignificant effects. However, at a higher pH, high pressure hastened the Maillard reaction whereas it caused the opposite at a lower pH. They suggested that pressure induced the ionization of the acid groups in the system resulting in a lower pH and a subsequent rate reduction. This pH dependency on the effect of pressure on Maillard reaction was confirmed by Moreno et al. (2003).

Pressure could also change the reaction profile of a system. When glucose-lysine solutions (with initial pH 10.1 and incubated at 60°C) were subjected to atmospheric pressure or 600 mega Pascal (MPa), significant quantitative differences on the reaction products were observed (Hill et al. 1998). High pressure also enhanced the formation of tetramethypyrazine under weak acidic conditions (Huang et al. 1996).

The volatile compounds produced by reacting glucose and lysine (pH 10.1) at 60°C with or without high pressure were compared (Hill et al. 1999). As shown in Table 20.1, high pressure greatly decreased the yields of the compounds indicated. It was suggested that pressure could retard volatile compound formation, enhance conversion of volatile to nonvolatile products, or cause alternative pathways for nonvolatile compound formation, resulting in fewer intermediate volatile products. Moreno et al. (2003) indicated that Amadori rearrangement products formed faster and subsequently degraded under high pressure, causing a subsequent increase of intermediate and advanced reaction products.

SPECIFIC MAILLARD REACTION PRODUCTS

Proline-Specific Products

The Maillard reaction of proline with reducing sugars yields different compounds depending on the reaction conditions. Much of the progress in the identification of proline-specific products and the elucidation of the formation mechanisms was made

Table 20.1. Comparison of volatile compounds formed at different pressures.

Compound	atmospheric pressure	600 MPa
pyrazines	5,215.7	166.6
pyranones	493.0	7.5
pyridines	467.2	26.6
pyrroles	401.6	18.7
furans & furanones	141.1	20.5
pyrrolizines	121.2	17.4
alicyclic ketones	48.5	6.0
indolizines	24.7	nd

Based on Hill et al. 1999.

by Tressl and coworkers (Tressl et al. 1993, 1985a, 1985b, 1985c; Helak et al. 1989a, 1989b). The major classes of identified compounds included pyrrolines, pyrrolidines, piperidines, pyrrolizines, and azepines. The proposed mechanism for the formation of these compounds, most of which were confirmed by labeling experiments, were described by Tressl and coworkers (1993).

A total of 16 compounds, composed of one pyrroline, 12 N-substituted pyrrolidines, and three 2-substituted piperidines, were generated when L-proline and simple sugars were heated for 1.5 hours at 150°C (Tressl et al. 1985a), as shown in Table 20.2. In another study (Helak 1989b), demonstrated that furylpyrrolidines and furylpiperidines were also obtained from the same model system. When proline undergoes Strecker-type reaction with cyclic enolones, 2-(1-pyrrolidinyl)-2-cyclopentenones are produced (Tressl et al. 1985c). With the development of more sophisticated techniques such as gas chromatography-olfactometry (GC-O), it is now possible to identify the odor-active compounds from a complex mixture of volatiles. The nitrogen-containing volatile compounds 2-acetyl-1-pyrroline (AP) and 2-acetyltetrahydropyridine (ATHP), also known as 6-acetyl-1,2,3,4-tetrahydropyridine, are considered as character impact compounds giving roasty odors to foods such as bread and popcorn (Schieberle 1991). On further investigation, the group of Schieberle also discovered the importance of 2-propionyl-1pyrroline (PP) and 2-propionyltetrahydropyridine (PTHP) in generating roasty notes (Hofmann and Schieberle 1998).

Another important group of proline-specific compounds identified was 2,3-dihydro-1H-pyrrolizines, with characteristic smoky and roasty aroma. These were obtained when L-proline was reacted with glyceraldehyde, erythrose, arabinose, glucose, and rhamnose for 1.5 hours at 150°C (Tressl et al. 1985b). In that study, among the 22 pyrrolizine derivatives generated, 19 were recognized as proline-specific compounds for the first time.

Table 20.2. Compounds identified in the Maillard reaction of proline and simple sugars.

Pyrroline	1-pyrroline
Pyrrolidine	pyrrolidine
	1-formylpyrrolidine
	w1-acetylpyrrolidine
	1-(1-pyrrolidinyl)2-propanone
	1-(1-pyrrolidinyl)2-butanone
	3-(1-pyrrolidinyl)2-butanone
	2-(1-pyrrolidinyl)2-pentanone
	3-(1-pyrrolidinyl)2-pentanone
	1-furfurylpyrrolidine
	1-(5-methylfurfuryl)pyrrolidine
	1-(5-hydroxymethyl)furfurylpyrrolidine
	2,5-dimethyl-4-(1-pyrrolidinyl)-3(2H)-furanone
Piperidine	2-acetylpiperidine
	2-propionylpiperidine
	2-(5-methyl-2-furyl)piperidine

Based on Tressl et al. 1985a.

Azepines were also isolated from the proline system. They are thought to be formed in a similar fashion as the 2-(1-pyrrolidinyl)-2-cyclopentenones generation (Tressl et al. 1985c). Eleven cyclopent (b)azepin-8(1H)-ones have been identified.

Asparagine-Specific Products

The reaction between asparagine and monosaccharides yielded 3-methyl-2(1H)-pyrazinones as asparagine-specific Maillard products (Shu and Lawrence 1995). These included 3,5-dimethyl-6-ethyl-2(1H)-pyrazinone, 3,6-dimethyl-5-ethyl-2(1H)-pyrazinone, 3,5,6-trimethyl-2(1H)- pyrazinone, and 3-methyl-5,6-diethyl-2(1H)-pyrazinone. Just recently, asparagine was also reported to be the main amino acid precursor of acrylamide, a potential cancer-causing agent (Mottram et al. 2002, Stadler et al. 2002, Becalski et al. 2003). Although it is easy to speculate that the decarboxylation and deamination of asparagine can readily form acrylamide, Yaylayan et al. (2003) reported otherwise. By using Fourier Transform Infrared (FTIR) and pyrolysis-GC/MS, they found that the thermal decomposition of asparagine produced maleimide instead. However, addition of reducing sugar leads to acrylamide formation. They suggested that the Schiff base formed from asparagine and reducing sugar stabilized itself by intramolecular cyclization, producing an oxazolidin-5-one intermediate. Under mild conditions, this intermediate can be decarboxylated to form azomethine ylide. Subsequent tautomerization and protonation can produce decarboxylated Amadori products, which can later form acrylamide upon cleavage of the C-N covalent bond under high temperatures.

Methionine-Specific Products

Strecker degradation plays a significant role in the formation of Maillard reaction products from methionine and reducing sugars. The Strecker aldehyde methional is often associated with potato-like flavor. Buttery et al. (1973) considered it as one of the major contributors to baked potato aroma. Methional was also identified in a potato-like model system (Mandin et al. 1999). Since glucose is necessary in the formation of methional, Shigematsu reaction does not occur in their reaction conditions. Shigematsu reaction was reported as an alterntive route to the formation of methional from methionine alone (Yu and Ho 1995). Methional was also found to be an important odorant in buckwheat honey (Zhou et al. 2002), sourdough rye bread (Kirchoff and Schieberle 2001), corn tortilla chips (Buttery and Ling 1998), and regular-fat and low-fat cheddar cheese (Milo and Reineccius 1997). In addition, Kumazawa and Masuda (2002) compared the volatile profiles of three green tea varieties and found that methional had high flavor dilution factors in all varieties. However, the highest value was observed for the Japanese tea Kamairi-cha. 2-(2-Methylthioethyl)-4,5-dimethyl-3-oxazoline and 2-(4-methylthiobutyl)-3,5,6-trimethylpyrazine were generated from methionine and 2,3-butanedione (Hartman and Ho 1984). The suggested mechanism was described by Ho (1996). Using pyrolysis-GC/MS analysis, Yaylayan and Keyhani (2001) investigated the fragmentation pathways in the methionine/glucose system. Aside from methional formation from Maillard reaction, they proposed that this Strecker aldehyde could also be formed from methionine alone. This is made possible by oxidative decarboxylation of methionine to form an imine followed by hydrolysis. Glucose, the sugar moiety, gives reactive dicarbonyls such as 1- and 3-deoxyglucosones. These Amadori compounds undergo C_3/C_3 cleavages to form acetol, glyceraldehyde, and pyruvaldehyde, which upon Strecker reaction leads to pyrazine formation. C_2/C_4 cleavages are also possible, producing glycoaldehyde and tetrose moieties. Glycoaldehyde incorporates C-1, C-2 and C-5, C-6 glucose carbon atoms whereas the tetrose moiety incorporates the last four carbon atoms.

Cysteine-Specific Products

Cysteine is generally recognized as a very important precursor to the formation of sulfur-containing flavor compounds in foods. Strecker degradation of this amino acid by dicarbonyl compounds in Maillard reaction produces reactive intermediates, such as ammonia and hydrogen sulfide, which can further participate in other reactions in the system.

Thermal degradation of cysteine in aqueous solution is highly pH dependent (Shu et al. 1985a, 1985b). A vigorous degradation was observed at pH 5.1, which is the isoelectric point of cysteine. At pH 2.2, the major components were 1,2,3-trithia-5-cycloheptene and 2-thiophenethiol. The 1,2,3-

trithia-5-cycloheptene was judged to resemble roasted onion and roasted meat odor.

Cysteine and ribose contribute immensely to the characteristic flavor of meat. The complex formation of meat flavor was discussed by Mottram (1998). Since numerous volatiles are usually involved, it is difficult to pinpoint the exact compounds responsible for the meat flavor. Therefore, Hofmann and Schieberle (1995) used aroma extract dilution analysis (AEDA) to determine the key odorants in a cysteine-ribose model system. The solution heated for 20 minutes at 145°C generated 29 odor-active volatiles with 2-furfurylthiol, 2-methyl-3-furanthiol, 2-phenyl mercaptan, and ethyl mercaptan as the most important contributors to the overall roasty, meatlike, sulfury odor. The flavor notes of these compounds as well as those of the other key odorants are indicated in Table 20.3. Since 5-acetyl-2,3-dihydro-1,4-thiazine had never been identified previously in model system or food, its structure determination was described (Hofmann et al. 1995). This intense roasty, popcorn-like odor compound has a similar low odor threshold as the more common roasty compounds, 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline.

Most model systems use ribose as the reducing sugar in Maillard reaction because this sugar is important in meat. However, ribose is usually not present in its free form in meat. To determine the effect of different forms of ribose in the formation of sulfur-containing compounds, Mottram and Nobrega (2002) investigated the products from cysteine and inosine 5′-monophosphate, ribose 5-phosphate, and free ribose. The inosine monophosphate system was relatively unreactive, producing a lower amount of volatiles such as thiols (mercaptoketones, furanthiols, and thiophenethiols) and disulfides (oxoalkyl, furyl, and thienyl disulfides).

Using the CAMOLA (carbohydrate module labeling or carbon module labeling) approach, Cerny and Davidek (2003) evaluated the formation pathways for the odor compounds from cysteine and ribose reacted for 4 hours at 95°C. The aroma compounds elicited from this system are listed in Table 20.4. New evidence suggests that the formation of 2-methyl-3-furanthiol, 2-furfurylthiol, and 3-mercapto-2-pentanone did not result from the ribose fragmentation pathways. Since 3-mercapto-2-pentanone and 2-mercapto-3-pentanone were not present simultaneously, 3-mercapto-2-pentanone did not form via the previously suggested pathway where 2,3-pentanedione and hydrogen sulfide were intermediates (Mottram et al. 1995). An alternative pathway was postulated instead.

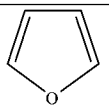
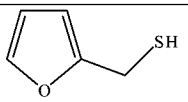
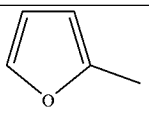
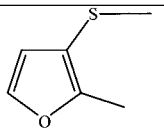
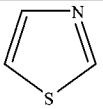
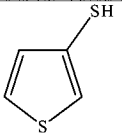
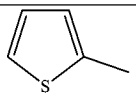
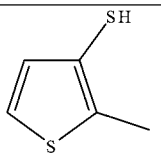
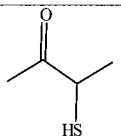
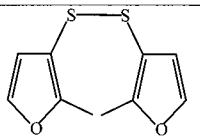
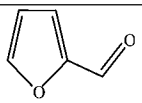
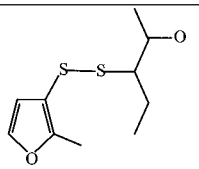
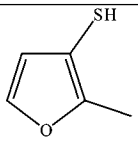
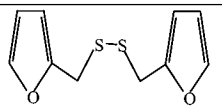
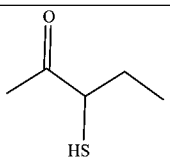
When cysteine was reacted with glucose or rhamnose, different flavor profiles were obtained by Hofmann and Schieberle (1997). Using aroma extract dilution analysis, they identified 34 odor-active volatiles in the glucose system in which, roasty and sulfury notes dominated. The responsible compounds included 2-furfurylthiol, 5-acetyl-2,3-dihydro-1,4-thiazine, 3-mercapto-2-butanone, 3-mercapto-2-pentanone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 2-(1-mercaptoethyl)furan. When rhamnose was used, caramel-like and spicy odor notes were more prevalent. The important compounds were 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-6-methyl-2(2H)-pyranone, 5-methyl-2-furfurylthiol, 2-furfurylthiol, and 5-acetyl-2,3-dihydro-1,4-thiazine. They also compared the key odorants formed from those generated by cysteine and ribose. In terms of the contribution to the overall odor, the compounds 2-furfurylthiol,

Table 20.3. Odor-active compounds in cysteine-ribose system.

Odorant	Description
2-furfurylthiol	roasty, coffee-like
2-methyl-3-furanthiol	meatlike, sulfury
2-phenyl mercaptan	roasty
ethyl mercaptan	roasty
3-mercapto-2-pentanone	sulfury, catty
5-acetyl-2,3-dihydro-1,4-thiazine	roasty, popcorn-like
3-mercapto-2-butanone	sulfury, rotten
bis(2-methyl-3-furyl)disulfide	roasty

Based on Hofmann and Schieberle 1995.

Table 20.4. Compounds identified in cysteine-ribose system.

Name	Structure	Name	Structure
furan		2-furfurylthiol	
2-methylfuran		2-methyl-3-(methylthio)furan	
thiazole		3-thiophenethiol	
2-methylthiophene		2-methyl-3-thiophenethiol	
3-mercapto-2-butanone		bis(2-methyl-3-furyl)disulfide	
2-furaldehyde		(2-methyl-3-furyl)(2-oxo-3-pentyl)disulfide	
2-methyl-3-furanthiol		bis(furfuryl)disulfide	
3-mercapto-2-pentanone			

Based on Cerny and Davidek 2003.

5-acetyl-2,3-dihydro-1,4-thiazine, and 2-acetyl-2-thiazoline did not seem to be affected greatly whether the sugar moiety in the system was a hexose or a pentose.

Histidine-Specific Products

Pyridoimidazoles such as 2-acetyl- and 2-propionylpyrido[3,4-d]imidazole are formed when histidine and glucose were heated in aqueous buffer or roast systems (Gi and Baltes 1995). Whereas 2-acetylpyrido[3,4-d]imidazole was produced by Strecker degradation of histidine with pyruvic aldehyde, as proposed in an earlier work (Gi and Baltes 1993), 2-propionylpyrido[3,4-d]imidazole was instead generated by the reaction of histamine and 2-oxobutyraldehyde via 2,5-dimethyl-4-hydroxy-3(2H)-furanone. When 1-methyhistidine, 2-methylhistidine, and 3-methyhistidine were reacted with glucose, volatile compounds were synthesized that were methylated at the imidazole ring and with preferred acetyl or propionyl residues at the pyridine ring.

Peptide-Specific Products

Peptides are present in various foodstuffs. Early investigations on the contribution of peptides to flavor generation were conducted by Chuyen et al. (1972, 1973). Aside from Strecker aldehydes, they identified a series of novel pyrazinones-2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkyl acid from the reaction of dipeptides with glyoxal at 100°C and pH 5.0. Rizzi (1989) also reported the formation of Strecker aldehydes and alkylpyrazines from small peptides and fructose.

Oh and others (1991) showed that the reaction of glycine and triglycine with glucose (180°C, pH 4–5, 2 hours) generated more pyrazines than diglycine and tetraglycine. As explained by Izzo and Ho (1992a), free glycine acts as a contributor to the structure of the pyrazine through Strecker degradation while bound glycine acts as an activator for sugar degradation. Oh and others (1992a) identified three new pyrazinones from glycine peptides and four from Gly-Leu and Leu-Gly (Table 20.5). The mechanism for the formation of 1-alkyl-2(1H)-pyrazinones from dipeptide and dicarbonyl compounds was illustrated (Ho 1996).

It is interesting to note that the reaction of Gly-Pro or Pro-Gly with glucose did not produce any pyrazinone but yielded pyrrolizines and pyridines instead (Oh et al. 1992b). The difference in the flavor compounds generated from the two systems was attributed to the presence of primary and secondary amino groups in Gly-Pro and Pro-Gly, respectively. This was related to the relative ease of sugar degradation and Schiff base formation.

Maillard Reaction of Deamidated Protein

Deamidation is a hydrolytic reaction that converts the amide of asparagine or glutamine to acid, forming the corresponding aspartic or glutamic acid residue. This process is accompanied by a liberation of an ammonia molecule. Food proteins are subjected to chemical or enzymatic deamidation to enhance their functional properties. Deamidation causes structural and conformational changes resulting in both improved solubility and surface hydrophobicity. These properties render deamidated proteins ideal as surface-active agents, ingredients that are essential in the food industry. Riha et al.

Table 20.5. Novel 1-alkyl-2(1H)-pyrazinones identified from dipeptides and glucose.

Dipeptide	Pyrazinone
Gly-Gly	1,6-dimethyl-2(1H)-pyrazinone
	1,5-dimethyl-2(1H)-pyrazinone,
	1,5,6-trimethyl-2(1H)-pyrazinone
Gly-Leu and Leu-Gly	1-methyl-3-isobutyl-2(1H)-pyrazinone
	1-isopentyl-2(1H)-pyrazinone
	1,6-dimethyl-3-isobutyl-2(1H)-pyrazinone
	1,5,6-trimethyl-3-isobutyl-2(1H)-pyrazinone

Based on Oh et al. 1992a.

(1996) reviewed the important aspects of nonezymatic deamidation of food proteins.

Food processing operations such as extrusion have been shown to cause nonenzymatic deamidation of proteins (Izzo et al. 1993a). Using wheat flour, they examined the effect of temperature, feed moisture, and pH on deamidation of gluten. Both temperature and moisture content were positively correlated with greater deamidation. This was attributed to more protein unfolding. Extremes of pH were also found to enhance deamidation.

As mentioned earlier, a molecule of ammonia is released during protein deamidation. This reactive molecule was found to affect the Maillard reaction of a complex system such as autolyzed yeast extract (Izzo et al. 1991, 1992b). The presence of ammonia enhanced browning but limited the amounts of volatiles, specifically pyrazines. It was hypothesized that the unstable ammonia was too reactive that browning resulted rapidly and as a consequence, pyrazine formation was slowed down. Since pyrazines are important flavor compounds with roasted and baked notes, Ho et al. (1994) deemed it necessary to clearly elucidate the role of deamidation in pyrazine formation. Using isotope labeling in the glutamine/glucose model system, they found that more than half of the pyrazines identified had ^{15}N that came from the amide chains of glutamine. This suggests that deamidation occurred with a significant effect on pyrazine formation because the liberated ammonia reacted more easily with dicarbonyls than the α-amino groups of glutamine.

Using a peptide model system, Zhang (1993) demonstrated that Ala-Asp generated more highly substituted pyrazines as well as a higher amount of total pyrazines while Ala-Asn produced more unsubstituted pyrazine and methylpyrazine. This shows that the acidic side chain of the peptide favored Strecker degradation of alanine while the amide side chain released ammonia leading to more unsubstituted products. To determine the effect of deamidation on Maillard reaction of a complex system, Izzo and Ho (1993b) investigated heated gluten samples with five residual amide levels. They observed quantitative differences in the aroma compounds and in general, more volatiles were present with higher amide levels. However, furans and dimethylpyrazine behaved differently. Although the amount seemed higher when more amides were available, these compounds increased again at the lowest amide levels. This was attributed to the structural changes caused by deamidation. Similarly, more browning was observed in samples with a lower degree of deamidation.

Since kinetic studies are important in determining the effect of processing conditions on the deamidation of food proteins, Zhang et al. (1993a, 1993b) investigated the kinetics and mechanism of soy protein and egg white lysozyme. They found that the overall deamidation reactions of both proteins followed an apparent first-order equation. The rate of the reactions was also influenced by the pH of the solution. However, the deamidation rate at pH above 5 was higher for lysozyme than soy protein. This was attributed to differences in the proteins' structure and properties.

INTERACTIONS OF MAILLARD REACTION AND LIPIDS

INTRODUCTION

Lipids are a common food component present intrinsically or added as an ingredient. Their widespread occurrence unavoidably causes them to impact Maillard reaction and to interact with its products. Major flavor changes happen consequently. The mechanism of flavor variation due to such interactions is being investigated in both model and food systems.

Degradation products from lipid oxidation and Maillard reaction give rise to certain aromatic compounds. Lipid oxidation results in the formation of aldehydes, ketones, and other compounds. On the other hand, Strecker degradation in Maillard reaction produces other reactive intermediates that can react with the lipid degradation products. The volatiles formed from these interactions include heterocyclic compounds containing oxygen, nitrogen, or sulfur, with long-chain n-alkyl substituents (Whitfield 1992).

The Maillard reaction and lipid oxidation products react to form pyrazines. Chiu et al. (1990) demonstrated the synthesis of pentylpyrazines or hexylpyrazines in a system containing ammonium acetate, acetol, and pentanal or hexanal. These alkyl-substituted pyrazines were also detected in potatoes (Carlin et al. 1986), corn-based systems (Bruechert et al. 1988), and fried chicken (Tang et al. 1983). Interaction between 2,4-decadienal, a linoleic acid oxidation product, and cysteine or glutathione yielded long-chain alkyl substituted

dithiazines and trithiolanes such as 2-methyl-4-butyl-6-pentylperhydro-1,3,5-dithiazine and 3-methyl-5-pentyl-1,2,4-trithiolane (Zhang and Ho 1989, Zhang et al. 1994). In the same study (Zhang and Ho 1989), they also observed the formation of a large quantity of 2-pentylpyridine in the model system containing glutathione instead of cysteine. The authors suggested that 2,4-decadienal was involved in a direct Schiff-base formation with the amino group in cysteine or glutathione, followed by electrocyclic reaction and aromatization to form pentylpyridine. The relatively stable glutathione rather than cysteine provided more primary free amino groups for the Schiff-base formation thus leading to the production of a larger amount of 2-pentylpyridine. This compound has been identified in deep-fat fried foods (Tang et al. 1983) and meat (Mottram 1985). Recently, 2-pentylpyridine has been recognized as the primary odor-active compound in soy protein.

Sulfur-containing heterocycles are important compounds in meat flavor, as discussed extensively by Mottram (1998). 2-Alkylthiophenes with C4 to C8 alkyl substituents were reported in beef (Min et al. 1979). A number of alkylthizoles have been identified in meat (Hartman et al. 1983, Tang et al. 1983). Numerous alkyl-3-thiazolines and alkylthiazoles were obtained from cooked beef (Elmore et al. 1997). It has been postulated that these compounds were formed from α-hydroxyketones or α-diones, hydrogen sulfide, ammonia, and aldehydes (Elmore and Mottram 1997). Some of the 3-thiazolines identified were 2-octyl-4,5-dimethyl-3-thiazoline, 2-hexyl-4,5-dimethyl-3-thiazoline, 2-pentyl-4-methyl-3-thiazoline, 2-heptyl-4,5-dimethyl-3-thiazoline, 2-octyl-4-ethyl-5-methyl-3-thiazoline, and 2-pentyl-4,5-dimethyl-3-thiazoline.

Factors Affecting Maillard-Lipid Interaction

Phospholipids are often part of a food matrix. The high amount of unsaturated fatty acids makes them more prone to oxidation. The presence of phospholipids has been shown to alter the configuration of volatile products in the cysteine-ribose system (Farmer et al. 1989). In this system, phospholipids caused a decline in the amount of sulfur-containing heterocyclic compounds including thiophenes, dithiolanones, dithianones, trithiolanes, and trithianes.

Edible oils play a role in pyrazine production. In the lysine-xylose/glucose systems, Negroni et al. (2001) observed opposite effects of edible oils on unsubstituted and substituted pyrazines. Decreasing amounts of unsubstituted pyrazines were obtained with olive oil, canola oil, and sunflower oil. In contrast, increasing levels of 2-methylpyrazine, 2,5-dimethylpyrazine, and 2,3-dimethylpyrazine were noted in olive oil, canola oil, and sunflower oil. The sensitivity of the pyrazines was suggested to be due to the differences in the degree of the unsaturation of the oils. This hypothesis should be further tested by using purified oils. Commercially available vegetable oils contain various amounts and types of phenolic antioxidants, which may have an impact on the degree of Maillard reaction. Many foods exist as emulsions. The emulsion properties also influence the perception of aroma compounds. van Ruth and others (2002) observed that lower lipid and emulsifier fractions and greater particle diameter of oil in water (O/W) emulsions enchanced the aroma release of alcohols, ketones, esters, aldehydes, terpene, and sulfur compounds.

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21
Traditional Laboratory Methods

Emira Mehinagic

Isolation
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Conclusion
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The first step in flavor analysis is the isolation and separation of flavor from the food matrix in order to obtain information that is representative of the studied material. Volatile components can be isolated from liquid or solid material by extraction or distillation. Next, they can be separated by liquid or gas chromatography (GC). Finally, these components are identified, by their chromatographic retention and different spectroscopic methods, and characterized by sensory methods in order to establish if they are "character-impact" odorants or not (Teranishi and Kint 1993).

Complete identification of flavor volatiles is rarely attained with a single sample preparation. Generally, a combination of two or more separation and identification techniques is needed. Fischer and others (1995), who compared chromatographic results from solid phase extraction, vacuum distillation, and simultaneous distillation/extraction of Cupuçoa fruit flavor, clearly demonstrated that the composition of the extracts is dependent on the isolation procedures employed. They showed that the fresher, volatile portion of fruit flavor was represented by the vacuum distillate while the heavier fruit notes, of higher polarity, were enriched in the extracts obtained by solid phase extraction. Moreover, Guntert and others (1998), who compared vacuum hydrodistillation with headspace extraction on different fruits, showed that dynamic headspace extracts are more dominated by the lower-boiling point components, while the vacuum hydrodistilled extracts are dominated by the higher-boiling point components.

ISOLATION

The first step in flavor analysis is isolation, with the main objective of separating selectively the volatile compounds from the matrix. The principal traditional isolation techniques are extraction (liquid-liquid extraction, solid-phase extraction, headspace extraction) and distillation (simultaneous distillation extraction, vacuum hydrodistillation). The choice of the technique depends on the food sample. For example, flavor from a solid matrix cannot be extracted directly by liquid/liquid extraction thus the solid material must first be prepared. The choice also depends on the nature of the volatiles. For instance, some volatiles can be difficult to extract because of their low solubility in organic solvents. During the isolation procedure, the thermal and catalytic decomposition of food components can occur (Teranishi and Kint 1993). The analysts must be certain that the isolation method will not generate compounds that were not present in the original sample. Moreover, many food aroma components are unstable and may

be oxidised by air or degraded by heat or extremes of pH. For these reasons, the choice of the isolation method is a very important step in flavor analysis.

Isolation of Components by Direct Extraction Procedures

There are numerous direct extraction procedures for the isolation of volatile components from the food matrix. The components are extracted by their distribution between two different phases. The techniques most employed are:

- Liquid-liquid extraction: The analyte is extracted from a liquid matrix to another immiscible liquid phase.
- Solid-phase extraction: The analyte is extracted from a liquid matrix to a solid phase by adsorption.
- Headspace extraction: The analyte is extracted from a solid or liquid food matrix to a gaseous phase.

Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is a process for separating components in solution by their distribution between two immiscible liquid phases (Robbins and Cusack 2003). This extraction method, frequently followed by concentration under nitrogen or in a rotary evaporator, was one of the earliest methods used to recover flavor compounds from foods.

LLE is a mass transfer operation, in which a studied liquid solution is in contact with an immiscible or nearly immiscible liquid solvent that exhibits preferential affinity or selectivity toward one or more of the components in the liquid solution. The basis for separation by this two-phase system is the selective distribution of substances between two liquid phases. Distribution is characterized by the partition coefficient, K, defined as the concentration in the low-density phase, C_L, divided by the concentration in the high-density phase, C_H:

$$K = C_L/C_H$$

Obviously, the choice of a suitable phase system is the key step in all partitioning work.

The selection of the solvent for extraction is one of the most important criteria in LLE. Many solvents are available but the main choices are usually hydrocarbons (isopentane, pentane, hexane), halogenated hydrocarbons (dichloromethane), and ethers (ethyl ether). The hydrocarbon solvents are very effective in cases where water and low-boiling alcohols must be rejected. These solvents are hydrophobic. On the contrary, when ethyl ether is used as a solvent, a considerable amount of water, as well as methanol and ethanol, will be extracted over a prolonged period.

Solvents differ in their extraction capabilities depending on their own and the solute's chemical structure. Once the functional group is identified, possible solvents can be screened in the laboratory.

The distribution coefficient and selectivity are the most important parameters that govern solvent selection:

- The distribution coefficient (K), as mentioned previously, for a component (A) is defined as the ratio of the concentration of A in the low-density phase to that in the high-density phase. Table 21.1 compares the efficiency of two solvents, acetone and acetic acid, on four different components. It shows that the distribution coefficients of benzene, butyl acetate, and hexane are higher with acetone, which means that this solvent is more efficient for the extraction of these components, while acetic acid is more adapted for the extraction of methyl acetate.
- Selectivity can be defined as the ability of the solvent to pick up the required component in the second phase as compared to other components. For example, polar solvents are selective for polar molecules.

The desired properties of solvents are a high distribution coefficient, good selectivity toward the solute, and little or no miscibility with the feed solution.

Other factors affecting solvent selection are boiling point, density, interfacial tension, viscosity, corrosiveness, flammability, toxicity, stability, compatibility with product, availability and cost.

For an existing process, replacing the solvent is usually a last resort because this would involve going back to a laboratory screening of the solvent and process optimization. However, changes in environmental regulations and economic considerations often induce improvements in processes in relation to solute recovery. In addition, the availability of specialized and proprietary solvents that score over conventional solvents in terms of performance and

Table 21.1. Distribution coefficients for the extraction of different components from the aqueous phase (water) using acetone or acetic acid as solvent (Robbins and Cusack 2003).

Component	$K_{acetone}$ (30°C)	$K_{acetic\ acid}$ (30°C)
Benzene	0.862	0.0984
Butyl acetate	1.127	0.705
Methyl acetate	1.153	1.273
Hexane	0.343	0.0167

economy for several extraction processes can provide further incentives for a change of solvent.

The temperature, pH, and duration of extraction can also influence the yield and selectivity. Operating pressure has a negligible effect on extraction performance and therefore most extractions take place at atmospheric pressure. Elevated temperatures are sometimes used in order to keep viscosity low and thereby minimize mass-transfer resistance. In some agrochemicals (e.g., orthene), pH is maintained to improve the distribution coefficient and minimize degradation of the product. Sometimes, the solvent itself may participate in undesirable reactions under certain pH conditions (e.g., ethyl acetate may undergo hydrolysis to acetic acid and ethanol in the presence of mineral acids). The duration of extraction is an important parameter in reactive extraction processes and in processes involving short-life components (e.g., the pigment carotene).

Even though LLE is frequently replaced by other solvent-free techniques, it is still used in flavor and aroma analysis, especially for the collection of preliminary data.

López and Gómez (2000) addressed some operational parameters in the application of LLE to extract aroma from wines. They compared several solvents (diethyl ether, n-pentane, freon-11, 1:1 ether/pentane, 1:1 ether/hexane, and dichloromethane) for the extraction of odorant terpenic components from "artificial wine" (12% by volume [v/v] ethanol in water). It was concluded that dichloromethane and 1:1 ether/pentane are the best solvents for the extraction of these analytes from wine.

Today, the reference technique for the extraction of volatile components from wine is continuous LLE (Villen and others 1995, Zhou and others 1996). In this method, all volatile compounds (low, medium, and high volatility) have a high partition coefficient to the organic phase. However, it requires solvent evaporation, which in some cases involves loss or degradation of some of the components and formation of others that were not originally in wine (Ortega-Heras and others 2002). In a related study, continuous LLE was employed by Rocha and others (2000) to extract free and releasable aroma from Portugal Bairrada white grapes. The sample (250 milliliters [mL]) and the extraction solvent dichloromethane (75 mL) were placed in an extractor at 50°C and the extraction was carried out for 25 hours. The dichloromethane extracts were cooled to −20°C to separate the frozen water from the organic phase and the organic phase was then dried and concentrated by distillation at low pressure prior to GC/mass spectrometry (MS) analysis. Chemometric analysis of the data allowed differentiation of several grape varieties and of the enzyme treatment studied.

Petersen and others (1998) compared the aromas of raw and boiled potatoes using a mild extraction. In this study, the LLE was carried out on a water suspension prepared from a mixture of 150 grams (g) of peeled shredded potato and 300 g of water. Then 200 g of suspension was gently treated with 100 mL 1:1 diethyl ether/pentane, and extraction was carried out on a magnetic stirrer for 40 minutes. Stirring was done at a low rate (200 revolutions per minute [rpm]) to avoid mixing the phases, since this would create an emulsion. After extraction, the flask was cooled and the nonfrozen organic phase was dried and concentrated under gently blowing nitrogen. GC/MS and GC-sniffing analysis of the extracts showed that the change in aroma during the boiling of potatoes depends on compounds both from lipid oxidation and from other types of reactions, for instance the Strecker degradation.

Such extractions, using organic solvents, are simple and direct but there are several valid objections to this method:

- The high-purity solvents are expensive. The organic solvent must be scrupulously purified (Ferreira and others 1993) because if the solvent is not clean, it could create an artefact.
- The perfect solvent does not exist and no solvent can extract all the compounds. For example, López and Gómez (2000) showed that the use of diethyl ether, which is employed as an extractor

of aroma from wine, does not result in a very high recovery of the following compounds: ethyl butanoate, 2-methyl propanol, 3-methyl butanol, hexanol etc., in comparison with freon 11, and dichloromethane. The method that is used does not extract all the compounds that it claims to.

- In general, the extracts are analyzed by GC, by injection in split mode, by getting rid of the excess solvent. This means that to reach the sensitivity required, the substances that are being quantified often have to be concentrated. During this concentration step, discrimination processes can take place. Not only can the impurities from the organic solvent be concentrated, but also only some of the compounds will be concentrated and some of the highly volatile substances will be lost. Thus, not all of the compounds claimed to be present will still be there (Grob and Muller 1987).
- Moreover, many organic solvents are toxic, inflammable, and pollutant.

All of these objections can be avoided if another type of solvent is used—supercritical fluids.

Supercritical fluid extraction (SFE): The high cost of organic solvents, the increasing public awareness of the health, environmental, and safety hazards associated with the use of organic solvents, as well as the possible solvent contamination of the final products, have all underlined the need for the development of new and clean technologies for the processing of food products (Mohamed and Mansoori 2002).

SFE is regarded as a promising alternative technique to other more conventional extraction procedures, chiefly because the dissolving power of supercritical fluids can be adjusted by regulating the pressure and temperature conditions employed. Carbon dioxide is the most commonly used supercritical fluid in the food industry because of its low critical temperature and pressure (T_c = 31.1°C and P_c = 72.8 atm). Moreover, this fluid is nontoxic, nonflammable, chemically stable, and available at high purity at low cost (Diaz-Maroto and others 2002). It is an inert gas that does not react with food constituents and is easily removable from the extract. This explains why carbon dioxide (CO_2) has been widely used for the aroma analysis of different food products (Polesselo and others 1993, Saito and others 1991, Mau and others 2003). In fact, one of the main advantages afforded by SFE is the retention of volatile substances at temperatures below 0°C using a CO_2-based cryogenic system and the transfer of analytes from the extraction solvent (supercritical CO_2) to the reconstitution solvent without the need for aggressive solvents and treatments (Larrayoz and others 1999).

The selection of the extraction pressure and temperature range is an important factor affecting the final composition of the extract and process yield, since the solubility of every component in the fluid will depend on these parameters.

Sonsuzer and others (2003) optimized the isolation of aroma compounds from *Thymbra spicata*, a thyme-like plant, using SFE extraction. The parameters to optimize were temperature (40, 50, and 60°C), pressure (80, 100, and 120 bar) and time (30, 60, and 90 minutes). Dependent variables were yield and monoterpene, sesquiterpene, and oxygenated mono-terpene contents. The most significant parameter was pressure. An increase in pressure increased the extraction yield. An increase in temperature produced a decrease in the solubility of oil components. The extraction of sesquiterpene and oxygenated compounds was more difficult due to their higher molecular weight and polarity, respectively, as compared to monoterpene.

Supercritical CO_2 is a poor extractor for polar substances. Therefore, for such analytes, the addition of modifiers or the use of other fluids, such as supercritical water, is advisable. Kubatova and others (2001) compared water-SFE and CO_2-SFE with hydrodistillation for the separation of aromatic essential oils from savory and peppermint. They showed that water-SFE was highly selective for polar oxygenated flavor compounds compared to CO_2-SFE and hydrodistillation. Extraction of savory with supercritical water removed almost 100% more thymol and carvacrol and 150% more borneol and linalool than hydrodistillation.

Solid-Phase Extraction

Solid-phase extraction (SPE) is defined as a process for extraction of the analyte from a liquid matrix to a solid support (adsorbent) by adsorption. The adsorbents used in SPE are bonded silica and various polymeric resins of varying hydrophobicity and with different selectivities. This technique can be directly applied to isolate odorants from liquid or liq-

uefiable samples, such as beverages, fruit pulp, and tissues. The procedure of extraction by SPE consists of four stages (Figure 21.1):

1. Conditioning of the adsorbent by an appropriate solvent
2. Introduction of the studied liquid matrix through the adsorbent
3. Recovery of the first type of analyte by passing an appropriate solvent
4. Recovery of the second type of analyte by another solvent

The advantage of this technique in comparison to LLE is that the recovery of analytes is done with a small quantity of solvent. Thus the obtained extract needs almost no concentration, which is necessary with LLE. The sensibility of SPE is very high.

SPE can be directly applied to isolate volatiles from liquid or liquefiable odoriferous samples, such as beverages, fruit pulps, and tissues. A typical contemporary application of SPE to aroma analysis was presented by Lopez and others (2002), who studied the extraction of minor and trace volatile compounds in wine. Wine samples (50 mL) were passed through a 200-milligram [mg]-SPE cartridge filled with Lichrolut-EN resin from Merck. The elution was carried out with 1.3 mL of dichloromethane. The extracts were directly analyzed by GC-Ion Trap-MS without further concentration. Twenty-seven important wine odorants, such as volatile phenols, vanillin derivatives, aliphatic lactones, nor-isoprenoids, minor esters and terpenols, were extracted and quantified. The recovery in the SPE isolation was higher than 90% for many extracted volatiles, except for guaiacol, vanillin, 2,6-dimethoxyphenol, and 4-vinylphenol.

SPE has also been applied to characterize butter aroma. Adahchour and others (1999) assessed the use of SPE cartridges packed with different sorbents, C18-, C8-, NH_2-, CN bonded silica, and SDP-1 (PS-OVB copolymer), to extract aroma compounds from this material after melting the butter and separating the aqueous phase from the fat. After desorption with 500 μL of methyl acetate, 1 μL aliquots were quantified and/or identified by GC/MS. The best overall recoveries were obtained with SDB-1 cartridges (80% average recovery).

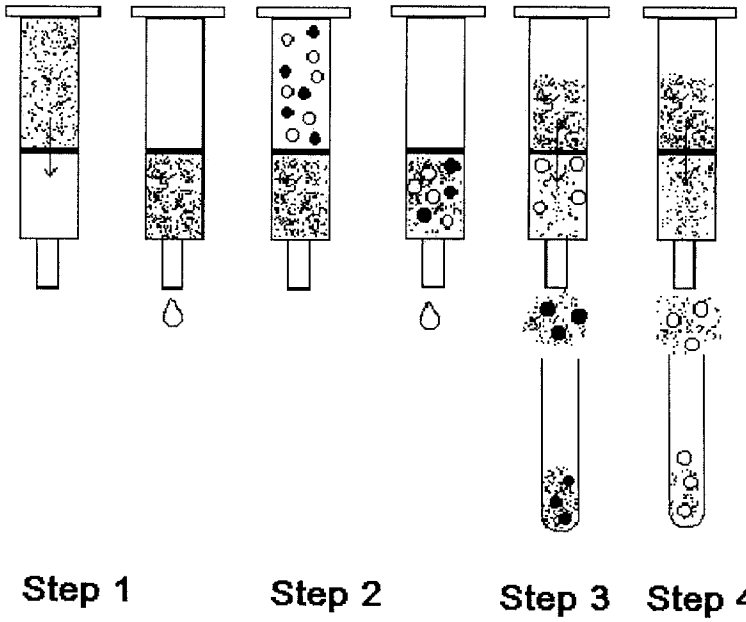


Figure 21.1. Fours stages of Solid-Phase Extraction: Step 1, Conditioning of the adsorbent by an appropriate solvent; Step 2, Introduction of studied liquid matrix; Step 3, Elution of interferences with an appropriate solvent; and Step 4, Elution of analytes by another solvent.

For samples such as fruits or other solids, SPE can be combined with isolation techniques such as distillation. Boulanger and others (2000) used the XAD-2 resin to recover the volatiles extracted previously by distillation from cupuassu pulp. The main resins were identified as linalool, α-terpineol, 2-phenylethanol, myrcene, limonene, ethyl-2-methylbutanoate, ethyl hexanoate, butyl butanoate, (E)- and (Z)- 2,6-dimethyl-octa-2,7-dien-1,6-diol, 2,6-dimethyl-oct-7-en-2,6-diol, and methoxy-2,5-dimethyl-3-(2H)-furanone.

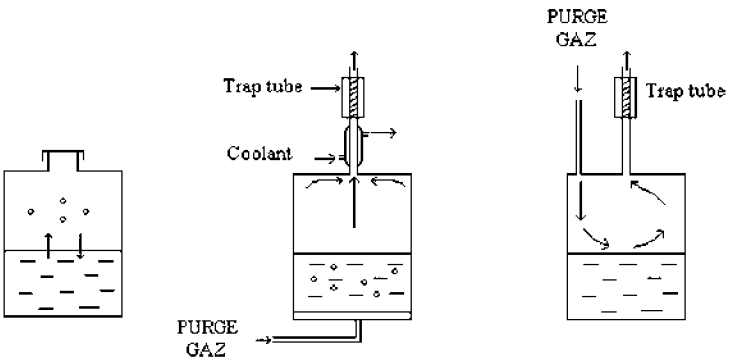
Mehinagic and others (2003) combined SPE with vacuum hydrodistillation in order to concentrate the aqueous extract obtained from this isolation technique on fresh apple fruit. The aqueous extract was passed through styrene divinylbenzene-based (ENVI Chrom P) SPE columns. The adsorbed volatiles were then recovered by elution with pentane/diethyl ether and analyzed by Gel Permeation Chromatography (GPC)-Flame Ionization Detector (FID) and GC/MS. In this study, SPE was used as a concentration technique and was compared to another concentration technique, liquid-liquid extraction. The study showed that both techniques gave very similar results, although the SPE was limited by the saturation of the SPE columns.

Headspace Extraction

Headspace (HS) extraction can be defined as a method used for obtaining information about the composition, the nature, or state of liquid and solid bodies by analysis of the gas phase with which they come into contact (Ioffe 1984). This technique has been applied widely in different scientific domains like medicine (Johansen and Felby 2000), geohydrology (Roe and others 1989), environmental science and technology (Cummins and others 2001), but the biggest application is still in food science (Macku and others 1988, Wang and others 1996, Young and Hovis 1990).

The technique of HS extraction of volatiles can be divided into three groups (Figure 21.2):

- Static HS: This procedure involves the equilibration of the volatile analyte within the sample with the vapor phase at a defined temperature. The method requires rigid control of the sample temperature, sample withdrawal, and other parameters like time and pressure (Nunez and others 1984). The concentration of the analyte in the phase does not change once the state of equilibrium is attained. However, the state of equilibrium is disturbed temporarily upon sampling. Therefore, the volume of the sample as well as the method of withdrawal must be chosen with precaution. The samples analyzed by this procedure can be liquid and solid solutions.
- Purge-and-trap HS: This procedure involves passing a carrier gas through the liquid material for a selected period of time. Generally, this gaseous effluent is passed through a suitable trapping medium, inert to the stripping gas,



a) static headspace b) purge-and-trap c) dynamic headspace

Figure 21.2. Diagram of three different methods of headspace extraction.

where the volatiles are trapped and subsequently eluted by a solvent or thermally into the gas chromatograph. This procedure is suitable for liquid products.

- Dynamic HS: This procedure is close to the purge-and-trap procedure except that the gaseous effluent is passed over and not through the material. The equilibrium between the condensed and gaseous phases depends on the flow of the stripping gas. The surface contact between gas and material is less and it may take longer for the analysis. However, this technique is suitable for extractions on solid matrixes that cannot be solubilized.

Static headspace: According to Raoult's law, in a closed system where a volatile component is in thermodynamic equilibrium between the liquid phase and the gaseous phase, the following equation holds (Soto 1994):

$$P_i = P^0_i \, x_I \gamma_I$$

where P_i = partial vapour pressure of the volatile component *i*, P^0_i = saturated vapour pressure of the pure component *i*, x_I = mole fraction of component *i* and γ_I = activity coefficient of component *i* in solution.

The value of the activity coefficient is constant when the concentration of component *i* in the gaseous phase is below 1% and Raoult's law can be simplified to Henry's law:

$$C_L/C_G = k$$

where C_L = concentration of the component in the liquid phase after equilibrium, C_G = concentration of the component in the gas phase after equilibrium, and k = the partition coefficient for the component. The k value is constant for a dilute analyte, a constant temperature, and an identical liquid matrix. Partition coefficients differ according to not only the properties of the analyte (polarity, molecular weight, volatility) but also the properties of the food matrix, which is complex and consists of water, proteins, lipids, polysaccharides, and salts.

The static HS technique fails when trace components or components with very low vapor pressure are analyzed. In this case, the concentration of the analytes in the gaseous phase can be increased by:

- Increasing the temperature: In general, raising the HS temperature decreases the partition coefficients (Kieckbusch and King 1979), with a linear relationship between *k* and the reciprocal of temperature (Przyjazny and others 1983). This relationship differs from one compound to another. The more soluble the volatile substance, the greater the change in solubility with a given temperature change. However, raising the temperature can increase the artefacts due to the enhanced chemical reactions in food samples.
- Adding salt: The solubility of many volatiles decreases in the presence of salts (Soto 1994). This phenomenon, known as the "salting-out" effect, produces an increase in the value of the activity coefficients (γ_i) with increasing salt concentration. The addition of salts causes a decrease in hydrogen bonding between the analyte and water as free water is sequestrated by the tight hydration shell surrounding the salt ions (Soto 1994).

Other factors can affect the extraction of volatiles:

- Increase the volume of the sample in the headspace vial to maximize the sensitivity of the method.
- Increase the equilibration time.
- Mix: the equilibrium will be attained more rapidly if the liquid phase is mixed during extraction.

The simplest way to analyze the composition of an aroma is by direct analysis of the portion of the air in contact with the odor source, without other sample treatments. However, the application of this technique is limited by a number of factors including low sensitivity (Stevenson and others 1996).

Nevertheless, when techniques and devices with adequate detection limits are available, and depending on the concentration of the analytes, static HS can be particularly suitable because of its simplicity. For example, Clarkson and Cooke (1996) showed that this technique was more efficient (detection limits and analysis time were better) than dynamic HS for the extraction and analysis of aroma compounds in the HS of commercial cigarettes.

Dynamic HS extraction: The static HS methods have several advantages. They are fast and the sample is hardly manipulated. However, their main disadvantage is their low sensitivity (Wampler 1997). The dynamic HS methods are becoming increasingly popular, and many authors have applied them to study the aromatic composition of food products. The dynamic procedure involves passing an inert

gas through or above the sample and collecting the stripped volatile constituents in a trap. The equilibrium between the food and the headspace is constantly removed resulting in improved sensitivity (Buttery and others 1996). This is why this method is more sensitive than static HS or some other extraction methods.

Variations of the dynamic HS method include:

- Sample purging, where the volatiles are bubbled or swept with a carrier gas
- Sample trapping, where the purged volatiles are trapped by physical (cold) or chemical adsorption (using various adsorbents, e.g., Tenax trap)
- The relative temperature and duration of purging and trapping
- The desorption method, either choice of solvent or thermal desorption

The choice of trapping method will influence the quality of analysis. Thus, cold traps have the disadvantage that water is collected with the volatile material. The most common sorbent traps are charcoal and porous polymers. The properties of charcoal enable a very wide range of organic compounds to be adsorbed from a sample because of its high specific surface area and resistance to heat up to 700°C without significant changes in structure. However, the use of this sorbent has some limitations that should be taken into account when HS extraction is performed:

- These adsorbents have a strong affinity for water, as opposed to nonpolar polymeric substances such as Tenax and graphitized carbon blacks.
- The temperature required for desorption is so high that some compounds are decomposed.

The most commonly used porous polymeric sorbent in flavor analysis is Tenax TA, a porous polymer resin based on 2,6-diphenyl-*p*-phenylene-oxide. This trap is designed specifically to capture volatiles and semivolatiles from gaseous, liquid, or solid phases.

Desorption of trapped analytes for subsequent analysis can be performed either by elution, with a small quantity of an appropriate solvent, or by using online automated desorption devices, which are more suitable for routine analysis. The choice of desorption method is dependent on the volatile compounds to be extracted. Buttery (1993) studied the liberation of *Z*-3-hexenal from fresh tomatoes using dynamic HS extraction with a Tenax trap. To elute trapped compounds, ether was used rather than the more common thermal desorption method, which could cause molecular rearrangement of *Z*-3-hexenal. However, thermal desorption has several advantages compared to solvent desorption, including improved detection limits, no interference of the solvent peak on chromatograms, and being a simpler procedure. Moreover, desorption can be automated, which allows the analysis of the evolution of different products over time. Dirinck and others (1989) used this technique, coupled with GC/MS, to study the aroma development in apples during the complete ripening process. They showed that in apples, aroma compounds, mainly esters, are formed gradually during ripening and can be used for prediction of the optimal harvest times for apples. They also showed that the dynamic HS sampling of the volatiles from intact fruits is a convenient procedure for the study of the influence of different external and internal factors on aroma development in ripening fruits.

Agelopoulos and others (1999) studied the temporal emission pattern of the volatile compounds released by the leaves of potatoes and broad beans by using dynamic HS coupled to GC/MS. The volatiles were trapped in Tenax TA traps and desorbed either thermally or by a solvent (diethyl ether). They showed that thermal desorption provided better detectability of volatile compounds than solvent desorption and therefore required reduced sampling times. The introduction of artefacts produced by degradation of the sorbent can be a major difficulty in dynamic HS extraction coupled to thermal desorption. Canac-Artega and Vaillou (2000) found that the interaction of volatile compounds, the trapping material, and water vapor can produce artefacts during the analysis of the aromas of dehydrated cheese and Parmesan cheese.

Distillation

If the level of extraction of volatiles is too small with HS extraction (e.g., when the product is rich in lipids), extraction by distillation is used. Steam distillation and hydrodistillation are traditional distillation procedures for the isolation of volatile aromatic compounds from food and detached parts of plants. Being simple and straightforward procedures, they

are still extensively applied for flavor characterization either alone or combined with other procedures. Distillation is usually carried out in two slightly different ways, as follows:

- In the first, the matrix to be extracted is mixed or suspended with water in a suitable vessel fitted with a condenser and, while the mixture is boiled, a condensate phase is collected. This procedure is called hydrodistillation. After the process, an organic, water-insoluble fraction can be separated from the aqueous phase.
- In the second procedure, steam is passed through a vessel containing a mixture of the matrix in water to yield a similar condensate. This procedure is called steam distillation.

Simultaneous Distillation Extraction

The analysis of flavors and fragrances requires the isolation of the volatile fraction from the matrix prior to GC analysis: direct extraction methods with solvent (LLE and supercritical fluid extraction) co-solubilize the nonvolatile components, which contaminate the injectors and limit the possibility of concentration.

Direct vacuum distillation followed by solvent extraction and concentration is tedious because of the high volume to be handled and the different steps that are time-consuming and affect the yields.

SDE, also known as the Lickens-Nickerson (1964) method (Figure 21.3) is a widespread distillation-based sample preparation method for the chemical analysis of fragrance and aroma.

The Lickens-Nickerson apparatus has been used on different products and modified by a number of laboratories, but the principle is the same (Schultz and others 1977, Godefroot and others 1981, Blanch and others 1993). A sample, along with distilled water, is contained in flask 1, and flask 2 receives a suitable volume of an extracting solvent denser then water (dichloromethane, chloroform, etc.). Flasks 1 and 2 are heated; the solvent and water vapors are conducted to the extractor body (3); where they are condensed over the surface of the cold tube (4). In this operation, aroma compounds are removed from the matrix by water vapor directly and transferred to the organic phase when the liquids condense together. Both water and solvent are collected in the extractor body after condensation and then return to their flasks, allowing continuous reflux. A modified apparatus allows the use of extracting solvents less dense than water, such as pentane or diethyl ether.

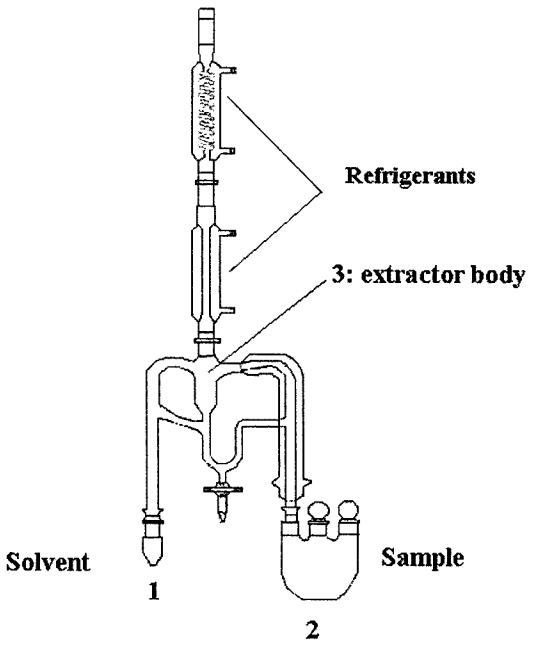


Figure 21.3. Lickens-Nickerson apparatus.

Factors affecting the extraction of volatiles by SDE are:

- Choice of solvent: Its density and selectivity (the same rules as described in section LLE are adopted in this case) are considered.
- Temperature: Both the sample and the solvent are heated in order to evaporate water from them from the flask and the temperature of heating for the sample must be carefully selected.
- Duration of extraction: Some odorant compounds can be destroyed and other compounds produced if the extraction is too long.

Andrade and others (1999) used SDE to analyze the aromatic composition of 15 varieties of cultivated mango, originating from different regions. After removal of the skin and kernel, the freshly macerated pulp of mango fruits (100 g) was mixed with water (20 mL) and submitted to SDE for 3 hours using pentane (2 mL) as the organic mobile phase. Then the organic phase was analyzed by

GC/MS. The data analysis showed that mango cultivars could be classified into three aroma groups. The first group rich in α-terpinolene (eight varieties, originating from Sri Lanka, Australia, and Florida), the second rich in δ^3-carene (three varieties, originating from Venezuela), and the third group rich in myrcene and (Z)-β-ocimene (four varieties, originating from India and Sri Lanka, respectively). Similar studies were carried out on the jackfruit (*Artocarpus heterophyllus Lam.*) grown in the Amazon region. Maia and others (2004) studied the difference between the aroma composition of "hard" and "soft" jackfruits. They used the same extraction procedure (3 hours of SDE with pentane as organic solvent) and analyzed the organic solvent by GC/MS analysis. The study showed that the aroma concentrate of "hard" fruits was dominated by isopentyl valerate (28.4%) and butyl isovalerate (25.6%) while the aroma concentrate of "soft" fruits was dominated by isopentyl isovalerate (18.3%), butyl acetate (16.5%), and ethyl isovalerate (14.4%). These differences were compatible with the fruits' morphological variation and previous observations. Another interesting study was described by Blanch and others (1996). They investigated the potential of the SDE technique for the rapid enrichment of wine aroma compounds. Several aspects concerning the extraction and concentration of volatiles from aqueous-alcoholic samples were studied, and three different operating modes were explored: SDE at normal pressure, SDE at reduced pressure, and SDE involving the concentration of dynamic headspace due to purging the sample with an inert gas. They concluded that the three investigated operation modes were suitable for the aroma analysis of wine. SDE at normal pressure provided recoveries ranging from 79 to 100% for some compounds previously reported as wine aroma constituents (e.g., isoamylacetate, ethyl hexanoate, terpinolene, 1-hexanol, and benzaldehyde). This operation method, however, demands a higher sample temperature than the other two operating modes, which is an important aspect to consider if thermolabile solutes are analyzed.

Various authors have also noted problems with SDE of the low recovery of compounds having high volatility, analyte losses with artefact formation and oxidation of flavor components not found by extraction-high vacuum distillation isolation (Farkas and others 1997, Stevenson and others 1996). Thus, Siegmund and others (1997) discovered that 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine, usually pointed to as an important aroma active compound in cooked and cured meat aromas, is an artefact formed during the distillations performed according to the Lickens-Nickerson SDE method.

Vapor Distillation

Vapor distillation can be carried out under atmospheric or reduced pressure in order to avoid the thermal degradation of products. The principle is the same: volatile compounds as well as water vapor are liberated from the product and led into one or more cooled traps. The extraction under atmospheric pressure is interesting because the rate of extraction of volatiles is higher and the compounds are directly condensed in water. However, the products must be heated so that the water can be evaporated and this produces many artefacts (degradation of some labile compounds, such as esters and lactones, and formation of new compounds). This technique is therefore reserved for the extraction of essential oils from spices and aromatic or medicinal plants. Saritas and others (2001) isolated the essential oils from aromatic lichens by hydrodistillation of fresh and dried plant parts. Combining GC/MS and ^{13}C-NMR techniques, they identified several terpenoid and aliphatic compounds in their extracts, including two unreported sesquiterpenes.

For other products, this technique is not recommended. It is preferable to use vacuum hydrodistillation. Figure 21.4 shows one of the pieces of apparatus proposed by Forss and Holloway (1967) for vacuum hydrodistillation used in the extraction of aroma from butter oil (Forss and Holloway 1967). This apparatus has also been used for the extraction of volatiles from fresh products such as apples (Mehinagic and others 2003) or mussels (Le Guen and others 2000). A quantity of the fragrance-generating sample, along with distilled water, is contained in flask 1 while flask 2 receives most of the condensed water cooled by the system of refrigeration. Some very polar compounds are condensed in flask 2 with water vapor and others are captured in traps plunged into liquid nitrogen at −196°C.

The application of this technique to model solutions showed that it can extract more than 80% of substances with boiling points lower than 150°C at concentrations of less than 1 parts per million (ppm) (Forss and Holloway 1967). Vacuum hydrodistillation is very interesting for studying fresh products

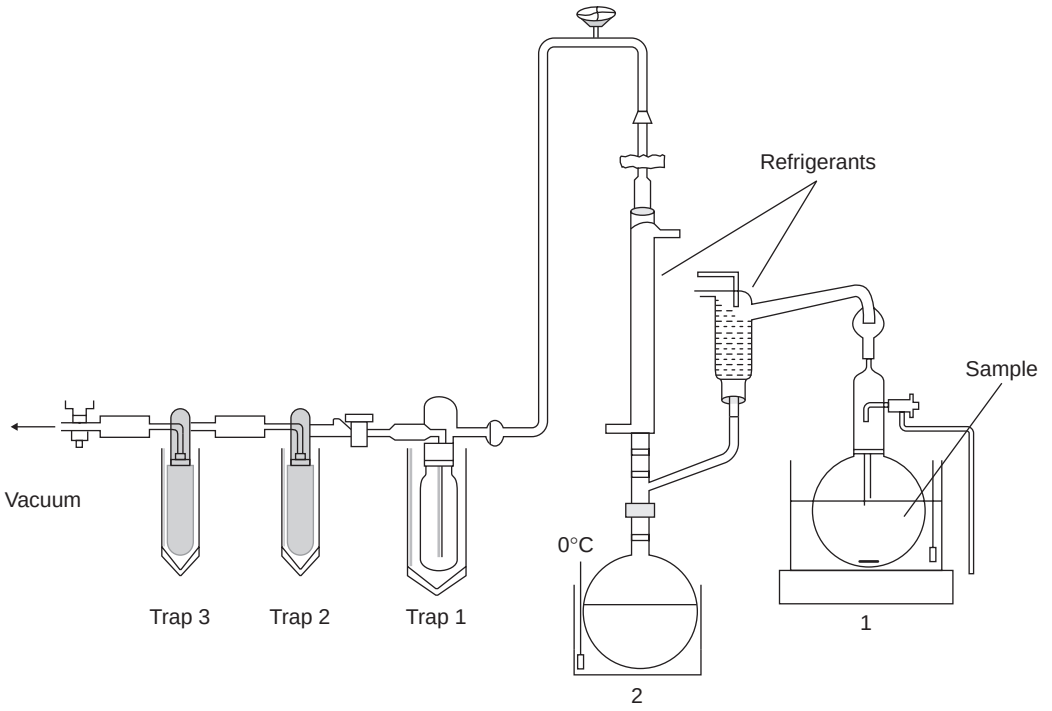


Figure 21.4. Apparatus for Vacuum distillation proposed by Forss and Holloway (1967).

because the sample does not need to be heated. In fact, in order to avoid cooked notes and artefacts, flavor analysis of fresh products, which are eaten in the raw state, needs to be carried out in a very gentle way. Güntert and others (1998) compared three extraction methods: vacuum hydrodistillation, dynamic headspace, and the simultaneous distillation extraction method on different fruits, including yellow passion fruit, strawberry, raspberry, pear, cherry, etc. They found that vacuum hydrodistillation shows clearly superior performance on fruits with respect to the sensory impression of the resulting extracts. Sensory evaluation of the vacuum headspace extracts of various fruits led to very fruit-typical descriptions and, consequently, the qualitative and quantitative flavor patterns of the analyzed fruits represented the genuine fruit flavors with no artefacts.

Moio and others (2000) combined vacuum hydrodistillation and LLE to identify key odorants from Gorgonzola cheese. Gas chromatography-olfactory (GC-O) analyses of distilled extracts concentrated by LLE showed that 2-nonanone, 1-octen-3-ol, heptanol, ethyl hexanoate, methylanisole, and 2-heptanone were the most relevant odorants in the aromas of natural and creamy cheeses.

Analysis

As stated earlier, the choice of the technique of isolation of volatiles depends on the physical and biochemical properties of the product, as well as on the molecular characteristics of the volatiles to be isolated. However, it must not be forgotten that the obtained extracts will be analyzed by different techniques that necessitate different conditions too. Characterization techniques fall into two categories: those based on separation science and those that simulate the entire sensory response. The first category comprises liquid and gas chromatography. The second one comprises sensory techniques, which use trained sensory panels to characterize volatiles, and electronic noses, which use arrays of gas sensors

with an associated pattern recognition technique for identification and quantification of complex mixtures of volatiles.

GC is suited to this role due to its excellent separating powers and extreme sensitivity (Eiceman and others 1992, Stevenson and others 1996). High-resolution columns (typically 25 meters [m] or longer) are mandatory in most applications using relatively nonpolar phases although more polar phases may assist with difficult separation.

Gas Chromatograph

GC is basically a separation technique, and the separation of compounds occurs when the extract is injected into a mobile phase (inert gas). The mobile phase carries the injected extract through a stationary phase, which is composed of chemicals that can selectively attract compounds. Every gas chromatograph includes a source of gas as the mobile phase, an inlet to deliver sample to a column, the column where separation occurs, an oven as a thermostat for the column, a detector to register the presence of a chemical in the column effluent, and a data system to record and display the chromatogram (Eiceman 2000). These parts of the chromatograph have been unchanged for over the last 40 years and the arrangement of the components of the chromatograph is presented schematically in Figure 21.5. The concept of GC as well as its instrumentation are discussed in detail in the literature (Jennings 1987, Eiceman 2000). Each of these components contributes to the overall efficiency of a GC separation. The carrier gas must be chemically inert and purified from moisture and oxygen because most columns do not tolerate these impurities when operated over 100°C. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of the detector, which is used. The suite of gas

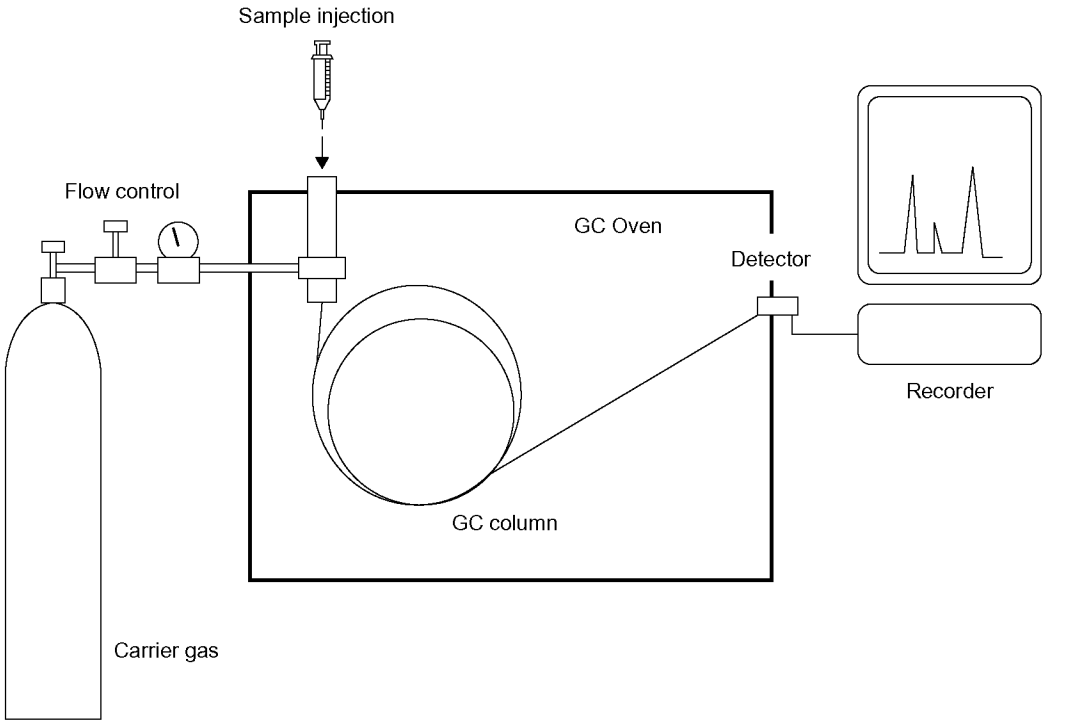


Figure 21.5. Diagram of gas chromatography.

chromatographic detectors includes the flame ionization detector (FID), thermal conductivity detector (TCD), electron capture detector (ECD), thermoionic detector, photoionization detector (PID), flame photometric detector, and some more unusual and expensive detectors like the atomic emission detector (AED). The most popular detector used in flavor and fragrance analysis is the FID because of its high sensitivity and universal applicability. Alternatively, selective detectors such as the nitrogen-phosphorus and the flame photometric detector can be used to detect nitrogen and sulphur compounds respectively in complex mixtures. These detectors can be used separately or in conjunction with the normal detector. High-resolution columns (typically 25 m or longer) are mandatory in postapplications using relatively nonpolar phases although more polar phases may assist with difficult separation (Sides and others 2000). The elution characteristics of a gas through a column of this type are quite complex and are affected by the flow of the inert gas, the chemical characteristics of the organic compound, the changing temperature, and the characteristics of the surface to which the gases bind.

Characterization of Compounds

The principle of GC is that volatile compounds, passed over a stationary phase, to which they have some tendency to bind, will be "slowed" compared to a gas, which passes over the same surface, but has no tendency to bind. The time that it takes for a compound to pass through the column is called its retention time. Under constant GC conditions, the retention time of a compound remains constant. This retention time is characteristic of the component and therefore it can be used to identify the component. Most frequently absolute retention time is unreliable, and so relative retention times are often calculated. Relative retention time is obtained by relating the retention time of an unknown compound to that of a standard compound or a series of standard compounds. The most commonly used system is that developed by Kovats and others (1958), who used n-paraffins as standards. These compounds have by definition an index equal to the number of carbon atoms multiplied by 100. The original Kovats indices were developed by using isothermal conditions. McRaynolds constants (McRaynolds 1966) are similar to Kovats indices but were developed by using temperature/programmed analysis. Jennings and Schibamoto presented a list of McRaynolds constants for a group of common flavor and fragrance compounds (Jennings and Schibamoto 1980).

The gas chromatography step provides considerable information about the identification of a compound. Tabulated lists of retention times for thousands of compounds are available both in book form and in computer libraries. However, the gas chromatography cannot completely characterize a compound, since more than one substance may have the same retention time.

A more definitive answer is obtained by coupling the gas chromatograph technique with mass spectroscopy, which is capable of providing a great deal of additional information about the eluted substances. In practice, while there are many pairs of compounds with similar retention times and many pairs of compounds with similar mass spectra, the combination of retention time and mass spectra usually provides a definitive identification of a compound.

The principle of mass spectroscopy is that a gaseous compound can be ionized and partially fragmented by an electron beam, and the mass to charge ratios (m/z) of the resulting charged fragments can be measured. All mass spectrometers have five major components including an inlet system, ion source, mass analyzer, detector, and signal processor. The first four components are under vacuum at high pressure that exceeds 10 millimeters of mercury (mmHg).

The inlet system's function is to introduce a small amount of sample (1 micromole or less) into the ionization source with a minimum loss of vacuum. The GC will serve as an inlet system, but it will also facilitate the separation of different compounds from the extract before their introduction to the mass spectrometer and ionization. During the most common ionization process, electron-impact (EI) ionization, the sample is bombarded with a stream of electrons that breaks the molecular chemical bonds. The resulting fragments (ions) are accelerated into a mass analyzer (most commonly magnetic or quadruple analyzer) and the ions are separated according to their abundance along a mass scale. Finally, a bar graph spectrum is generated. Many excellent books giving more details about this technique are available (Watson 1985, McFadden 1973, Silverstein and others 1974).

Because compounds fragment in a set pattern according to their chemical structure, the mass spec-

trum of the unknown component can be identified by comparing it to a library of known spectra. Mass spectral libraries are available commercially (e.g., Wiley and National Institute of Standards and Technology [NIST]). Unfortunately these spectral collections do not contain many volatile compounds important to flavor analysis and that is the reason why many companies develop their own spectral libraries.

Many studies have used GC/MS to analyze the complex aroma of all food products, such as wine (Villen and others 1995), vegetables (Andrade and others 2000, Rocha and others 2000, Petersen and others 1998), or dairy products (Badings and Neeter 1980, Gallois and Langlois 1990, Sablé and Cottenceau 1999). However, it must be remembered that many, if not most, of the volatile compounds in a typical chromatogram are not aroma active. Volatile compounds that contribute to aroma can be localized in the gas chromatogram of the flavor extract and determined on the basis of their odor activities by GC-O (Scheiberle 1991). This method involves simultaneous "sniffing" of the effluent from the GC column and identifying of the eluting compounds by odor. In the 1980s, the following two principal GC-O methods were developed to divide volatiles into odorants with high aroma impact and into volatiles with secondary importance (presumably nonodor active): Combined Hedonic Aroma Response Measurement (CHARM) (Acree and others 1984) and Aroma Extract Dilution Analysis (AEDA) (Urlich and Grosch 1987). AEDA is performed with few trained assessors (two or three). The stepwise-diluted extracts of a sample are sniffed until the dilution at which no compounds are perceived anymore (Cerny and Grosch 1992, Holscher and Steinhart 1992). While CHARM analysis is usually carried out by 8 to 10 assessors who are also asked to give additional information about the intensity and the duration of the olfactometric perception of each compound (Prost and others 1998, van Ruth and others 1996). These techniques are useful tools for a precharacterization of all volatile compounds. However, rank orders of the intensities of odor-active compounds received from two methods may diverge (Abbott and others 1993) and the obtained results must be interpreted with precaution (Stephan and others 2000).

In addition, some more appropriate GC-O methods are described in literature like Osme (Guichard and others 1995, McDaniel and others 1990) or the finger span method (Etievant and others 1999), which give information about the perception of odorants from the medium air. Thresholds in air are responsible for the classification of the aroma impact of the volatiles.

CONCLUSION

A suitable choice of an isolation technique is essential for the accurate, reliable characterization of the chemical composition of fragrance and aroma. Some of the general techniques discussed in the previous sections, LLE, distillation, and SPE, are still employed occasionally for fragrance and aroma analysis. However, the current tendency is to replace these techniques with technologies that are simultaneously less aggressive to analytes and capable of dealing with ultra-low concentrations of analytes in samples. The contemporary applications are focused on several variations of dynamic HS or solid phase microextraction (SPME) sampling, or more recently on HS-SPME.

The extracted compounds are identified generally by gas chromatography, which gives a wide range of possibilities. The combination of gas chromatography with mass spectrometry in the 1950s was a breakthrough in analytical aroma research and the beginning of the identification of a multitude of volatiles from different food products. By addition of a sniff-detector, the GC-O technique made it possible to characterize the volatiles and to identify odor active compounds.

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22
Recent Developments in Flavor Measurements

Jean-Luc Le Quéré

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INTRODUCTION

Measuring aroma means analyzing volatile compounds that are sensed in the nose at the olfactory receptors either via the orthonasal (odor) or retronasal (aroma) routes when foods are eaten. Instrumental analysis of aroma molecules has been the subject of important specialized treatises (for the most recent literature on the subject see (Ho and Manley 1993, Marsili 1997, Mussinan and Morello 1998, Stephan and others 2000, van Ruth 2001a, Reineccius 2002, Deibler and Delwiche 2004). The aroma compounds are mainly hydrophobic and instrumental analysis of volatiles must, therefore, consider, as a first step, an extraction method suitable for separating these volatiles from the food matrix. Part of this chapter will focus on recent advances made with sorptive extraction methods: solid-phase microextraction (SPME) (Lord and Pawliszyn 2000) and stir bar sorptive extraction (SBSE) (Baltussen and others 1999).

As no single method yields a "true" picture of a food aroma (Reineccius 2002), isolation and analysis of aroma remain challenging (Teranishi 1998). Moreover, the extraction step may lead to artifacts, and the total volatile content in most cases is very difficult to relate to a sensory profile determined by a panel or experienced by a consumer. Therefore, it appears much more efficient to concentrate efforts on the identification of those compounds that are really relevant to the perceived flavor. Because no universal extraction method exists, it appears essential to choose a method that yields an extract representative of the sensory properties of the food (Abbott and others 1993a, Etiévant and others 1994, Etiévant and Langlois 1998). In order to identify the key volatiles that contribute significantly to food flavor, gas chromatography coupled with olfactometry (GC-O) is very often used (Leland and others 2001, Lee 2003). GC-O and associated extract representativeness will be part of this chapter.

However, it is particularly difficult to predict an aroma perception as it is still not known how the various volatiles combine to produce an overall sensory impression. Moreover, interactions between taste and aroma (Noble 1996) and interactions of trigeminal sensations with taste and aroma (Green 1996) occur and play an important role in global flavor perception. However, methods that allow direct analysis of flavor molecules released in the mouth

during consumption have been developed in the last years (Taylor and Linforth 1996, Roberts and Taylor 2000). Development of instrumental techniques and data obtained recently for volatile flavor compounds will be presented with the aim to explain more closely the relationship between aroma perception and food volatiles composition.

Finally, specific instrumental techniques have been developed for the global analysis of food flavor. The methods currently used in quality control of food flavor are still usually based on sensory evaluation by a panel of experts. These panels are able to monitor the quality of a particular food, to detect defects, and to compare samples for classification purposes. Nevertheless, obtaining fast results at low cost using instruments could be advantageous. The so-called "electronic noses" based on gas sensor technology, despite some important drawbacks for some of them (Schaller and others 2000a), are theoretically able to perform some classification tasks (Schaller and others 1998), and some applications for the analysis of foodstuffs have been developed (for instance see Mariaca and Bosset 1997, Schaller and others 1999 for cheese applications). Two other global analysis methods based on mass spectrometry (MS) seem interesting for classification purposes. The first one analyzes total headspace using a mass spectrometer, without any prior GC separation (Vernat and Berdagué 1995). This method is often referred to as a mass-based electronic nose. Alternatively, headspace sampling may be replaced by solid-phase micro extraction (SPME) of food volatiles (Marsili 1999). Both sampling methods, followed directly by mass spectrometry, have found applications for the rapid characterization of foods like cheese, for example (Schaller and others 2000b, Pérès and others 2001, Pérès and others 2002a). The second method is pyrolysis mass spectrometry (Aries and Gutteridge 1987), where a small food sample is pyrolyzed at up to 500°C. The resulting volatile fraction, characteristic of the flavor but also of the matrix composition, is analyzed by a mass spectrometer. For all the rapid instrumental methods used for classification, a pattern or fingerprint is obtained for each sample, and extensive data treatment, either by conventional multivariate statistics or artificial neural networks, is necessary for classification and quality control purposes (Aries and Gutteridge 1987, Pérès and others 2002b).

SORPTIVE EXTRACTION METHODS

All the extraction procedures used to isolate the volatile fraction from the food matrix should be adapted to the analysis of trace levels of lipophilic molecules generally present in a polyphasic medium, while minimizing losses of highly volatile molecules and preventing modification of compounds or the formation of artifacts.

Solid-phase microextraction (SPME), first developed for the extraction of volatile organic compounds in water (see Lord and Pawliszyn 2000 for a recent review of the technology), has been applied recently to the isolation of aroma compounds from food (Harmon 1997, Kataoka and others 2000, Pillonel and others 2002, Reineccius 2002). SPME partitions analytes between a liquid or a vapor phase and a thin solid-phase adsorbent, of which there are several choices in terms of polarity and film thickness (Kataoka and others 2000), coated on inert fibers, generally associated with a syringe that serves as a direct injection device (Harmon 1997, Kataoka and others 2000). The method, which is an equilibrium method, can be performed either in the direct extraction mode (immersion of the fiber in sample matrix, generally in an aqueous solution or suspension) or in a headspace configuration. It can be automated very easily, but the extraction of the solutes depends on polarity, volatility, partition coefficients, sample volume, temperature, and the nature of the adsorbent-coating material. Therefore, the technique exhibits a certain degree of selectivity, but with the advantages of sensitivity, ease of use, no solvent, and small sample volume (Harmon 1997, Kataoka and others 2000, Pillonel and others 2002, Reineccius 2002). Nevertheless, each extraction step, i.e., extraction mode (direct or headspace), selection of fiber coatings, extraction setup (concentration, time, agitation, temperature), desorption, gains through a careful optimization for each application (Bicchi and others 2000, Kataoka and others 2000, Ferreira and de Pinho 2003). SPME, used for the first time for the analyses of food volatile compounds in the mid 1990s (Yang and Peppard 1994, Pelusio and others 1995, Chin and others 1996), has since been used in significant applications on food aroma (Kataoka and others 2000, Pillonel and others 2002, Le Quéré and Etiévant 2003, and references

cited therein). Analyzing volatiles directly by immersion of the fiber in highly complex matrices could damage the fiber, and SPME is therefore used almost always in the headspace mode. Comparison of direct SPME and headspace SPME of Camembert cheese volatiles obtained after cryo-trapping of the aqueous phase under vacuum showed only a slight reduction in sensitivity using headspace SPME compared to direct SPME (Jaillais and others 1999). Compared to other headspace extraction procedures, it is very often concluded that SPME is more appropriate for routine quality control due to its simplicity, repeatability, and low cost (Cavalli and others 2003). That is probably why the method has been widely used in recent works on food aroma (Le Quéré and Etiévant 2003 and references cited therein).

The main limitation of SPME is the relatively low extraction yield due to the relatively small amount of sorbent available on the syringe needle (typically approximately 0.5 μL). A novel extraction technique that uses up to 200 μL of the sorbent PDMS (polydimethylsiloxane) was developed to overcome this drawback (Baltussen and others 1999). The new technique called stir bar sorptive extraction (SBSE) consists of a glass-coated magnetic stir bar with a cylindrical coating of PDMS (typically 5 millimeter [mm] film thickness, 10 mm length) that is spun in an aqueous medium for a predefined time (Baltussen and others 1999). After completion of the extraction step, the stir bar is transferred in a thermodesorption system and desorbed at the head of a GC column after cryo-refocusing of the extracted material (Baltussen and others 1999). A complete set of coated stir bars (called Twister(tm)) and a thermodesorption system are commercially available from Gerstel GmbH (Gerstel, Müllheim on the Ruhr, Germany). As expected, the recoveries were higher for SBSE than for SPME (Baltussen and others 1999), and the detection limits were found in the low nanograms per liter (ng/L) range for a wide selection of volatile and semivolatile analytes (Baltussen and others 1999). SBSE has been used for the measurement of volatiles from a wide variety of liquid foods (Pillonel and others 2002, and references cited therein) including recently brewed coffee (Bicchi and others 2002) and wine (Kittel and others 2004).

As an extension to SBSE, headspace sorptive extraction (HSSE) has been developed (Tienpont and others 2000) to overcome the limitation of headspace-SPME in terms of extraction capacity. Limits of detection in the ng/L range have been obtained for the analyses of volatiles of some food samples (Tienpont and others 2000). HSSE bars coated with approximately 55 μL PDMS (commercially available from Gerstel GmbH) are suspended in the headspace of the sample (Bicchi and others 2002) and after sampling completion, the bars are thermally desorbed in a thermal desorption unit connected to a gas chromatograph (Bicchi and others 2002). As expected, when comparing headspace extractions of coffee (Bicchi and others 2002) and olive oil (Cavalli and others 2003) volatiles, HSSE bars showed a higher concentration capacity than SPME fibers due to the higher amount of polymeric coating. However, like SBSE, HSSE needs a thermal desorption unit to be handled and therefore requires a significant investment, compared to SPME if used in manual mode (automation is possible at cost price). Nevertheless, because SBSE and HSSE coated bars are less subject to deterioration than SPME fibers, they can be applied easily to the analyses of both headspace and liquid (Bicchi and others 2002). Direct thermal desorption of food samples is also possible (Pfnür and others 2003), but seems more suited to the extraction of semivolatile compounds (Cavalli and others 2003).

KEY AROMA COMPOUNDS

Representativeness

As already outlined, because there is no universally applicable method, none of the extraction techniques described here or in the previous chapter yields an aroma isolate that truly represents either qualitatively or quantitatively the aroma profile of a food (Reineccius 2002). It is therefore necessary to choose the isolation procedure best suited to address the problem faced: determining the complete aroma profile, identifying key odorants or off flavors, monitoring aroma changes with time in foods or prediction of sensory properties (Reineccius 2002). When the ultimate aim of a particular study is the identification of the compounds that are important for flavor (the key odorants), the most reliable results will be obtained if the odor of the extract resembles closely that of the food itself (Etiévant and others

1994, Etiévant and Langlois 1998). It is for instance advisable to prevent oxidation during the extraction step by addition of a suitable antioxidant, especially when oxidation of sensitive compounds may alter the odor of the extracts (Escudero and Etiévant 1999). Different sensory methods, which necessitate a trained sensory panel, can be used to check the sensory representativeness of the food extract odors (Etiévant and others 1994). When an estimation of the relative importance of key constituents in a single sample is required, a similarity test is preferred. The panelists are asked to score the similarity of the odor of the extracts obtained by different methods to the odor of the food itself used as reference on an unstructured 10-centimeter (cm) scale. This approach was applied to various foodstuffs including wine (Abbott and others 1993a, Etiévant and others 1994, Bernet and others 1999), cheese (Etiévant and others 1994, Le Quéré and others 1996), ham (Guillard and others 1997), butter (Guyot and others 1998), tomato (Etiévant and Langlois 1998), champagne (Escudero and Etiévant 1999), coffee (Sarrazin and others 2000), vinegar (Charles and others 2000), mussels (Le Guen and others 2000), oyster (Pennarun and others 2002), edible algae (Le Pape and others 2004), black currant (Boccorh and others 2002), and apple (Mehinagic and others 2003). The similarity test can be completed by a descriptive analysis of the extracts (Moio and others 1995, Le Quéré and others 1996, Priser and others 1997, Guyot and others 1998, Sarrazin and others 2000) or even by a quantitative descriptive analysis (QDA) of the extracts compared to a QDA of the food samples (Abbott and others 1993b, Le Guen and others 2000, Pennarun and others 2002, Mehinagic and others 2003, Le Pape and others 2004).

When different food samples have to be compared, triangle tests and overall matching tests are preferred. The different samples are presented as control samples and extracts from the samples, presented in random order, have to be matched with controls. This approach was initially done on beer extracts (Abbott and others 1993b). A key point in these evaluations of representativeness is the choice of a suitable matrix for testing the olfactory character of the extracts. For fat-containing food like cheese or butter, the best results have been obtained when the extracts are added to an emulsion, i.e., a matrix similar to food in terms of fat composition (Etiévant and others 1994, Guyot and others 1998). Since, generally, a combination of techniques should be used to obtain a reasonably complete view of an aroma profile (Reineccius 2002), it is noteworthy that substantial efforts have been made recently toward sensory evaluation of headspace or SPME extracts. Thus, oyster volatiles desorbed from the Tenax trap used in dynamic headspace have been collected in flasks containing water (Pennarun and others 2002). Edible red algae volatiles have been desorbed from the dynamic headspace Tenax trap and collected in evacuated brown flasks (Le Pape and others 2004), and solvent-free extracts from apples have been collected by preparative gas chromatography in Teflon bags half filled with nitrogen (Mehinagic and others 2003). The easiest and most promising technique in this field is probably "direct GC-olfactometry" (i.e., without a chromatographic column) where a complete headspace or SPME extract is directly evaluated at the sniffing port of a gas chromatograph (Lecanu and others 2002). This has been recently applied for cheese (Lecanu and others 2002), orange juice (Rega and others 2003a, 2003b) and apricot extracts (Guillot and others 2003).

Gas Chromatography-Olfactometry

The separation technique that uses a human nose as a detector and known as gas chromatography-olfactometry (GC-O, sometimes referred to as "GC-sniffing"), has received considerable attention during the past 20 years in aroma research (see for example Blank 1997, Leland and others 2001, Reineccius 2002, Lee 2003). The selectivity of this specific detector is based only on the odorous properties of the individual compounds separated by high-resolution gas chromatography. As the most abundant volatiles may have little, if any, odor of significance in a food (Mistry and others 1997), GC-sniffing has been an invaluable tool for identifying key compounds in aroma extracts.

The first aim of the technique is to discriminate the odorous compounds from the many background volatile components. The so-called "aromagram" constructed from the chromatogram obtained by simply smelling a GC effluent (Blank 1997, Reineccius 2002) is a potential interface with sensory analysis, as odor descriptors detected at the GC sniffing port can be compared to the descriptors generated in sensory evaluation of the original food.

This method is particularly efficient for identifying off flavors. Selection of key odorants or character-impact compounds in a food is another objective of GC-sniffing. Quantitative approaches (the true GC-olfactometry) based on odor detection thresholds or on odor intensity have been developed and are the subject of specialized treatises (Mistry and others 1997, Leland and others 2001, van Ruth 2001b, Reineccius 2002, Lee 2003).

Three different methods have been developed for GC-olfactometry: (1) dilution analyses based on determination of detection thresholds, (2) detection frequency methods, and (3) intensity measurement methods. Original dilution methods, CHARM (for combined hedonic aroma measurement) analysis developed by Acree and coworkers (1984) and aroma extract dilution analysis (AEDA) developed by Grosch and coworkers (Ullrich and Grosch 1987), are essentially screening methodologies since the methods, based only on detection thresholds determination, violate certain sensory rules, and psychophysical laws (Grosch 2001, Reineccius 2002, and references cited therein). They can be used to identify those single odorous compounds that are most likely to contribute to the complex odor of a food. Originally developed by McDaniel and coworkers (McDaniel and others 1990), the Osme method is basically a cross-modal technique aimed at measuring the perceived odor intensity of eluting volatiles. In OSME and other cross-modality matching methods (Guichard and others 1995, Etiévant and others 1999), results are not based on odor detection thresholds, and only one concentration of the extract is evaluated by a panel, contrarily to dilution methods where several dilutions of the extract are evaluated. Results can be subjected to statistical analysis and more consistent results are obtained when panelists are trained (Callement and others 2001). The detection frequency methods, originally developed by Roozen and coworkers (Linssen and others 1993), and referred to as nasal impact frequency (NIF) or surface of nasal impact frequency (SNIF) since the work of Chaintreau and coworkers (Pollien and others 1997), also use a group of assessors who simply have to note when they detect an odor in a single GC run (i.e., also at only one concentration). The GC peaks being detected as odorous by the greatest number of assessors are considered to be the most important. Not being based on real odor intensities, the method has important drawbacks, especially when all the odorous compounds are present above their sensory threshold for all the assessors (Reineccius 2002). However, a study on gewürztraminer wines from Alsace showed that the odor intensities measured by OSME using the finger-span cross modality matching method were well correlated to the detection frequencies (Bernet 2000, Etiévant and Chaintreau 2001). According to these authors, the theoretical saturation limitation when using detection frequencies is practically reachable for only 10% of the detected odors (Etiévant and Chaintreau 2001).

Nevertheless each of the methods described above has its advantages and weaknesses. Only two studies have compared all the methods for their performance (Le Guen and others 2000, van Ruth and O'Connor 2001). In both cases, the results obtained with the different techniques were found to be very similar and well correlated. Finally, the choice of a GC-O method depends on the objective of the study, on the quality of the panel and on the time scheduled for the analyses (Le Guen and others 2000). Dilution techniques are clearly time-consuming, intensity methods give better results with a trained panel (Le Guen and others 2000, Callement and others 2001) while detection frequency methods are the least demanding, but also the least precise (Le Guen and others 2000). A comparative critical review may be found in Etiévant and Chaintreau (2001).

The aim of any GC-O experiment is to determine the relative odor potency of volatiles present in an aroma extract or fraction and to prioritize compounds for identification then usually performed using GC coupled to mass spectrometry (GC/MS). Mass spectrometry is also used for quantification purposes through the use of a stable isotope dilution assay (Milo and Blank 1998, Blank and others 1999, and references cited therein). Such a precise quantification is required for the determination of odor activity values (OAVs) generally calculated when using AEDA (Grosch 1994, 2001). OAVs, calculated as the ratio of concentrations to odor thresholds, despite their limitations in terms of psychophysical validity (Mistry and others 1997), give a good indication of the respective contributions of key odorants to the aroma of foods. They are the basis of the first attempts of using recombination studies to validate impact odorants sensorially in model foods (Grosch 1994). Aroma-recombination studies are the important last step in sensorially validating

the analytical data obtained by GC-O and for quantification of key odorants of food (Mistry and others 1997). Many examples of models used in recombination experiments may be found in a recent review (Grosch 2001). Preparation of aroma models was found simpler for liquid foods than for solid foods, because in that case it is not easy to reproduce the composition and distribution of the nonvolatile fraction of the food matrix (Grosch 2001). However, for cheese models for instance, either bland unripened cheese (Grosch 1994, Preininger and others 1996, Kubickova and Grosch 1998) or specially designed odorless model cheese (Salles and others 1995) have been successfully used to incorporate potential key odorants. Thus, the branched-chain volatile fatty acids, 4-methyloctanoic and 4-ethyloctanoic acids, were confirmed to be essential to the typical goaty note of goat cheese (Le Quéré and others 1996) and their retronasal aroma thresholds were determined using a cheese model (Salles and Le Quéré 1998, Le Quéré and Salles 2001, Salles and others 2002). GC-O allows odor evaluation of individual compounds, but a large loss of sensory properties is encountered when odorants are mixed (Grosch 2001). To understand the perceptual interactions of odorants, recombination studies in model foods or psychophysical experiments are necessary. Masking or enhancing properties of important odorants may be evaluated during GC-O with a new method called OASIS for original aroma simultaneously input to the sniffing port (Hattori and others 2003). It consists in evaluating complex odors at the sniffing port by delivering an original aroma directly at the sniffing port while a GC-O experiment is running. It is then possible to know how the individual components separated in the GC and sensed at the sniffing port influence the original odor notes. The authors have demonstrated that even high odor threshold compounds may affect an original Japanese green tea aroma (Hattori and others 2003).

The GC-O methods that have been developed during the past 20 years, combined with aroma extracts, headspace, or even SPME (Dufour and others 2001), have facilitated the identification of potent odorants in numerous foodstuffs. Hundreds of scientific papers have been published on the subject and it is out of the scope of this chapter to review all of them.

DYNAMIC METHODS FOR FLAVOR CHARACTERIZATION

Supposing that the "best" extraction and identification methods are used, trying to correlate the overall levels of flavor components in a food to the sensory perception experienced when eating this food is very often unsuccessful. In other words, it is not enough to know the exact composition of food in terms of flavor compounds to understand perfectly the perception of its flavor. Perception of flavor is a dynamic process (Piggott 2000). During the consumption of food, the concentration of aroma compounds at the olfactory epithelium varies with time as they are released progressively from the food matrix during chewing. Release kinetics depends on the nature of the food matrix and on individual mastication patterns. Sensory methods, such as time-intensity, have been used to study the time-related aspects of flavor perception (Piggott 2000).

Release of Volatiles *in vivo*

Methods that measure volatiles directly in the mouth or in the nose have been developed to obtain data that could reflect the pattern of aroma molecules released from food and that are effectively present at the olfactory receptors during consumption, reviewed recently in an edited book (Roberts and Taylor 2000). Among the various approaches aimed at sampling aroma from the nose (nosespace), the collection of expired air samples on Tenax® traps provided the first robust results (Linforth and Taylor 1993, 1994). Applied to cheddar cheese, GC-O of buccal headspace showed a number of volatile compounds that have been suspected to contribute primarily and most likely to the original flavor (Delahunty and others 1996). It was presumed that the buccal headspace extract was representative of the aroma compounds that a consumer perceives during consumption (O'Riordan and Delahunty 2001).

By overlapping the sampling time periods, release curves can be constructed and temporal changes reflecting relative concentrations of volatiles at a particular moment during consumption can be determined (Linforth and others 1996). Correlation of accumulated data with sensory time-intensity data has been demonstrated (see for instance Delahunty and others 1996).

Real time *in vivo* flavor release was demonstrated some time ago using mass spectrometric breath-by-breath analysis with an optimized membrane separator interfaced to a mass spectrometer operated in electron impact mode (Overbosch 1987). Sensory time-intensity data measured in parallel for the perception of 2-pentanone in vegetable oil showed a clear adaptation effect, the stimulus being present in exhaled air long after the perception ended (Overbosch 1987). The method was also used by Soeting and Heridema (1988) and was extensively reviewed by Overbosch (Overbosch and others 1991). However, membrane separator techniques suffer from selectivity problems and from a clear lack of sensitivity (Taylor and others 2000).

More recently, atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been developed to monitor aroma release during chewing (reviewed by Taylor and others 2000). Air from the nose (nosespace) is sampled directly into the APCI-MS source through an interface making real time breath-by-breath analysis routinely possible (Linforth and others 1996, Taylor and Linforth 1996, Roberts and Taylor 2000, and references cited therein). Therefore, by combining time-intensity sensory studies with nosespace analysis, it is now possible to relate temporal parameters of aroma release to perception (de Kok and Smorenburg 1999, Linforth and others 2000, Salles and others 2003). Perceptual interactions of aroma with sapid compounds may also be studied by this method through controlled delivery of both aroma and sapid molecules to panelists (see Cook and others 2004 for recent published results). The APCI-MS method has been extensively reviewed in detail in specialized treatises (Roberts and Taylor 2000, Taylor 2002, Taylor and Linforth 2003). APCI sources may also be connected to a tandem mass spectrometer (MS-MS) such as an ion trap with the selectivity and structural capabilities benefits of MS-MS (Haahr and others 2003, Sémon and others 2003).

Another powerful chemical ionization method is proton-transfer-reaction mass spectrometry (PTR-MS). Originally developed by the Lindinger group (Lindinger and others 1993) for on-line trace gas analysis, it consists of a three-chamber system. In the first chamber, a nearly pure H_3O^+ ion is generated by electrical discharges in H_2O vapor. A small electric field drives H_3O^+ ions through an orifice into a drift tube, where chemical ionization takes place, while neutral volatiles are introduced into the drift tube. Volatile compounds with proton affinities exceeding that of water (166.5 kilocalorie per mole [kcal/mol]) ionize by proton transfer from H_3O^+ and are accelerated into the third chamber, the mass spectrometer. Specificity of PTR-MS compared to other chemical ionization approaches is that the generation of the reactant ion and the chemical ionization process are spatially and temporally separated. Individual optimization is therefore possible and quantification is made easier (Yeretzian and others 2000a). Since the early works on headspace flavor volatiles that used PTR-MS (Yeretzian and others 2000a, 2000b), applications developed rapidly and the first specific international conference was organized in 2003 in Innsbruck, Austria (First International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications, January 18–23, 2003). Results may be found in the current literature on headspace (Blank and others 2003, Mayr and others 2003), nosespace (Mayr and others 2003, Roberts and others 2003, Roberts and others 2004), and model mouth (van Ruth and others 2003, van Ruth and others 2004) applications.

In vitro Measurements: Model Mouth Systems

Mechanical devices that aim to mimic the processes that occur in the mouth during eating have been developed (Piggott 2000, and references cited therein). These "model mouths" are often variants of dynamic headspace analysis, but their aim is to obtain time-resolved data similar to those obtained during *in vivo* studies. The various parameters like temperature, airflow, mastication rate, and addition of artificial saliva can be varied to study their effects on volatile flavor release (van Ruth and others 1995, van Ruth and Roozen 2000, Rabe and others 2002). The main advantages of model mouths are the large quantities of food samples that can be handled, overcoming some sensitivity problems encountered when monitoring volatiles at low concentrations (Taylor 2002), and the suppression of inter-individual variations, always encountered *in vivo*, that can be detrimental to a robust interpretation of the data. Release of volatile flavor compounds from the retronasal aroma simulator (RAS), originally developed by

Roberts and Acree (1995), has been compared with flavor release *in vivo* using APCI-MS detection in both cases (Deibler and others 2001). While delivering higher concentrations of volatiles than from human breath, the RAS gave a good approximation of time-averaged flavor release in the mouth, with volatile compounds present at similar ratios (Deibler and others 2001). The model-mouth device originally developed by Roozen and coworkers (van Ruth and others 1994) has been used to investigate the relationships between the gross, nonvolatile, and volatile compositions and the sensory attributes of Swiss-type cheeses (Lawlor and others 2002). Eight flavor attributes were found to be correlated with subsets of volatiles, amino acids, free fatty acids, and gross compositional constituents, with, for instance, the nutty flavor of Emmental that was positively correlated with the concentrations of propanoic acid, ethyl acetate, and 2-pentanone (Lawlor and others 2002). In a recent study, the model mouth system has been compared to the RAS in terms of the effects of oral physiological characteristics on the release of aromas as a function of the physicochemical properties of model emulsions (Geary and others 2004). Both have been found suitable for the study of oral parameters on aroma release (Geary and others 2004) with confirmed limits for the temporal dimension of the release (Deibler and others 2001, Geary and others 2004). Real time data, comparable to those obtained *in vivo*, have been obtained recently with a computerized apparatus that follows the temporal dimension of flavor release from liquid food (Rabe and others 2002, Rabe and others 2003).

Flavor release and flavor perception are dynamic processes and must be studied using dynamic methods (Piggott 2000). Dynamic techniques have been developed to study the parameters of flavor release from foods. Parallel increased applications of dynamic sensory methods provide a better understanding of food flavor. However, further work is needed to improve our knowledge of various interactions arising at different levels in the process of food consumption, such as, interactions among food ingredients (Taylor 2002), and interactions at the perceptual levels such as taste-aroma interactions (Noble 1996, Given and Paredes 2002, Taylor 2002), or trigeminal interferences (Green 1996, Given and Paredes 2002), as these play a fundamental role in overall flavor perception.

GLOBAL AND FAST ASSESSMENT OF FLAVOR

The methods currently used to evaluate and control the quality of food flavor are still essentially based on sensory evaluation by a panel of experts. These trained panels are able to handle such difficult tasks as quality monitoring through descriptive analysis, off flavors detection, and comparison of samples for classification purposes. It could be interesting for such tasks to substitute humans by instruments that could give quicker answers at reduced costs.

"Electronic Nose"

Evaluation of aroma release from food using gas sensors, the so-called "electronic noses," is theoretically feasible (Mielle 1996, Hodgins 1997, Schaller and others 1998). Electronic noses are generally composed of arrays of nonspecific gas sensors that are based on different physical principles (Mielle 1996, Hodgins 1997, Schaller and others 1998). The most common sensors are semiconducting metal oxides and conducting organic polymers, and they all give rise to nonspecific responses with typical patterns. Therefore, pattern recognition software, using either standard multivariate data analyses or artificial neural network technology, must be used for data treatment and final presentation of the results (Hodgins 1997, Schaller and others 1998). The electronic nose is particularly attractive for quality control applications where conformity/nonconformity answers are expected (Mielle 1996). Discriminative studies have been conducted on all types of food with some success (Schaller and others 1998, Monge and others 2004, and references cited therein). However, some problems occurred with the repeatability of the system that could be possibly related to the product itself, the sampling technique, or the moisture content of the air used for sampling, precluding its use in routine tests (Schaller and others 1998). Metal oxide semiconductor technology, despite some poisoning problems affecting the sensors, seems more reliable than conducting organic polymer sensors that showed a poor sensitivity to volatile components, the main problem of these sensors lying however with their instability (Schaller and others 2000a). However, recently, the ripening of Danish Blue cheese was successfully monitored by means of an electronic nose that contained 14 conducting polymer (polyaniline)

sensors; results were found to be highly correlated to those of sensory analysis and GC-MS analysis of volatile compounds during a 5- to 12-week ripening period (Trihaas and others 2003). The close control of the experimental sampling conditions (quality of dry air with a humidity <0.5% and equilibration time at controlled temperature) might explain this success (Schaller and others 1998). Nevertheless, despite some success in some classification tasks when using perfectly controlled sampling conditions (see for instance Monge and others 2004, for a recent application on flavored pectin gels), electronic noses hardly meet the requirements of the food industry in terms of precision, reproducibility, sensitivity, and stability (Mielle and others 2000). Moreover, the sensors are known to deteriorate or can be poisoned, therefore changing their response. Even with frequent calibration, the inherent weaknesses of the technique make perenniality of the built databases problematic. Giving a global response, these instruments cannot be used to identify single odorants or to differentiate samples with subtle differences in distinctive sensory attributes. Therefore, in off flavor studies where identification of the off flavor compound is a prerequisite and in quality control assessment they may be used successfully only after recognizing their inherent weaknesses (Mielle and others 2000, Reineccius 2002).

Mass Spectrometry-based Systems

For classification purposes, two other global and fast analytical methods, based on mass spectrometry, have been used for food products and seem more powerful and reliable than electronic noses. The first consists of a global analysis of a headspace sample by an MS operated in electron ionization mode, without any GC separation (Vernat and Berdagué 1995, Pérès and others 2003). The feasibility of the method was originally demonstrated for rapid classification of four rather different French cheeses (Vernat and Berdagué 1995). This method is often referred to as a "MS-based electronic nose" (Schaller and others 2000b). The mass patterns obtained, considered as fingerprints of the food products analyzed, also need data treatment, either by conventional multivariate analyses or artificial neural networks. SPME may be used as a preconcentration technique instead of headspace sampling (Marsili 1999). Applied to rapid characterization of cheeses, SPME has been demonstrated to be a very efficient preconcentration technique, superior to dynamic headspace analysis in terms of repeatability, simplicity, and compatibility with an autosampler (Schaller and others 2000b). However, contradictory results have been published when SPME (Pérès and others 2001) yielded less satisfactory results than those obtained by dynamic headspace analysis (Pérès and others 2002a). The better performance of the dynamic headspace method in that case was attributed to the absence of signal drift (aging of the SPME fibers causes drift, as demonstrated by Pérès and others 2001) and to automation of the sample injection into the mass spectrometer, a problem that can be easily overcome by automating the SPME injection. A new review on the subject has recently appeared (Pérès and others 2003).

Developed in the 1980s for food applications, direct pyrolysis-MS is another method that delivers "fingerprints" that can be used for classification/authentication purposes (Aries and Gutteridge 1987). With this method, a tiny food sample is pyrolyzed rapidly at up to 530°C and the resulting volatile fraction, characteristic of the flavor but also of the matrix breakdown, is analyzed immediately by a mass spectrometer operated in low energy electron ionization mode. Here again a complex mass pattern is obtained for each sample and several data preprocessing steps are often necessary to select a reduced number of mass fragments that allow satisfactory classification. Curie-point pyrolysis-mass spectrometry with associated multivariate data analysis techniques is considered as a powerful classification tool in microbiology for the recognition of microorganisms (Talon and others 2002, and references cited therein) and food science (Aries and Gutteridge 1987, Pérès and others 2002b, and references cited therein). A clear advantage of the method is that it provides a specific fingerprint of the food matrix that can be potentially related to textural parameters (Pérès and others 2002b). In a similar approach, the proton-transfer-reaction mass spectra (PTR-MS) of the static headspace of Mozzarella cheese were found to display a discrimination power comparable to sensory descriptive analysis (Gasperi and others 2001).

CONCLUSION

Recent developments in techniques used for flavor measurements include extraction methods based on

adsorption of aroma molecules on polymers. These sorptive extraction methods (SPME, SBSE, HSSE) are easy to use and fully automatable. Pertinent complementary sensory information can be obtained by combining gas chromatography of representative flavor extract with olfactometry. Nevertheless, the relationships among aromas present in a foodstuff and sensory perception of that food are not straightforward. It is still not well understood how the various flavor-active components combine to produce a particular sensory perception. Recent developments in dynamic instrumental methods that can follow the *in vivo* sequential release of the aroma molecules are valuable tools that can account for the time-dependent balance of the flavor compounds released from the food matrix in the mouth. With more complete and accurate information, combined flavor chemistry and sensory evaluation should help us understand the relationship between flavor stimuli and perceived flavor and explain the mechanisms of flavor perception.

Authentication of foodstuffs and routine fast analyses are other challenges. Tools developed recently that combine analytical instrumentation for global assessment of flavor with multivariate data analyses have demonstrated their usefulness for classification purposes.

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Part IV
Beef Quality

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23
Sensory Evaluation of Beef Flavor

Rhonda K. Miller

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INTRODUCTION

Beef flavor is an integral component of the overall acceptability of beef, and it is a very complex and dynamic sensory component. Initial beef flavor in a product changes with fresh and frozen storage, is influenced by microbial growth, is altered due to chemical components and their reactions that are inherent in muscle fibers or adipose cells, can be effected by the environment, is influenced by cooking and cooking method, and can be altered by suppressing or masking some components and enhancing other attributers. Beef flavor can be segmented into general categories of positive- and negative-flavor attributes. However, what truly constitutes a positive- and/or negative-attribute is very subjective and differs by individual. The major component of beef flavor—cooked beefy/brothy—can be generally agreed upon as the most important positive flavor attribute in beef flavor. It is the beefy/brothy flavor that is associated with beef and is the major flavor attribute in canned beef broth or in broth made from cooking beef in water. However, beef flavor is composed of multiple attributes and is a blend of aromatics, basic tastes, mouthfeel, and aftertaste. When consumers eat beef, they tend to identify beef flavor as one sensory component: beef flavor. However, when an off component of beef flavor is presented in beef, consumers can identify that "something is wrong" and some may be able to identify or express the attribute. So this is the challenge. In reality, beef flavor is a very complex sensory element. It is identified using three of the five senses, and it has multiple components. Chemically, quantifying the chemical constituents of beef flavor is very challenging as gas chromatograph and mass spectroscopy (GC-MS) can help identify thousands of individual chemical compounds that make up beef flavor. But to the consumer, beef flavor is one attribute.

In the sensory detection of beef flavor, there are multiple tools that can be used. Each tool has strengths and weaknesses, and the interpretation of the sensory results provides useful information. The key for the sensory professional is to understand the objective of their test and then to select the sensory tool that will adequately measure beef flavor without external biases. In this chapter factors affecting beef flavor and the sensory evaluation of beef flavor will be discussed. First, components of beef flavor will be defined, and then specific aspects of environmental and sample preparation will be discussed to assure that the sensory evaluation of beef flavor is not influenced by these factors. Then, the use of discriminative, descriptive, and consumer sensory tests to evaluate beef flavor will be presented.

COMPONENTS OF BEEF FLAVOR

Beef flavor is identified through the olfactory senses as odor and flavor aromatics, through the taste receptors on the tongue as basic tastes, and through the trigeminal nerve as mouthfeel. After consumption, aftertaste

and mouth-feel can also contribute to beef flavor. In addition, there are multiple factors that contribute that can potentially impact beef flavor. Table 23.1 presents a general beef flavor lexicon. This lexicon was first presented by Johnsen and Civille (1986) for detection of warmed-over flavor in beef or lipid oxidation components. The base lexicon has been adjusted to include attributes that have been used or identified in beef and is not all inclusive. The lexicon can be easily adjusted to a product specific basis using Civille and Lyons (1996) to include attributes that are product specific. Using this lexicon segments beef flavor into sensory components that assist in understanding the multiple facets of beef flavor.

Chemically, beef flavor is comprised of the beefy/brothy/lean component that is derived from the lean or muscle fiber of the beef cut. Beefy/brothy flavors are detected as odor and/or as flavor aromatics by the olfactory receptors in the nasal cavity. There are other flavors associated with the lean or muscle fiber component of beef. These odor and/or flavor aromatics also are volatile compounds and have been described as cowy, grainy, serumy/bloody, livery, browned, and burnt. The basic tastes of salt and sweet are generally recognized as derived from the lean of beef. Salts, such as sodium and potassium, are components of the muscle fiber and contribute to the salty basic taste of beef. Sweet basic tastes are derived from glycogen and glucose compounds in beef. For example, meat from species that are higher in glycogen is usually described as sweeter (i.e., horse meat is sweeter than beef).

Within the lean component of meat are heme-containing compounds, mainly myoglobin and some free iron, that are water-soluble compounds of the muscle fiber. These components have been associated with serumy and livery aromatics and metallic mouthfeel in beef. An example of this flavor in chicken is the higher serumy flavor of dark chicken meat versus white chicken meat. This same flavor difference can be identified between beef muscles.

The lipid component of beef also contributes significantly to beef flavor. The odor and/or flavor of cooked beef fat contribute to overall beef flavor. This lipid component is what is associated with the species-specific flavor or the flavor that identifies a meat piece as being beef and is referred to as cooked beef fat aromatic. This species-specific flavor is mainly due to the unique fatty acid composition of beef. Beef fat is not only associated with cooked beef fat odor and/or aromatic, but the fat component also can contribute other flavors as volatile compounds can be stored in the water-soluble component of lipid cells that are derived from the animal diet. These compounds may impart odor and/or flavor aromatics to beef flavor. These compounds are usually animal diet-derived and some descriptors are grassy, musty, boiled corn, and nutty. These aromatics are very diet-specific, and the presence of these types of compounds is easily influenced by seasonality, forage quality, soil conditions, and availability of alternate forages or feedstuffs.

The lipid component of beef also can be associated with off flavor that is a result of lipid oxidation. The aromatics associated with this chemical reaction are cardboardy, painty, fishy, and livery aromatics. Sometimes these flavor changes are consolidated into the term rancid or rancidity. However, as lipid oxidation proceeds in beef, cardboardy flavor aromatics usually slowly increase and with later stages of oxidation painty and fishy aromatics become apparent. Livery aromatics increase as lipid oxidation proceeds as well. Therefore, in beef products where changes in flavor are due to lipid oxidation, measuring individual attributes of flavor changes provides a more detailed description of the rate and extent of lipid oxidation.

Beef flavor also can be impacted by microbial growth. In aerobic packaging systems, spoilage microorganisms can impart putrid, rotten-egg, or sulfur type aromatics to beef. In vacuum-packaged beef, growth of spoilage microorganisms has been shown to increase soured aromatics. Other off flavors may be identified during fresh storage that affect beef flavor such as nutty, musty, acidic, buttery, and yeasty.

Environmental factors can affect beef flavor. A classic example of this is the effect of plastic flavors associated with packaging materials on beef. During storage in plastic films, beef can absorb plastic aromatics from the packaging materials.

Cooking and cooking methods can influence beef flavor. During cooking, serumy/bloody aromatics are slowly replaced by cooked beefy/brothy aromatics. Also, with increased degree of doneness, livery aromatics increase.

It is apparent that beef flavor is a highly complex entity and it can be influenced by a wide variety of factors. The sensory evaluation of beef flavor should

Table 23.1. Lexicon terms used to evaluate beef flavor originally developed by Johnsen and Civille (1986) and expanded with attributes from Civille and Lyons (1996).

Attribute	Description or definition of attributes
Odors	Defined as the volatile compounds detected by the olfactory senses in the nasal cavity during smelling
Cooked beef lean	The aromatic associated with cooked beef muscle meat
Cooked beef fat	The aromatic associated with cooked beef fat
Browned	The aromatic associated with the outside of grilled or broiled beef
Serum/bloody	The aromatic associated with raw beef lean
Grainy/cowy	The aromatic associated with cow meat and/or beef with grain/feed character is detectable
Cardboardy	The aromatic associated with slightly stale beef, refrigerated for a few days only and associated with wet cardboard and stale oils and fats
Painty	The aromatic associated with rancid oil and fat
Fishy	The aromatic associated with some rancid fats and oils
Livery/organy	The aromatic associated with beef liver and/or kidney
Flavor Aromatics	Defined as the volatile compounds detected by the olfactory senses in the nasal cavity during chewing the sample in the mouth
Cooked beef lean	The aromatic associated with cooked beef muscle meat
Cooked beef fat	The aromatic associated with cooked beef fat
Browned	The aromatic associated with the outside of grilled or broiled beef
Serum/bloody	The aromatic associated with raw beef lean
Grainy/cowy	The aromatic associated with cow meat and/or beef with grain/feed character is detectable
Cardboardy	The aromatic associated with slightly stale beef, refrigerated for a few days only and associated with wet cardboard and stale oils and fats
Painty	The aromatic associated with rancid oil and fat
Fishy	The aromatic associated with some rancid fats and oils
Livery/organy	The aromatic associated with beef liver and/or kidney
Basic Tastes	Defined as the compounds detected by the taste receptors on the tongue
Sweet	Taste on the tongue associated with sugars
Sour	Taste on the tongue associated with acids
Salty	Taste on the tongue associated with sodium ions
Bitter	Taste on the tongue associated with bitter agents such as caffeine, quinine, etc.
Mouthfeel	Defined as the senses detected by the trigeminal nerves in the mouth and throat that are feeling factors
Astringent	The chemical feeling factor on the tongue, described as puckering/dry and associated with tannins and alum
Metallic	The chemical feeling factor on the tongue associated with iron and copper
Aftertaste	Defined as any of the above sensory attributes or new attributes that leave residual sensations and can be flavor, basic tastes, mouthfeel, or other attributes after swallowing or expectorating a sample

include all components of beef flavor, provide an opportunity to describe flavor attributes that may emerge with time, and provide an environment that will not interfere in sensory detection of beef flavor attributes.

TESTING ENVIRONMENT AND SAMPLE PREPARATION

The testing environment and sample preparation can easily influence beef flavor. Controls for the sensory testing environment and sample preparation have been discussed in Chapter 6, Sensory Methodology of Muscle Foods. These components must be considered for all sensory testing, and a protocol must be defined and considered prior to any sensory evaluation, regardless of the sensory tool being used, but beef flavor can be easily influenced by storage and preparation. As the goal of any sensory evaluation, Tables 23.2 and 23.3 provide a checklist of factors to consider, standardize, or control in test samples for sample storage and sample preparation, respectively. Items presented in these tables are not all inclusive, but they are presented to provide guidelines on factors that may influence sensory verdicts for beef flavor. For individual evaluations and specific products, additional items may need to be considered, added, or deleted. The major point is that consideration and discussion of sample storage and preparation issues occur and a full understanding, elimination, or standardization of their effects on factors of beef flavor must be included in the design of the sensory study.

After standardization and attention to consistent sample storage and preparation, standardization of the sample presentation to sensory panelists is critical to assure that sensory attributes detected by the panelists are the true attributes of the sample and

Table 23.2. List of important parameters and examples of how these parameters may influence beef flavor and beef flavor of samples during storage.

Item	Examples of how sensory attributes could be affected
1. Raw material meat source	Raw materials can vary due to animal of origin and harvesting influences.
a. Age of animal	Meat from older animals is tougher, darker in color, and more intense in flavor for some attributes; meat from very young animals is more tender, lighter in color, and has lower flavor attributes; should consider what is normal for your product.
b. Sex of animal	Meat from intact males may differ in flavor attributes and be more variable in tenderness and/or tougher than meat from castrated males or females; meat from females differs in fat content and/or connective tissue depending on relationship of growth to onset of puberty.
c. Nutritional background of animal	Meat from animals fed grass can be more variable in flavor and tenderness than meat from animals fed grain. Type of forage and length of time fed a specific forage item may influence flavor attributes.
d. Quality grade of carcass	Meat from carcasses with higher fat content differs in flavor and can differ in tenderness or the variability of tenderness than meat from animals with lower fat content.
e. Yield grade of carcass	Meat from carcasses with a higher percentage of fat may differ in tenderness and flavor than meat from carcasses with a lower percentage of fat.
f. Elimination of sources with obvious defects (bruises, abscesses, excessive trim, quality defects such as pale meat, dark meat, blood splash, etc.)	Quality defects can influence multiple meat palatability attributes, such as meat that is pale due to animal stress prior to harvesting may be drier, less flavorful, and tougher than meat from animal not stressed.

Table 23.2. Continued

Item	Examples of how sensory attributes could be affected
2. Definition of handling and/or trimming specifications	Top rounds denuded on one day and used in a study with top rounds not trimmed of external fat from another day may result in flavor differences as microbial growth occurs on the surface of meat during storage. The sour flavor in meat induced by growth of spoilage microorganisms during vacuum packaging may be higher in the denuded top round as the external surface may not be sufficiently trimmed prior to obtaining samples for sensory evaluation.
3. Sampling technique of raw material	Meat removed from a carcass by professional meat cutters on the fabrication line of processing facility will usually be more consistent than meat removed by inexperienced personnel in a meat cooler.
4. Age or length of storage of raw material	Meat stored for 5 days versus meat stored for 28 days will most likely differ in tenderness, flavor, and juiciness due to differences in microbial growth, lipid oxidation, and postmortem proteolysis in the meat during storage.
5. Storage container of raw material	Packaging materials can impart flavors. Beef stored in different plastic liners may differ in flavor attributes.
6. Gaseous atmosphere during storage	Gaseous atmosphere affects microbial growth and specific microorganisms can impart specific flavors. Meat stored in vacuum packages can develop sour flavors that are the result of acid-producing spoil microorganisms, whereas meat stored aerobically can have sulfur, putrid flavors that are imparted by spoilage organisms.
7. Particle size during storage (i.e., whole muscle vs. comminuted)	Once meat tissue has been lysed, it is more susceptible to lipid oxidation and microbial growth, and these factors can affect beef flavor.
8. Temperature during storage of raw material	Temperature affects rates of lipid oxidation and microbial growth that subsequently can impact beef flavor.
9. Potential temperature fluctuations during storage	Temperature affects rates of lipid oxidation and microbial growth that subsequently can impact meat flavor even with small fluctuations during frozen or refrigerated storage.
10. Processing day	Raw material source, temperature, and worker expertise can vary across processing days, and these factors can affect any of the aforementioned items.
11. Batch within processing day	Batches differ due to different raw material and potential handling, temperature, equipment, and worker differences, and these factors can affect any of the aforementioned items.
12. Transportation parameters	Temperature fluctuations during transportation and loss of packaging integrity can affect rates of lipid oxidation and microbial growth as previously discussed.
a. Containers	Containers can impart specific aromatics, either protect or not protect packaging integrity and/or affect temperature stability.
b. Temperature	Standardized temperature during transportation is critical. Continuous temperature recorders can verify proper temperature controls.
c. Length of time	Standardized length of time during transportation or standardized conditions are critical.
d. Special considerations	Any factor that may either impart flavor, alter packaging integrity, affect temperature, or result in an increase in rate of lipid oxidation and microbial growth needs to be preexamined and controlled.

Table 23.3. Examples of factors that can affect sample preparation and examples of how these factors may influence beef flavor.

Item	Examples of how sensory attributes may be affected
Temperature of meat prior to cooking	Frozen or meat at the freeze/thaw temperature of 28°C will require longer cooking times that can decrease juiciness and tenderness and subsequently impact beef flavor attributes—higher livery, burnt, and brown aromatics.
Sample thickness and size	Thicker meat portions require longer cook times to reach a standard internal degree of doneness. Larger or smaller meat portion sizes may also require more or less cooking time, respectively, that will alter the juiciness, tenderness, and flavor of the meat.
Sample fat trim	Fat is an insulator and can impact temperature transfer rates, and therefore, cook time and cook yields may impact meat palatability.
Cooking method	Dry and wet cooking methods, type of grill (electric versus gas; serrated versus flat; two-sided or one-sided heat source), air flow (conventional versus convection versus impingement), distance from heat source, environment (enclosed versus open), and humidity impact meat palatability attributes.
Cooking temperature	The temperature of the environment or the service temperature of the oven or grill impacts the rate of cooking and will subsequently impact beef flavor. For example, higher temperatures may result in higher burnt/browned and livery aromatics while lower temperatures may increase cardboardy aromatics.
Cooking endpoint temperature	Meat flavor, tenderness, and juiciness attributes change as meat is cooked from low or rare to high or well done degrees of doneness. Note that endpoint temperature and visual degree of doneness may not always coincide.
Monitoring of endpoint cooking temperature	Type of thermocouples and placement of thermocouples may impact the accuracy of monitoring cooking endpoint temperature.
Cook time	Longer cook times can result in drier, tougher meat due to dehydration from longer exposure to a heat source. Beef flavor may be lower in serumy/bloody, cowy/grainy and higher in cooked beefy/brothy, livery, and browned/burnt aromatics.

that are not present due to sample presentation. The size and number of samples presented to each panelist needs to be standardized. When evaluating beef flavor, multiple attributes are being assessed. Therefore, the number of samples evaluated in a sensory day should be tightly controlled and the number of samples should minimize taste panel sensory fatigue. Additionally, the serving size should be sufficient for panelists to evaluate all attributes. Two serving portions are most commonly used. The serving size for whole-muscle meat products is usually two or three 1.27-centimeter (cm) cubes. For ground beef patties, one or two wedges that may represent 1/6, 1/4, or 1/3 of the patty may be served including the edges or 1.27- or 2.54-cm squares where the edges are excluded may be served. In some cases, the entire ground beef patty may be served and the panelist cuts the size of sample that they are accustomed to eating. Ground beef patties also may be served on a bun and panelists eat the sample as they would when eating a sandwich. For processed meat samples, such as sausages or frankfurters, a whole sausage or frankfurter may be served especially when texture attributes are being evaluated, but 2.54-cm sections may be served when only flavor attributes are being determined. The interpretation of the results of the sensory evaluation is dependent on the sample size and form served to panelists. The

sample size should be considered in relationship to the purpose of the sensory evaluation and the sample size needed for adequate evaluation by the panelists.

Sample temperature and potential temperature fluctuations of samples within and between panelists is a critical issue. As aromatic compounds volatilize at higher temperatures, if sample temperature fluctuates, sensory panelists may detect differences in the amount of a beef odor and/or flavor attribute between samples, but the difference is actually due to sample temperature differences. It is important to standardize sample serving temperatures within and between sensory days to remove this potential effect. Samples should be identified for sensory panelists using random, three-digit codes to eliminate subconscious selection of a sample due to identification. The serving container should be neutral in color, nonabsorbent and not impart flavor or odors to the sample. For example, glass or white, glazed china containers will not have container-specific aromatics and will be visually neutral. Plastic containers can be used, but they should be opaque or neutral in color and tested to assure that odors or flavors associated with the container are not present. For beef samples being evaluated for odor and flavor, a glass custard dish with a concave glass watch glass top provides an opportunity for odor evaluation by gently shaking the sample, then moving the glass back to smell the sample. The container can be preheated to a standard temperature to reduce temperature fluctuations during evaluation and to not burn nasal tissues during evaluation.

Samples should be served to panelists in random order as there are strong first order bias in central location consumers and trained sensory evaluation tests (either higher or lower ratings in comparison to samples evaluated later) and last order biases for long-term in-home use consumer tests especially for preference data. Order effects can either be randomized across samples or balanced (that is, every sample can be evaluated in each order and order combination sufficiently to account for the effect in the statistical model as a block). Visual attributes of a sample can affect sensory verdicts due to expectation errors. For example, panelists may expect meat cooked to a rare degree of doneness to have higher serumy flavors than meat cooked to a well-done degree of doneness. Therefore, the panelists' evaluation is not independent, but is influenced by the visual attributes of the sample. In this case, the use of red lights during evaluation will assist in controlling this effect.

Guidelines for cookery and sensory analysis of meat (AMSA 1995; ASTM 1978) should be used as standardized techniques for the sensory evaluation of beef flavor.

DISCRIMINATIVE TESTS

Discriminative tests are used to discriminate or to tell overall differences between one or more products. There are multiple tests to select from and each test has strengths and weaknesses. In the meat science literature, discriminative tests are not commonly used. They are more commonly used in industry to detect differences between one or more samples. Discriminative tests are useful tools and may not be directed at determining differences in one attribute, like beef flavor, but differences between two or more products. The common discriminative tests used to determine differences in beef flavor will be discussed and information on the type of data and decisions that can be made from each test will be presented. Trained sensory panelists or consumer sensory panelists can be used for discriminative tests. Trained sensory panelists will generally be able to determine smaller differences between samples than consumers, whereas, consumer responses more closely mirror differences from the general population.

There are two categories of difference tests: overall difference tests and attribute difference tests. Overall difference tests determine if sensory differences exist between two samples, and attribute difference tests are more specific and ask if samples differ in specific sensory attributes. Triangle tests, two-out-of-five test, duo-trio tests, simple difference test, "A" – "not A" tests, difference-from-control tests, sequential tests, and similarity tests are examples of difference tests. Directional difference tests, pairwise ranking tests, simple ranking test, rating approach, multisample difference tests, and balanced incomplete block (BIB) design-ranking tests are examples of attribute difference tests. Although all of these tests could be used to determine differences in beef flavor, generally, triangle tests, duo-trio tests, and simple difference tests are used. These three tests will be discussed for use in determined differences in beef flavor.

Triangle tests use three samples coded with random three-digit codes, and the panelist is asked to select the sample that is different. Two samples are alike and a third sample is different. Multiple panelists, either trained or consumer, are used and the order of the three samples is randomized, and the sample that differs is alternated between the two samples. Using a predetermined significance level, the correct number of samples versus the total number of tests evaluated is used to determine if differences existed between the two samples. Tables to determine significance are presented in Meilgaard and others (1999). Panelists have a 33% chance of guessing the correct answer in a triangle test, and the greater the number of tests conducted, the more the guessing factor influence is decreased. For beef flavor determination, the two samples being evaluated would be presented in the test following the aforementioned controls. If panelists detected differences based on the predetermined significance level and number of samples served, then it can be concluded that the two samples differed in beef flavor. The challenge with this test is determining what aspect of beef flavor the panelists are evaluating. In the question, the sensory professional may ask the panelists to differentiate samples based on "beef flavor," therefore, leaving the interpretation of what beef flavor is up to the panelists. This provides a general evaluation of beef flavor and may be sufficient for the study. Another approach is to ask a specific question concerning beef flavor. For example, in evaluating irradiated versus nonirradiated ground beef patties, a triangle test could be conducted with consumer panelists to determine if irradiation affects beef flavor or if "rancid-type flavors" differ. The sensory professional should make sure that the panelists understand what "rancid-type flavors" are. These tests can provide valuable information regarding whether samples differ in beef flavor, but they may not provide information on what aspect of beef flavor differs or is affected or how much difference exists in beef flavor between samples.

For duo-trio tests, panelists are given a reference or control sample. They are then given two samples and are asked to identify the sample that is the same as the reference or control sample. Panelists have a 50% chance of selecting the correct sample. Again, the number of correct responses are calculated and compared to the total number of evaluations completed. The probability that the samples differ is determined using tables from Meilgaard and others (1999).

In simple difference tests, panelists are provided two samples and asked if the samples differ or if they are similar. Panelists may be provided one or up to four possible combinations of presentations for the two samples (1 then 2, 2 then 1, 1 then 1, and 2 then 2). The total number of correct responses for each of the four combinations is determined. The placebo effect is compared to the treatment effect using x^2-analysis. The x^2-statistic is calculated by:

$$x^2 = \sum \frac{(O - E)^2}{E},$$

where O is the observed value and E is the expected number. The determined x^2-value is compared to a x^2 table at a probability of 0.05. If the calculated value is greater than the table value, a significant difference exists between the two samples.

Duo-trio and simple difference tests, as previously discussed for triangle tests, can provide viable tools for determining differences in beef flavor between two samples. However, the challenge is determining what attributes of flavor differ. It may not be important to know what aspects of beef flavor differ between two samples, but if differences in specific flavor attributes need to be quantified, descriptive sensory tests can be used.

DESCRIPTIVE TESTS

To describe and quantify the intensity of sensory attributes in meat samples, descriptive sensory tests are an effective tool and they are particularly effective tests for quantifying differences in specific beef flavor attributes. Descriptive tests use trained sensory panels to rate the intensity of attributes that are product specific. The type of test, the development of the attributes to be evaluated, and the rating scale may differ across tests, but the overall objective of descriptive tests are to describe and quantify sensory attributes.

Three types of descriptive tests can be used to determine beef flavor: Meat Descriptive Analysis, Spectrum® Descriptive Analysis, and Quantitative Descriptive Analysis. Free-choice profiling has been used on a limited basis to determine differences in beef flavor. This method can be used as an effective tool in assessing beef flavor differences in meat products.

Descriptive tests use a dictionary or family of attributes called a lexicon. The attributes in a lexicon are defined so that then each attribute can be quantified. A lexicon can be product specific or a general product lexicon can be used as a base and other attributes added as defined by trained panelists. A general beef flavor lexicon is presented in Table 23.1. Attributes can be identified by using existing published lexicons, attributes identified from Civille and Lyons (1996), by free profiling where individual panelists develop their own descriptors, or by identification through ballot development sessions with a trained descriptive attributes sensory panel.

Ballot development sessions can be a very useful tool in descriptive sensory evaluation for identifying the beef flavor attributes. In ballot development sessions, panelists that have shown the ability to describe and provide input into individual sensory attributes are used. The panel leader presents products that are similar to the products that will be used in the study. Sensory panelists are asked to evaluate the product and provide ideas and input into the sensory attributes that they detect. The panel leader can provide standard lexicons for similar products or use resources like Civille and Lyons (1996) that provide a large list of sensory attributes, definitions, and references. Care should be taken to not depend too much on standard lexicons because product-specific attributes may be overlooked. Products should be presented to the panelist that represent the range and average products for the study. The panel leader should help direct the session, but they should not stifle creativity and discussion. A guideline for the sequence of events that could be followed during a ballot development session is presented in Table 23.4. Some descriptive analysis tests do not require extensive ballot development sessions (Quantitative Descriptive Analysis), whereas other methods (Meat Descriptive Analysis and Spectrum Descriptive Analysis) use extensive sessions and a standardized ballot.

Meat Descriptive Analysis: This descriptive method was developed by meat scientists to evaluate the palatability of whole muscle red meat products. Palatability attributes for red meat are defined as juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness, and flavor intensity. These attributes are measured using 8-point scales. The American Meat Science Association has published guidelines for use of this method (AMSA 1978, 1995). This is the predominant sensory method used in the scientific literature to determine differences in red meat palatability; however, note that in the original ballots presented in AMSA (1978) and then again in AMSA (1995), the beef flavor attributes are described as beef flavor intensity and are rated using 1 = extremely bland and 8 = extremely intense. This method is a very effective tool for determining juiciness, muscle fiber tenderness, connective tissue amount, and overall tenderness between samples when the sensory panelists have been adequately trained, but the beef flavor intensity attribute is very general. This method is not highly sensitive to flavor intensity differences, and if the objective of a study is to understand flavor differences, the Spectrum Method for Descriptive Flavor Attributes, the Tragon Quantitative Descriptive Analysis Method, or the Free Profiling Method are better sensory tools. In my laboratory, the Meat Descriptive Ballot has been altered to provide more meaningful beef flavor sensory information. This ballot is presented in Table 23.5. Note that the beef flavor attributes of cooked beef brothy and cooked beef fat have been added. In addition, attributes for off flavor attributes are listed. Panelists identify off flavor attributes by listing the number and then the intensity rating of the attribute. The problem with rating only beef flavor intensity is that beef flavor is a multicomponent attribute and evaluating only one generalized attribute is not consistent and repeatable and is difficult to define.

For further processed meat products and ground meat, the Meat Descriptive Attribute method can be used; however, attributes need to be changed to reflect the product being tested. Initial juiciness, sustained or final juiciness, initial beef flavor, and sustained or final beef flavor are examples of sensory attributes used for ground beef. For further processed products, attributes for the texture and flavor of the product should be used. The Spectrum Method for Descriptive Flavor Attributes, the Tragon Quantitative Descriptive Analysis Method, or the Free Profiling Method may be more appropriate methods for assessing sensory attributes for further processed meat products.

After completion of the sensory evaluation, data can be analyzed using Analysis of Variance to determine treatment differences for each descriptive attribute. Principal Component Analysis and other multivariate analyses may be used to understand the

23.4. An example of the sequence of events for ballot development sessions for beef flavor in a whole-muscle beef ste

Session goal	Exercises Used in the Session
Familiarize panelists with beef flavor product.	1. Presentation of a baseline beef steak—use cooking methods as in study; flavor lexicon for discussion.
Initiate and stimulate identification of flavor attributes.	2. Introduce one or two references for beef lexicon to begin anchoring on a familiarize panelists to concept of lexicon and attribute identification.
Continue initiation and stimulation of identification of beef flavor attributes.	1. Presentation of the baseline beef steak. Ask panelists to describe the flav narrow down the descriptors into categories that are similar between pan get consensus on some attributes.
Introduce one to two products into categories that are variations from the baseline product.	2. Begin using attributes that are being defined to evaluate one or two steak variations from the baseline but that are representative of steaks that wil the study. Continue to encourage and provide a free discussion of the fla
Continue with identification of the flavor attributes in the product as in Session 2.	1. Present the baseline product. Ask panelists to describe the flavor. Utilize identified in Session 2. Continue to fine-tune these descriptors. Bring in attributes to clarify what the specific attribute is and how to identify it.
Introduce the baseline product and the new ingredient in its pure form and begin identifying the flavor attributes of the ingredient.	2. Examine one or two additional products that are variations from the base to discuss flavor descriptors, identifying new descriptors, or altering exis descriptors. 3. Taste the new ingredient in its pure form. Begin identifying the flavor des ingredient.
Repeat Session 3 until panelists are familiar and comfortable with the attributes as defined.	1. Same as Session 3. 2. As panelists gain experience, confidence, and repeatability on each attrib attributes references and continue to expand the ballot.
Identify references for the flavor.	1. Present the baseline product. Review the current list of descriptors being 2. Introduce and examine baseline examples of each descriptor and begin a descriptors for each panelist.
Introduce scaling for each descriptor.	1. Present the baseline product. Review the current list of descriptors. 2. Utilize references of each descriptor to continue anchoring panelists on 3. Introduce product with the varying concentrations of the ingredient and with the descriptors as defined. 4. Continue identification of alternate descriptors.
Develop a final ballot.	1. Progressively continue sessions until a final ballot is defined and panelis with the descriptors and the scale.

Table 23.5. Meat descriptive attribute ballot for whole muscle meat as defined by AMSA (1995).

Name _____ Session _____ Date _____

Sample	Juiciness	Muscle Fiber Tenderness	Overall Tenderness	Connective Tissue Amount	Overall Flavor Intensity	Cooked Beefy/ Brothy	Cooked Beef Fat	Off Flavor Characteristics
Warm-up								

JUICINESS		MUSCLE FIBER & OVERALL TENDERNESS		CONNECTIVE TISSUE AMOUNT		FLAVOR INTENSITY		OFF-FLAVOR CHAR.			
								X	Other (describe)	M	Metallic
8	Extremely Juicy	8	Extremely Tender	8	None	8	Extremely Intense	A	Acid	MU	Musty
7	Very Juicy	7	Very Tender	7	Practically None	7	Very Intense	B	Bitter	N	Nutty
6	Moderately Juicy	6	Moderately Tender	6	Traces	6	Moderately Intense	BR	Browned	P	Putrid
5	Slightly Juicy	5	Slightly Tender	5	Slight	5	Slightly Intense	C	Cardboard	SA	Salty
4	Slightly Dry	4	Slightly Tough	4	Moderate	4	Slightly Bland	CH	Chemical	SB	Serumy/bloody
3	Moderately Dry	3	Moderately Tough	3	Slightly Abundant	3	Moderately Bland	CW	Cowy	SD	Soured
2	Very Dry	2	Very Tough	2	Moderately Abundant	2	Very Bland	F	Fish-like	SO	Sour
1	Extremely Dry	1	Extremely Tough	1	Abundant	1	Extremely Bland	L	Liver	SW	Sweet

combined effect of all attributes on differences in the meat products tested.

The Spectrum® Method: This method was developed by Gail Vance Civille to use across a wide variety of products and it is easily adapted for use in assessing beef flavor. This method uses a universal intensity scale from 0 (none) to 15 (extremely intense) to quantify beef flavor differences. The universal scale standardizes the level of intensity for any attribute and is used to standardize panelists when using the scale. The universal scale defines a 2 as the soda flavor in salt-less saltine crackers, and the apple flavor in Mott's applesauce is a 5. The orange flavor in Minute Maid frozen orange juice reconstituted as defined on the package is a 7, and the grape flavor in Welch's grape juice is a 10 for intensity. The cinnamon flavor in Big Red Gum is a 12 on this intensity scale. The scale is then used on specific attributes where the attributes are defined using ballot development sessions or a standard lexicon as presented in Table 23.1 for beef flavor. Note that an attribute defined as other is always included on the ballot. If panelists identify an attribute that is not on the ballot, they provide a descriptor and then rate the attribute for intensity. This provides an avenue for the identification of attributes not defined during ballot development that become apparent during testing. Tables 23.6 and 23.7 are examples of Spectrum® Method ballots used to evaluate beef flavor in whole-muscle beefsteaks and ground beef. This sensory method is easily adapted across meat products and is useful for whole-muscle and processed meat products. These data are analyzed as defined for the Meat Descriptive Analysis, and differences in specific attributes can be determined or multivariate analyses can be used to examine changes of multiple attributes in meat products.

Quantitative Descriptive Analysis Method: This sensory method is commonly used to determine beef flavor differences between samples and is an especially valuable tool used in industry for value-added or processed beef products. It is a useful tool for determining beef flavor differences. The Tragon Corpo-ration developed the Quantitative Descriptive Analysis Method or QDA Method for descriptive analysis. In this method, a large pool of trained sensory panelists is used. For a particular meat product, panelists who can discriminate beef flavor attributes are selected. Ballot development and training sessions are similar as previously described for Meat Descriptive and Spectrum methods except the panel leader is more of a facilitator and does not take as active a role in directing the discussion. References for attributes are provided, and products that emulate products used in the study are presented to the panelists, but panelists are free to develop their own descriptive terms. Descriptive terms are rated using a 15-cm line scale. Panelists will independently rate each product for descriptive attributes and then are provided feedback from the panel leader as to their consistency and performance. Beef flavor attributes can easily be evaluated using this method. The multiple components of beef flavor can be used as defined in Table 23.1 or attributes as defined by the panelists will be assessed. As panelists independently evaluate each product and do not discuss data, the terminology or the samples after each taste session, the terminology may differ from project to project. The panel leader provides the feedback to the panelists independently. Data are analyzed statistically using the QDA analysis package, and data are reported in a "spider web." The statistical package organizes and categorizes similar beef flavor attributes into attributes that are presented in the spider web. By looking at differences in intensity of attributes across different products in the test, beef flavor differences can be determined. In Figure 23.1, a spider web for beef flavor of enhanced and nonenhanced beef steaks is presented. The interpretation of data presented in Figure 23.1 is that enhanced beef steaks are higher in beefy/brothy, salty taste, serumy flavors, and overall aroma than control steaks. However, enhancement did not increase the beef fat flavor and slightly decreased livery flavor in beef steaks when compared to the control steaks. This type of data presentation is easy to interpret and provides a good snapshot of the attributes that are changing or that do not differ across treatments.

Free-Choice Profile Method: The Free-Choice Profiling Method give panelists the freedom to develop their own descriptors and scales. Panelists do not need to be uniformly anchored and efforts are not made to assure panelists use the scale similarly. Panelist training sessions include only a short session where the sensory procedures are explained. Panelists have not been biased by training and are considered "consumers." Sensory samples are served to the panelists, and they are asked to evaluate the products for beef flavor. They then describe the attributes of beef flavor and rate the intensity as

Table 23.6. Descriptive attribute ballot for beef flavor determination in whole-muscle beef steaks using the Spectrum® Method of descriptive analysis.

Name _____	Date _____	Session _____	
Sample Number	_____	_____	_____
AROMATICS			
Cooked Beef/Brothy	_____	_____	_____
Cooked Beef Fat	_____	_____	_____
Serumy/Bloody	_____	_____	_____
Grainy/Cowy	_____	_____	_____
Cardboard	_____	_____	_____
Painty	_____	_____	_____
Fishy	_____	_____	_____
Liver	_____	_____	_____
Soured	_____	_____	_____
Browned/Burnt	_____	_____	_____
Dirt	_____	_____	_____
Medicine	_____	_____	_____
Old/Putrid	_____	_____	_____
Plastic	_____	_____	_____
Other (describe)	_____	_____	_____
FEELING FACTORS			
Metallic	_____	_____	_____
Astringent	_____	_____	_____
BASIC TASTES			
Salt	_____	_____	_____
Sour	_____	_____	_____
Bitter	_____	_____	_____
Sweet	_____	_____	_____

Table 23.7. Descriptive attribute ballot for beef flavor determination in ground beef patties using the Spectrum® Method of descriptive analysis.

Name _____ Date _____ Session _____

SAMPLE ID Number	Warm-Up				
AROMATICS					
Cooked Beef/Brothy					
Cooked Beef Fat					
Serumy/Bloody					
Grainy/Cowy					
Cardboard					
Painty					
Fishy					
Liver					
Soured					
Browned/Burnt					
Other (describe)					
FEELING FACTORS					
Metallic					
Astringent					
BASIC TASTES					
Salt					
Sour					
Bitter					
Sweet					
AFTERTASTE					
Astringent					
Fat Mouthfeel					
Bitter					
Browned/Burnt					
Sour					
Sweet					
Other (describe)					
AFTER FEELING FACTORS					
Lip burn					
Metallic					

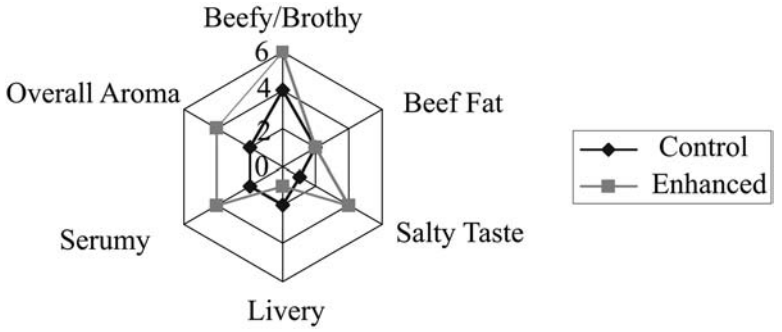

Figure 23.1. A "spider web" for Quantitative Descriptive Analysis for enhanced and nonenhanced beef steaks.

they perceive it. The data are analyzed using the Generalized Procrustes Analysis where it collates sensory terms that are similar. The sensory professional then interprets differences in beef flavor attributes. This method does not require the extensive time to train and standardize panelists. Panelists' performance is not evaluated and ballot development sessions are not needed. Retaining trained sensory panelists is not needed. Although this method is not commonly used to determine beef flavor differences in beef products, it is an increasingly used sensory tool for food and an acceptable sensory tool for ascertaining beef flavor differences in beef products.

CONSUMER EVALUATION OF BEEF FLAVOR

Consumer protocol, ballot design, and consumer selection has been discussed in other chapters in this text. Beef flavor is easily included in consumer evaluation of beef acceptability. As consumer studies are a method of assessing consumer preference or acceptance, beef flavor is obviously a component of consumer acceptance. Beef flavor like/dislike should be included on the ballot as a question and structured similarly to the questions for overall like/dislike. Questions regarding the intensity of beef flavor also are viable questions to ask consumers. By asking a flavor like/dislike question followed by a question asking consumers to rate the intensity of beef flavor, relationships between how much beef flavor in the product drives consumer acceptability can be ascertained. Care should be taken in asking consumers questions about specific beef flavor attributes as defined by trained sensory panelists. Consumers may not understand the attribute and while they may rate the attribute, the data will be meaningless. Beef flavor questions are appropriate for both in-home and central location tests and also are very useful when evaluating further processed or value-added beef products.

CONCLUSION

In conclusion, beef flavor is an important sensory attribute when conducting sensory evaluation of beef. While there are multiple sensory tools available for assessing the level of beef flavor, the fact that beef flavor is not a single attribute, but multiple attributes, makes the sensory evaluation of its components challenging. While discriminative tests can be useful sensory tools for determining differences in beef flavor between beef products, they many times do not provide quantitative differences in specific attributes. Descriptive sensory tools, mainly Meat Descriptive Attribute, Spectrum® Descriptive Attribute, and Quantitative Descriptive Attributes testing methods are the most common tools used to assess beef flavor differences. Some use of the Free-Choice Profile Method can be used, but it is not common. Consumer sensory evaluation of beef can provide important information on consumer acceptance of beef products. Ascertaining acceptability of beef flavor as a component of a consumer test is a viable sensory tool and should be used when conducting consumer evaluation of beef and beef products.

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Handbook of Meat, Poultry and Seafood Quality
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24
Beef Quality and Tainting

James M. Martin

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INTRODUCTION

The quality characteristics of beef, as with any other meat are very important to the ultimate eating satisfaction of the consumer. Various factors such as tenderness, juiciness, color, and flavor may be influenced by changes occurring during the conversion of muscle to meat. Similarly, the postslaughter fabrication and handling steps by the muscle foods processor can affect the ultimate eating quality of fresh and previously frozen beef products. Beef, as with most fresh meats, provides an excellent medium for the support of microbial growth. Microbial growth if permitted to continue will render meats unacceptable to consumers. The ultimate goal of the beef industry is to produce high quality products that consumers will enjoy and purchase again. The subject of tainting or spoilage is therefore of critical concern to the muscle foods industry. Muscle deterioration begins soon after the process of slaughter as the result of microbial, chemical, and physical processes. These processes must be controlled or possibly eliminated, or they will render beef unfit for consumption. As this chapter progresses, we will examine the causes and consequences of beef tainting in fresh and frozen product. Furthermore, the discussion will include various protective measures that can be used to produce top quality beef products. When beef is evaluated for its sensory characteristics, all of the previous factors and other possible factors should be considered. The material that follows is not a review of the scientific literature, rather a general compilation of available information. The author acknowledges the vast contributions made to this topic by a variety of researchers and textbook authors. There is a list of several references provided from which the information contained in this chapter was gleaned so that the reader can investigate this topic further if they so desire.

TAINTING/SPOILAGE INTERVENTIONS

The extension of shelf life is essential for storage of most fresh and processed meat products. By any standard, fresh meat must be considered to be one of the more perishable foods. Preventative measures to stop tainting and spoilage of muscle foods must be applied quickly after slaughter. The means of meat deterioration are diverse and include several processes.

Meat preservation involves application of measures to delay or prevent certain changes, which make meat unusable as food or which downgrade quality characteristics. Most of the edible tissues of healthy animals up to the time of slaughter are either sterile or contain very low concentrations of microorganisms. Postmortem muscle experiences a sudden cessation of the body's defenses against invasion and growth of foreign microorganisms. Apparently there is very little if any residual bacteriostatic or bacteriocidal properties in the muscles of freshly slaughtered animals. The process of hide removal, evisceration, and carcass fabrication operations can result in microbial

contamination of the meat. Perhaps the most universal preventative measure against tainting in beef or any fresh meat is postmortem carcass cooling or refrigeration, and subsequent freezing.

REFRIGERATION AND FREEZING

Once the slaughter process is completed, the internal temperatures of animal carcasses generally range between 30 and 39°C. This postmortem body heat must be removed during initial chilling. As a general rule, the internal temperature of the thickest portion of the carcass should be reduced to 5°C or less. Beef carcasses are initially chilled in blast (forced air) coolers or chill tunnels at temperatures generally ranging from −10°C to 0°C. If the temperature is reduced below −2°C, the carcasses will begin to freeze, which changes the physical state of the muscle tissue as well as endogenous enzymatic and chemical changes. There are some considerations with regard to carcass chilling. These factors include specific heat of the beef carcass, carcass size, amount of external fat (which acts as insulation), and the temperature of the chilling environment. Due to the fat cover of most beef carcasses, the chilling process may take up to 48 hours or longer to reach an internal chilled temperature of 5°C or lower.

The amount of shrinkage or moisture loss incurred during initial chilling must be held to a minimum for both economic reasons and quality retention. Given adequate cooling of hot beef carcasses and cuts, the deterioration of fresh chilled beef is generally due to surface changes. The natural surface consists of fat and connective tissue, and during cooling the consistency of the latter changes so that further loss of water by evaporation is restricted. Conversely, muscle surfaces continue to lose water at a fairly rapid rate, and this desiccation leads to an increased concentration of salts at the surface, which causes oxidation of the muscle pigment to a brown color of various intensities.

Once beef is removed from the cooler, moisture tends to condense on the cool surfaces, especially when the relative humidity of the atmosphere is high, a phenomenon known as "sweating." This effect can promote microbial growth, if the product is temperature abused (<4.44°C) for periods of about 2 hours or longer. It is important to consider that effective refrigeration along with various protective coverings allow longer storage or shipping of beef. Most beef carcasses are fabricated into subprimal cuts that are vacuum packaged and boxed for storage and shipping. Proper packaging materials for fresh retail cuts are important to maintain desirable color. The eye-appealing bright cherry-red color most retail fresh beef displays is dependent on several variables including proper packaging and display temperatures. Additionally, proper packaging can help prevent contamination during retail display.

The duration for which fresh meat can be stored in refrigeration depends on certain characteristics of the muscle. For example, pork, poultry, and fish possess more highly unsaturated fats than beef or lamb and tend to be more susceptible to the development of oxidative rancidity or "warmed-over" flavor. The length of time meat may be kept safely under refrigerated storage in the home is determined by previous handling conditions and refrigerator temperature. However, even under ideal home refrigeration temperatures, fresh meat should be consumed within about 4 days of purchase to not compromise full sensory quality. To prevent further quality deterioration and subsequent spoilage, fresh meat that will not be consumed within this time frame should be frozen. Some deterioration will occur during slow freezing in home refrigerators, but such losses in quality are preferable to bacterial spoilage and discoloration, which would render the beef product useless.

The effectiveness of freezing in reducing the rate of muscle spoilage has long been understood. The advantages of temperatures below the freezing point of fresh meat, about 28°C, are recognized as an excellent method for slowing the rate of spoilage. The process of freezing at a rapid rate has little detrimental effect on flavor, color, odor, or juiciness of meat after cooking. However, over time in frozen storage, a gradual decrease in the sensory qualities of odor and flavor can develop. Several questions should be answered when assessing the ultimate quality of any frozen fresh meat. The manner of freezing, the reason for freezing, the kind of packaging, and the time in frozen storage all affect quality characteristics during frozen storage. Generally, good quality can be maintained for several months (4–12) of frozen storage if some precautions are observed. Tight-fitting and moisture-proof packaging and consistent storage temperatures (0°C or lower) are important factors.

Frozen storage that is prolonged in improperly packaged beef products (please refer to Chapter 28)

can result in "freezer burn" or surface dehydration. This phenomenon is the result of water loss from the affected surfaces. The resulting appearance will manifest itself as grey patches on the products surface. Variations in frozen storage temperatures can accelerate the onset of freezer burn. This condition can cause accelerated lipid oxidation or the warmed-over flavor most consumers would find objectionable. Surfaces that are desiccated provide sufficient surface area for interaction with oxygen that leads to volatile flavor compounds that produce objectionable odors and flavors.

The development of oxidative rancidity can be of concern during frozen storage. This is generally recognized as a sensory problem with regard to odor and flavor. Frozen storage does not prevent but rather slows the onset of oxidative rancidity. Generally, beef stored in lower frozen temperatures will manifest slower rates of rancidity onset. Oxidative rancidity occurs most rapidly at −2°C to −4°C and almost ceases below −30°C. Fresh pork and poultry meat are at a greater risk for rancidity development in frozen storage primarily due to the characteristics of the fat. Meats with greater degrees of unsaturation tend to develop this negative quality attribute faster than more saturated meats such as beef.

MICROBIAL TAINTING

Beef, as are all fresh meat products, is a very perishable commodity. It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter. However, when the cut of fresh meat arrives at the retail level, especially if it is in ground form (i.e., hamburger) there can be varying numbers and types of microorganisms found. There can be many avenues by which the fresh beef cut becomes contaminated with microorganisms.

After being stunned and hoisted, beef animals are exsanguinated by severing the jugular vein with what is generally referred to in industry as the "stick knife." A dirty stick knife can introduce organisms into the bloodstream, before cessation of heart activity, where they can be deposited in some areas of the carcass. Organisms from the beef hide may also be deposited onto the carcass or onto freshly cut surfaces. Bovine manure and other organic material tend to remain on the hides of even the most well kept animals. Transfer of these indigenous organisms can cause contamination. Additionally, some hide microorganisms can become airborne and contaminate adjacent carcasses or cut surfaces. Punctures in the gastrointestinal tract may deposit large inoculums of microorganisms onto the surface of beef carcasses. Meat plant workers can also be a source of bacterial contamination during the slaughtering and fabrication procedures. However, it must be stressed that as the carcass leaves the slaughter floor there are several steps the beef industry currently has in place to minimize the chance of fecal or other contamination to the beef carcass. These procedures include steps such as steam vacuuming, passing carcasses through steam cabinets, and real time identification of organic residue by biophotonic technique, spraying the carcasses with bactericidal organic solutions, and washing with hot water. Another concern is the placing of fresh beef cuts into nonsterile containers, which inoculate the cut with whatever organism may be present in the container. This can be an effective source of contamination of ground or minced meats.

After slaughter, and subsequent slaughter floor decontamination procedures, beef carcasses are thoroughly chilled and later moved into the food distribution channels. Carcass fabrication procedures to render primal and retail cuts allows increasingly higher numbers of microorganisms to contaminate the product surfaces. The fate of these microorganisms depends upon the ultimate use of the meat. Fresh meat has a high water content corresponding to a water activity of approximately 0.99. This environment readily supports and even promotes the growth of contaminating microorganisms, which may be present on the meat surface. Prompt effective carcass chilling creates a selective environment and permits growth of only those microorganisms that are capable of growing at temperatures approaching the freezing point of meat. Vacuum packaging of meats in oxygen-impermeable films results in a further constraint that reduces the microorganism's ability to grow and proliferate.

Microbiological tainting of fresh meats can involve many different organisms. Viruses, molds, yeasts, and bacteria all factor in the spoilage of fresh meat products. Viruses are very small organisms that do not usually contribute to meat spoilage but may be harmful for meat plant workers or consumers. Molds are multicellular organisms that display a variety of colors and are typically recognized by their "fuzzy" cotton like appearance. Molds can develop

numerous very small spores that are spread by air currents and other means, which causes mold growth to appear on other meat surfaces. Yeasts, as mold spores, can be spread through the air or by other means and will contaminate meat and equipment surfaces wherever they settle. Bacteria are unicellular and vary in appearance from elongated and short rods to spherical or spiral forms.

Fresh meat begins to undergo change from the moment of slaughter. The tissues no longer respire due to oxygen deficiency, and reserve glycogen will be metabolized beneath the tissue surface via fermentative pathways, which results in the accumulation of lactic acid and concomitant lowering of the pH. The extent of this pH drop will mediate the ability of the tainting organisms to develop, grow, and proliferate on the carcass surface. Therefore, the spoilage organisms present on dark-cutting beef (high pH) should be different from those present on pale soft and exudative pork (low pH).

Meat products for the most part require refrigerated temperatures to maintain sensory integrity. Therefore, organisms that can grow at refrigerated temperatures cause most types of meat spoilage. These spoilage organisms are generally referred to as psychrotrophs. The problematic attribute of these bacteria is their ability to grow at refrigerated temperatures (<5°C). Different organisms have different abilities to render meat unusable for consumption. Usually the first manifestation to the consumer is some type of visible defect. These defects may include milky exudates, excessive free liquid in the vacuum or over-wrapped package, slime, and generalized off odors. Additionally, there can be discoloration that can be green, yellowish, or brown. Moreover, there are other organisms that can produce quantities of organic compounds such as amines, esters, and acetoin that are further regarded as possessing high spoilage potential.

Microbial spoilage is generally considered either aerobic or anaerobic, depending on the conditions under which it occurs. This also depends on what type of organism causes the spoilage. Slime formation, unappealing flavors and odors, and color changes usually occur as the result of contamination with aerobic bacteria and yeasts. Environmental conditions such as temperature and water activity dictate which species of bacteria or yeasts cause slime formation. Many of these bacteria can produce oxidizing compounds, which then convert oxymyoglobin or the bright cherry red color of fresh beef to unattractive gray, green, or brown colors.

Spoilage will develop with certain variations depending on the species of bacteria that are predominant. The following discussion will concentrate on specific groups of organisms that are grossly responsible for tainting and spoilage of beef.

After the slaughter processes are completed, most carcass surfaces tend to have high microbial populations of fecal and soil origin. The majority of these organisms are nonpathogenic. These organisms are referred to as spoilage bacteria, because their presence subsequently leads to diminished sensory qualities such as appearance and odor. Spoilage organisms that thrive in refrigerated temperatures are classified as psychrophiles. These organisms grow best at temperatures lower than 20°C. There are several genera of this type of bacteria that can be present and grow on carcass and cut surfaces at refrigerated temperatures. These include *Pseudomonas, Moraxella, Psychrobacter*, and *Acinetobacter. Aeromonas, Altermonas, Shewanella, Micrococcus, Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Flavobacterium*, and *Proteus* and may accompany these bacteria, in smaller numbers.

Fresh beef is susceptible to fungal spoilage by the following genera of molds: *Thamnidium, Mucor,* and *Rhizopus*. These molds are prone to develop "whiskers" on beef; *Penicillium* produces green patches; *Sporotrichum* and *Chrysosporium* produce "white spots"; and *Cladosporium* is a common cause of "black spots" that can be seen on aged beef carcasses. Storage temperatures below −5°C apparently inhibit the growth of molds. Yeasts that have been recovered from refrigerator-spoiled beef include the genera *Candida* and *Rhodotorula*. In contrast to spoilage that can occur on beef carcasses, only bacteria spoil ground beef. The following generas are responsible for the most spoilage problems: *Pseudomonas, Alcaligenes, Acinetobactor, Moraxella,* and *Aeromonas*.

Pathogenic bacteria are those that cause illness. There are two types of illness associated with food poisoning—food intoxication and food infection. Food intoxication results from the consumption of bacterial toxins produced in the food, while food infection occurs as the result of consumption of bacteria, which then produce toxins in the alimentary

system. Food that appears perfectly appetizing can contain bacterial toxins that are relatively odorless and tasteless, and therefore readily consumed.

Currently, there is considerable effort being made to implement a system to track beef products from the farm, processing plant, retailer, and finally to the table. Currently, most beef products, if recalled due to food-borne illness can be traced back to the processor, but not to the producer or individual animal. Most food-borne illness linked to beef consumption are associated with ground beef. However, fresh beef tainting can also encompass all other beef cuts. Most pathogenic organisms associated with fresh beef tainting live in the intestinal tract of healthy animals. The more prevalent pathogenic organisms are:

- *E. coli* 0157:H7: This pathogen occurs in the gastrointestinal system of beef animals. The early 1980s saw this organism emerge as a source of serious illness in humans who had eaten undercooked ground beef. *E. coli* 0157:H7 causes acute bloody diarrheas, abdominal cramps, and hemolytic uremic syndrome, which may develop into chronic kidney failure.
- The typical mode of product contamination occurs during the skinning and eviscerating part of the slaughter process. Current technology to detect the disease in humans has improved, thus increasing the number of reported cases. It should be noted that increased frequency of testing at the processing plant and strict sanitation and hygiene programs provide the consumer with the safest beef products possible.
- *Salmonella*: *Salmonella* has been recognized as a primary cause of gastroenteritis in humans for over 100 years. This organism is widely dispersed with humans and animals being the primary reservoirs. A recent report of a study discovered 45% of the rumen contents of healthy cattle were found to contain *salmonella*. The Food Safety and Inspection Service (FSIS) of the USDA has started a *salmonella* testing program for slaughter plants and ground beef processors.
- *Campylobacter*: This organism may be the greatest cause of acute bacterial diarrheas in humans. Meat animals such as beef carry the organism in their intestinal tracts. These bacteria can contaminate many types of raw meats, and is no longer considered an emerging pathogen. Advances in technology have made it more effective to test for *Campylobacter*, thus control of this pathogen can be enhanced.

SUMMARY

In summary, tainting or microbial spoilage can result in slight or catastrophic reduction in beef quality characteristics, especially odor and flavor. The processes associated with slaughter and fabrication of beef carcasses can contaminate the products with a variety of microorganisms. There are several interventions that can be implemented into the production chain that will effectively reduce the rate of product deterioration. Methods such as chilling, freezing, packaging, organic acid sprays, and steam treatments are commonly used by the beef industry today. Through these intervention steps, tainting or further spoilage can be reduced so the consumer can enjoy the attributes of a most nutritious food.

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Microbiological and Sensory Properties of Beef

Jack Thomas

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INTRODUCTION

While beef has always been an important and popular mainstay of our diet, its popularity has reached new levels in recent years. Demand for beef steadily increased from the beginning of the twentieth century until about 1976 (*USDA Agricultural Fact Book* 2002). Then high production costs and competition from other animal proteins, most notably chicken, lessened the demand for beef. Beef needed to reposition itself in the market. Through the joint efforts of producers, meat companies, and researchers, intense activities focused on returning beef to the center of the dinner plate. New consumer-friendly products were, and continue to be developed, but most importantly, the beef industry renewed its focus on quality. Marketing efforts that promoted both new products and high quality traditional beef cuts brought consumers back to beef. They rediscovered how flavorful beef can be. As a result, demand for beef has increased dramatically in the past few years. Much of this growth has been in the food service industry, especially in the white tablecloth restaurant trade. Also, the recent popularity of high protein, low carbohydrate diets has helped maintain the high demand for beef.

The road to today's record high demand has not always been smooth. The 1993 outbreak of foodborne illness in the northwestern U.S. from *Escherichia coli* 0157:H7 (Centers for Disease Control and Prevention 1993), forced the industry to focus on the microbiological safety of beef. Since then, the beef industry has spent over $3 billion developing and implementing methods aimed at reducing the incidents of food poisoning arising from the consumption of beef. Through joint efforts of the federal government and industry, meat inspection was completely revamped with the passage of the Pathogen Reduction; Hazard Analysis and Critical Control Point Systems Act (*Federal Register* 1996). Hazard Analysis and Critical Control Point (HACCP) programs were developed for all meat slaughter and processing facilities. Because of this renewed focus on food safety and the success of HACCP, there has been a decrease in pathogenic bacteria in meat products. An added bonus has been a reduction in the general microbiological load on beef and a concomitant increase in shelf life of beef retail cuts. Additional efforts aimed at educating consumers on safe handling of food at home have also been important in reducing food poisoning outbreaks.

SOURCES OF BACTERIA IN BEEF

The microbiology of beef has been studied for more than 100 years. Ayers (1955) wrote an excellent review on the microbiology of meat. He cited articles published as far back as 1886 where researchers were concerned about bacteria in beef. Obviously, the concern over the ability of bacteria in meat to possibly cause disease in consumers and waste through spoilage is not new. Ayers (1955) and other researchers (Sofos and others 1999) have reported

on the sources of bacteria found in beef. Prior to the slaughter process, the meat from a healthy animal should be sterile. It is through the processes of immobilization, exsanguination, hide removal, evisceration, handling, and further processing into wholesale and retail cuts that bacteria may come in contact with beef tissues.

The hides of cattle are loaded with bacteria (Ayers 1955, Sofos and others 1999). While the total bacterial load on the hides is influenced by the amount of mud and manure present and time of year, suffice it to say that the external surface of beef hides carries a high bacterial load and also a wide variety of bacterial species. Even with extremely careful hide removal, some of these bacteria will be transferred to the exposed surfaces of the carcass. The transfer can come from direct contact of the exterior surface of the hide with the carcass or indirectly through workers and equipment. Also, since the kill floor environment is generally warm and humid, bacteria that become airborne may survive and later contact exposed carcass surfaces (Sofos and others 1999).

The intestinal tract of cattle is also loaded with bacteria. Bacteria from the intestinal tract may come in contact with the carcass surface if the intestinal tract is ruptured during evisceration. Also, feces voided during slaughter and rumen contents spilled through the esophagus may be additional sources of contamination. After the carcass is chilled, further contamination may occur during the processing into wholesale and retail cuts. This contamination will likely be from the transfer of bacteria on the carcass surface to newly exposed lean surfaces. Also the possibility exists of freshly cut surfaces being contaminated by equipment and workers.

Ayers (1955), Savell and others (1981, 1986), and Dixon and others (1991) all reported on the microflora of beef carcasses. Generally speaking, numerous bacterial species may initially be present and often in high numbers. Fortunately, most bacterial species found in beef are relatively benign, and do not survive postmortem preservation practices. When it comes to pathogenic and spoilage bacteria of importance, relatively few species are responsible for most damage.

The pathogens of most concern are *Campylobacter jejuni, Salmonella* spp., *Escherichia coli* 0157:H7, and *Listeria monocytogenes.* Fortunately, these bacteria are only present in very low counts on a limited number of carcasses. Unfortunately, very few of these bacteria are necessary to cause sickness, and possibly death, when people consume contaminated products. Therefore, methods to control bacterial growth on beef must consider the growth requirements of these major pathogens. Since pathogen control is of utmost importance, preservation often involves altering the environmental conditions under which beef is stored. The controlled storage conditions inhibit pathogenic bacterial growth but may allow growth of some spoilage bacteria. The spoilage bacteria that usually end up dominating the microflora are *Pseudomonas* spp. or *Lactobacillus* spp.

METHODS OF PRESERVATION

In addition to requiring an adequate nutrient supply, bacteria's ability to grow depends on their environment. Variations in temperature, oxygen, pH, and water availability, and other environmental factors will determine not only how well bacteria can grow, but which species are capable of growing under those conditions. Obviously, beef meets the nutrient requirements of most bacteria. Although somewhat limited, it does supply carbohydrates, which many bacteria utilize for energy. More importantly, it has an abundant supply of protein and bacteria that can metabolize amino acids (putrefactive bacteria) find beef to be an especially favorable growth medium. Beef is also high in water and has a pH (pH approximately 5.7), which is well within acceptable limits for most bacteria.

While it is desirable to limit the growth of all bacteria in fresh beef, controlling the growth of pathogenic bacteria is of primary importance. Many of the pathogenic bacteria are mesophilic (body temperature) and require temperatures between 10°C and 40°C for optimal growth. Obviously, one of the most effective methods to prevent the growth of many pathogenic bacteria is to store meat at cold temperatures. It is common in developed countries to store meat at temperatures of −2°C to 2°C as soon after slaughter as possible. While pathogenic bacteria may still be present, their ability to grow is greatly inhibited by the cold temperature and the competition for nutrients from bacteria that can grow at refrigeration temperatures. Mechanical refrigeration has been in widespread use in industry for more than 100 years. However, it was only after World War II that mechanical refrigeration became readily available at the

household level. Unfortunately, refrigeration is still not available in most of the developing countries of the world. Anyone who has visited the "wet" markets in Indonesia, China, Africa, or other developing areas notices meat products being stored at ambient temperatures. The only recourse is to consume this meat before the pathogenic bacteria have time to grow to dangerous levels and to very thoroughly cook the meat prior to consuming.

During refrigerated storage, *Pseudomonas* spp. becomes dominant because they are the most capable of growing at refrigeration temperatures under aerobic conditions (Newton and Rigg 1979, Savell and others 1986). Shelf life depends on the initial psychrotrophic (cold-loving bacteria) load and storage temperature (Greer and Jeremiah 1981). These workers reported a significant inverse relationship between retail display case blower temperature and bacterial generation time (temperatures close to 0°C greatly decreased bacterial growth rate). Chandran and others (1986) also reported lower levels of bacteria on steaks stored in the dark at 0–1°C versus steaks stored in lighted display at 2–5°C.

Refrigeration was the primary method utilized to preserve fresh meat until the 1960s. This is when vacuum packaging of wholesale and primal cuts of beef began. While the development and acceptance of vacuum packaged beef cuts took many years, and the process is somewhat complicated, the concept is simple—limit the oxygen available at the meat surface during refrigerated storage and the ability of *Pseudomonas* spp. (or other aerobic psychrotrophs) to grow is greatly limited. *Lactobacillus* spp. then becomes the dominant microflora (Newton and Rigg 1979, Savell and others 1986, Dixon and others 1991). The advantage is that *Lactobacillus* spp. grow very slowly at refrigeration temperatures and this greatly extends the storage life of beef. Newton and Rigg (1979) reported an inverse relationship between oxygen permeability of meat packaging film and growth of *Lactobacillus* spp. Gill and Penney (1988) reported that putrid spoilage caused by aerobic bacteria was reduced by increasing CO_2 concentrations. Even though lactic acid bacteria will eventually dominate within the vacuum package, their metabolic by-products are not as offensive as the by-products produced by putrefying aerobic psychrotrophs.

As effective as refrigeration and vacuum packaging have been at controlling bacterial growth in beef, they are not perfect. With the food poisoning outbreaks from *E. coli* 0157:H7 and continued problems with pathogenic and spoilage bacteria, the beef industry began searching for other intervention methods to further reduce the bacterial numbers in beef.

One obvious step to reduce carcass contamination is to reduce the bacterial load on the exterior of cattle prior to them entering the processing room. Processing plants routinely wash the live animals to eliminate the visible hide contamination (manure and mud). Although washing does not remove all bacteria on the hides, it does reduce the number. Also, eliminating manure and mud reduces the possibility of contaminating equipment and the carcass surface during hide removal. Bowling and Clayton (1992) patented a process to chemically remove hair from cattle hides. The process is applied after exsanguination and prior to the start of hide removal from the carcass. Schnell and others (1995) reported that chemical dehairing reduced visible contamination (hair and feces) on carcasses. Castillo and others (1998) found that chemical dehairing was effective at reducing levels of inoculated *Salmonella* spp., *Escherichia coli* 0157:H7, and *Listeria monocytogenes.*

Reagan and others (1996) deliberately contaminated beef carcasses with bovine fecal matter at a level of $>10^4$ colony-forming units (cfu)/square centimeters (cm^2) to evaluate the efficacy of washing, trimming, hot water washing (74 to 87.8°C), and rinsing with 0.3 to 2.3 parts per million (ppm) ozone or 5% hydrogen peroxide solution at reducing bacterial loads. All intervention steps reduced the numbers of bacteria. However, trimming and washing or hot water rinsing were the most effective. In a similar study, Gorman and others (1995) found hot water rinsing was as effective at reducing bacteria numbers as chemical treatments consisting of 5% hydrogen peroxide, 0.5% ozone, 12% trisodium phosphate, or 2% acetic acid. Hardin and others (1995) evaluated washing carcasses with 35°C water followed by rinsing with either a 2% lactic acid or 2% acetic acid solution. Again, all treatments were effective but lactic acid spray was superior. However, it should be noted that the water temperature used was considerably lower than that used in the previous studies (Reagan and others 1996, Gorman and others 1995). Phebus and others (1997) reported on the use of steam pasteurization, steam vacuuming, trimming, washing, and lactic acid to reduce

bacterial loads on beef carcasses. They reported all treatments effectively lowered bacterial counts, but were most effective when used in combination. Strict sanitary procedures and a 55°C, 1% lactic acid solution was used by Dixon and others (1991) to effectively reduce the bacteria numbers on beef and extend the shelf life of steaks manufactured from these carcasses. Other researchers (Hamby and others 1987, Quartey-Papofio and others 1980, Prasai and others 1991, Woolthuis and Smulders 1985) have shown reductions in bacterial counts on beef carcasses by treating with lactic and/or acetic acid. Acuff and others (1987) concluded that decontamination with organic acids is most effective when done immediately after slaughter. Other chemical applications that have shown effectiveness at reducing bacterial numbers on beef carcasses include acidified sodium chlorite and activated lactoferrin. Castillo and others (1999) reported sodium chlorite activated with citric acid reduced counts of inoculated *Escherichia coli* 0157:H7 and *Salmonella typhimurium* 4.5 log cycles on beef cuts removed on the slaughter floor. This treatment was more effective than phosphoric acid-activated sodium chlorite or washing with water. Acetic acid solutions are corrosive to equipment and can pose a safety hazard. Acidified sodium chlorite is more user friendly. Throughout the beef industry, most microbial interventions will consist of an acid treatment (lactic, acetic, or acidified sodium chlorite) in combination with steam vacuuming and/or steam pasteurization.

Lücke (2000) reported on the practice of inoculating meat with lactic acid producing bacteria, commonly referred to as probiotics, to control the growth of spoilage and pathogenic bacteria in meat. Lactic acid bacteria do not compete very well with gram-negative pseudomonas and therefore are not likely to be effective in preserving aerobically packaged fresh meat. However, probiotics may be beneficial in vacuum-packaged meat. Strains of lactic acid bacteria that are only slightly putrefactive may be used to suppress the growth of naturally present highly putrefactive lactic acid bacteria.

Ionizing radiation also is effective at reducing microbial numbers in meat. However, it is not currently feasible to apply this to a beef carcass. Currently, its greatest promise is in reducing bacteria numbers in frozen ground beef patties.

The beef slaughter, processing, and retail industries operate on very tight profit margins. Each industry segment is continually searching for ways to increase their production efficiencies. An obvious way to increase efficiency at the retail level is to process vacuum-packaged primal cuts into packaged retail cuts at a central location and then distribute these retail ready cuts to stores for consumer purchase. This system allows concentration of skilled labor into one or perhaps a few locations. Skilled labor is not needed at the retail store, since the only preparation necessary is to remove the packaged retail ready cuts from their shipping container and place them in the display case. In addition to improved labor efficiency, centralized processing offers the potential for increased quality control, including improved sanitation. With all of these advantages, it would seem that all beef offered for retail sale would be centrally processed. However, the shelf life of conventionally packaged (foam tray over wrapped in oxygen-permeable film) retail beef is too short for the process to be successful. After processing and packaging, the cuts still have to be shipped and possibly stored at the retail market before being placed in the display case for sale. Once in the display case, the cuts need to maintain their consumer acceptability for 3 to 5 days. According to Tewari and others (1999), centrally processed and packaged retail cuts must be able to maintain their quality for 20 to 30 days of storage prior to being placed in the retail display case.

Even though vacuum packaging offers increased storage time, it is usually applied only to wholesale or primal cuts. It is not an acceptable method of packaging for individual retail cuts. Because of the reduced oxygen levels during vacuum storage, the meat pigment myoglobin remains in the unoxygenated state. The dark color, usually described as purple, associated with unoxygenated myoglobin is unacceptable to consumers. Also, there is formation of oxidized myoglobin, referred to as metmyoglobin. Metmyoglobin is brown in color and is not acceptable to consumers. Oxidation of myoglobin to metmyoglobin naturally happens during storage and retail display of beef. It will usually be the first quality defect noted during retail display and is not associated with microbial activity. If small amounts of metmyoglobin are formed during vacuum storage, the metmyoglobin reducing activity of the muscle tissue may convert it to myoglobin upon exposure to oxygen. When the meat surface is exposed to oxygen, oxymyoglobin is formed. Oxymyoglobin is the pig-

ment responsible for the bright red color that consumers associate with high quality beef. Altering atmospheric conditions under which the meat is stored so that high quality appearance is maintained while bacterial growth is limited is necessary for the concept of centralized processing to be realized. The process utilized is generally referred to as modified atmospheric packaging (MAP).

With MAP, the atmosphere can be modified within each package or a number of conventionally packaged cuts can be placed in a container, which then has its atmosphere modified. Regardless of the exact method used, the modified atmosphere will usually contain a high level of CO_2 to aid in inhibiting bacterial growth. In systems where MAP is applied to a container holding several cuts, the cuts will be wrapped in an oxygen-permeable film so that when they are removed from the container oxygen can interact with myoglobin. One disadvantage to this system is the necessity for a large container capable of maintaining a high CO_2 concentration. More importantly, depending on the amount of metmyoglobin formed at the meat surface during storage, it may take up to 2 days for oxymyoglobin to be formed after exposure to air (O'Keefe and Hood 1982). That makes this system difficult to employ at retail.

Systems where MAP is applied to each package usually have an atmosphere containing both a high level of CO_2 and O_2. This allows for inhibition of bacterial growth while maintaining acceptable beef color. It should be noted that this combination of CO_2 and O_2 is a compromise and the shelf life of cuts packaged under these conditions are not as great as steaks stored under high CO_2 and/or low O_2. Gill and Jones (1994) reported steaks packaged in CO_2 plus O_2 atmospheres began to deteriorate in appearance after 12 days. Color degraded prior to acheiving sufficient numbers of bacteria to have any flavor effects. They noted that the useful storage life of steaks stored in high CO_2 packages is limited by the onset of bacterial spoilage and this may take several weeks. Beef stored in high O_2 environments is also more susceptible to fat oxidation and the resultant undesirable flavors.

Since it is not currently possible to slaughter cattle aseptically, carcasses and the resultant wholesale and retail cuts will carry a bacterial load. These bacteria will eventually affect the odor, color, flavor, and overall acceptability of the meat. Additionally, intervention steps aimed at reducing the bacterial load must also be assessed not only on their ability to limit bacteria but on their effect on sensory characteristics of beef.

Pseudomonas spp., which eventually dominate on aerobically packaged meat, are putrefactive. While these bacteria will utilize any available glucose for energy, they ultimately metabolize amino acids. This will lead to production of putrid odors, slime, and undesirable flavors. Greer and Jeremiah (1981) and Greer and others (1983) showed that time for putrid odor production was dependent on initial psychrotrophic load and storage temperature. Nassos and others (1988) reported that odor and appearance decreased in acceptability with increasing storage time. Newton and Rigg (1979) reported undesirable putrefactive odors were detected when *Pseudomonas* spp. reached levels of 10^6cfu/cm^2. They found that putrid odors were detectable after 2 weeks of storage at 0°C in highly oxygen-permeable packages. However, meat from these packages was still acceptable in flavor after 11 weeks of storage. Savell and others (1986) found steaks cut from beef knuckles stored in highly oxygen-permeable film scored low in color desirability even after 1 day of retail display. These low scores were associated with aerobic plate counts of 10^6cfu/cm^2 and a high percentage of *Pseudomonas* spp. making up the microflora. Egan and Shay (1982) inoculated sterile, vacuum-packaged meat samples with a homofermentative strain of lactobacillus. They found that objectionable odors and flavor developed when lactobacillus levels reached 10^8cfu/cm^2. Many other workers (Acuff and others 1987, Dixon and others 1991, Savell and others 1986) have reported that vacuum packaging prevented growth of most bacteria and greatly increased storage time of beef primals. Dixon and others (1991) showed retail steaks removed from loins and ribs stored for up to 80 days in vacuum packaging were acceptable in color and overall appearance for 2 days of retail display. Egan and Shay (1982) also reported that vacuum packaging greatly extended storage time, however, even vacuum-packaged sterile meat samples had a limited storage time due to development of undesirable flavors.

Quartey-Papofio and others (1980) conducted a study designed to evaluate the effectiveness of potassium sorbate and acetic, formic, and propionic acids as meat sanitizers. They evaluated acid concentra-

tion, pH, and exposure time on 11 different microorganisms. While acetic acid at pH 1.0 was more effective than acetic acid at pH 2.0, they concluded the high amount of hydrogen chloride (HCl) required to lower the pH was impractical. All further tests were conducted at normal pH and at pH adjusted to 2.0. Potassium sorbate, either alone or in combination with the other acids, offered little benefit in reducing bacterial numbers. They found 2% formic acid or a combination of 1% formic acid and 1% acetic acid were the most effective at eliminating bacteria. However, meat treated with formic acid (up to 2% solution) turned brown shortly after treatment. The degree of discoloration was believed to be dependent on concentration. They attributed this to oxidation of the iron in myoglobin and the resultant formation of metmyoglobin. To overcome this discoloration, they added 1% or 5% ascorbic acid to the 2% formic acid solution. The mixture of 1% ascorbic acid did not improve color. But, the addition of 5% ascorbic acid resulted in minimal discoloration and an improvement in antimicrobial activity. Bell and others (1986) tested inoculated strips of beef by dipping them in various concentrations of acetic acid (0.6%, 1.2%, 1.8%, or 2.4%) or acetic acid with formic acid (0.6% acetic with 0.046%, 0.092%, 0.184%, or 0.23% formic acid). They found an increase in discoloration with an acetic acid concentration greater than 1.8% and with all acetic acid treatments exposed for 10 minutes versus 1 minute. When 0.6% acetic acid was combined with formic acid concentrations of 0.046% or greater, color was less desirable than controls. Taste panelists were able to detect differences in flavor between control samples (dipped in distilled water) and samples treated with 1.2% acetic acid. However, panelists were not able to distinguish flavor differences between control samples and samples treated with 0.6% acetic acid combined with 0.046% formic acid.

Woolthuis and Smulders (1985) treated calf carcasses with up to 4.0% (vol/vol) lactic acid. Solutions up to 1.25% did not produce unacceptable color and solutions up to 2% had no effect on flavor of roasted *Longissimus* cuts. However, solutions of 4% resulted in a slightly sour taste. They also reported high concentrations of lactic acid created rusty brown spots on carcasses. They concluded that 1.25% lactic acid solutions could effectively be used to reduce bacterial numbers and have little or no effect on lean color and flavor. Mikel and others (1996) treated beef strip loins with an equal mixture of 2% lactic acid and 2% acetic acid. They reported that muscle ultrastructure was degraded with the acid treatment. However, shear force value was not affected. They also found no differences in Hunter color values, fat oxidation, or cooking loss. Prasai and others (1991) sprayed 1% lactic acid on carcasses immediately after hide removal, after evisceration, or at both times. Samples for bacterial enumeration were removed from the carcasses just prior to entering the chill cooler (0 hour) and after 72 hours of chilled storage. In all 0-hour samples and in all but one 72-hour samples, lactic acid treatment reduced bacterial numbers. Generally, the greatest reductions in bacterial counts were on carcasses treated after evisceration or after a combination of both hide removal and evisceration. Treating after evisceration alone was more effective than treating only after hide removal. Loins removed from treated and control carcasses were stored for 14 days in vacuum package and evaluated for color, odor, and overall acceptability. No differences were detected for these sensory attributes. Hamby and others (1987) treated carcasses with intermittent sprays of water, lactic acid, or acetic acid (alone and in combination) for up to 12 hours following slaughter. They reported no differences in odor, color, or purge from treated and control samples. Acuff and others (1987) removed strip loins from chilled beef carcasses that had not been treated with any decontamination procedure postslaughter. The loins were treated with either a 1% lactic acid solution, 1% acetic acid solution, or a solution containing 1% lactic acid, 2% acetic acid, 0.25% citric acid, and 0.1% ascorbic acid. None of the treatments had a consistent advantage over others for lean color, fat color, surface discoloration, or odor.

Microorganisms play an important role in storage, preservation, consumer acceptability, and safety of beef products. Undesirable changes in beef's appearance often occur prior to bacterial numbers reaching levels sufficient to cause spoilage. Nonmi-crobial color changes are used by consumers to determine product freshness. Probably unknown to most consumers, this offers them a margin of safety between undesirable color and unacceptable bacterial numbers. However, pathogenic bacteria do not need to be present in large numbers to be dangerous and pose a human health hazard. Therefore, efforts to reduce and eliminate pathogens are necessary. Industry programs aimed at reducing pathogens have the added benefit of lowering numbers of all

bacteria. This has not only led to beef being safer, but also to increasing its shelf life. These changes have come without lowering of beef's appearance or eating qualities.

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26
Quality Measurements in Beef

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INTRODUCTION

In modern society, consumers of any products expect an optimal quality/price relationship and reliable quality. Meat is not the exception. Meat quality varies within and between animals of the same breed, sex, age, weight, and environment. Moreover, muscle composition is an important factor that influences pre- and postmortem biochemical processes and therefore meat quality.

There are four major categories to describe meat quality: hygiene, physiology, technology, and sensory attributes. To determine the quality of meat, characteristics such as color, tenderness, water-holding capacity, and sensory evaluation are used in the meat industry. Moreover, since one of the most important quality attributes for meat is tenderness, quality determination can be based on attributes that affect meat tenderness; these are postmortem proteolysis, intramuscular fat, connective tissue, and the contractile state of the muscle.

Protein, water, and other tests have been standardized by the Association of Official Analytical Chemists (AOAC). However, measurements such as pH are not well standardized. The importance of pH in meat is tremendous. Meat in the pH range of 5.4–5.6 has the most desirable properties for table cuts. Higher pH results in color changes. Also, high pH meat has a poor microbial stability, and the cooked flavor is inferior. Therefore, optimization of potential online methods to measure beef quality is a crucial area of research in the meat industry.

Finally, the meat industry is looking for reliable methods that will correlate sensory attributes and instrumental analysis. However, sensory analysis has its drawbacks, and these need to be considered. Some of the parameters to consider are level of training, size of the panels, size of the samples, internal temperature reached, and instrument used.

CARCASS QUALITY

Meat quality can be divided into intrinsic and extrinsic factors. Intrinsic factors include the type of cut, color, fat lumps, fat rims, marbling, and fat content. The extrinsic factors include price, origin, and information on animal production (Grunert and others 1996). Parameters that are used to determine quality

of a carcass include slaughter weight, carcass yield, fatness classification, muscle, fat, bone, and intramuscular fat content (Sañudo and others 2004). The carcass weight is used in conjunction with ossification to estimate the effect of growth on palatability. Also, the carcass hanging method is considered in grading meat. Marbling is used to assess palatability, in the same way as ultimate pH, ageing, and cooking method (Thompson 2002). One more consideration is the percent of weight losses during frozen storage. Losses vary from 0.05 to 1.2%, initially, but after 6 months of frozen storage, losses can be up to 4.6% (Mendez 1999).

Another quality factor is microbial quality. Muscle foods are described as spoiled if organoleptic changes make them unacceptable for the consumer (Ellis and Goodacre 2001). Spoilage bacteria can contaminate carcasses, which results in reduced shelf life of meat causing economic losses (Venkitanarayanan and others 1997). In addition, carcasses can be contaminated with fecal bacteria if personnel observe poor hygiene practices (Bolling and others 2002).

The color of meat differs among species. For beef, the *longissimus* muscle is very color stable but the *psoas major* very unstable. Color stability is independent of hemoglobin content. The rate of discoloration under aerobic conditions depends on the rate of oxygen diffusion and consumption, the autoxidation of the pigment, and the rate of metmyoglobin reducing activity (Klont and others 1998). To evaluate color and discoloration of carcasses an 8-point muscle color is used with the following anchors: 1—bleached red, 5—slightly dark red, and 8—very dark red (Tan 2004).

Muscle type plays a major role in the postmortem tenderization process. Three mechanisms have been suggested to explain the differences in ageing rate among muscle types. One is the levels of proteases and inhibitors; second, sensitivity of muscle proteins to proteolysis; and third, osmotic pressure. In addition, an increased tenderness with larger sarcomere lengths has been reported (Klont and others 1998). Electrical stimulation is used in the meat industry to improve meat tenderness. Electrical stimulation prevents muscle from shortening but also might disrupt the physical structure of the muscle (Hwang and others 2003).

The quality attribute most commonly measured in fresh meat is pH. Prediction of ultimate pH will allow the meat processor to maintain the quality of meat (Monin 1998). To measure pH the reference method of ISO 2917 is suggested. This method involves piercing a hole in the meat sample with a knife and inserting an electrode (O'Neill and others 2004).

Meat appearance determines purchase decision and acceptability. This attribute depends on color, amount of visible fat (marbling), and wetness (exudation). Visible fat involves intramuscular fat (marbling) and intermuscular fat. Appearance is an important quality factor used in some carcass grading systems. High marbling may or may not be desirable. Intermuscular fat is important for consumer acceptability of meat commodities containing several muscles (lamb legs, pork chops, or ham slices). Quantification of intermuscular fat can be performed using video-image analysis, ultrasound echography, X-ray computerized tomography, and magnetic resonance imaging (MRI). The USDA's marbling scorecard includes 1: devoid, 2: practically devoid, 3: traces, 4: slight, 5: small, 6: modest, 7: moderate, 8: slightly abundant, and 9: moderately abundant. Additionally, there are five evaluation standards for marbling: 5 = excellent, 4 = good, 3 = average, 2 = below average, and 1 = poor (Yoshikawa and others 2000).

SENSORY ATTRIBUTES AND EVALUATION

Any sensory evaluation requires the training of panelists. To conduct a successful sensory panel, there are several requirements: environmental, panel, and product conditions (Miller 1998). Questionnaires are developed according to the objectives of the testing. For example, consumer questionnaires for uncooked products include color, visible fat amount, size, thickness, overall aroma, and freshness. For cooked products, the sensory characteristics evaluated by consumers include overall appearance (color, visible fat amount, size, juiciness), overall flavor (beefy/meaty, freshness), overall texture/visual (ease of cutting, juiciness, degree of doneness), and overall texture/oral (tenderness, firmness, juiciness, chewiness, and oiliness) (Muñoz 1998). The major descriptive attributes for whole-muscle are juiciness, muscle fiber tenderness, connective tissue, and overall tenderness (Miller 1998).

Sensory Attributes

The mastication process involves two processes: contact with the teeth and size reduction by the teeth. The masticatory system of humans is highly

responsive to changes in food texture. This sensitivity has a protective element since high forces can damage the teeth or the jaw joint. The ability to sense food texture intraorally evolved 200 million years ago. In this sense, we can observe that every human society seems to have a variety of food texture terms (Lucas and others 2002). Sensory parameters to assess sensory attributes in meat have been developed (Table 26.1). A particular scorecard includes four sensory attributes: hardness, springiness, juiciness, and fat sensation. Each attribute has a different scale, anchors, and reference food (Ruiz and others 2003).

Sensory Evaluation

Sample Preparation

Sample size for muscle pork is a cube of 1.27 cm and should be given in at least two serving portions per sample. For ground meat products, panelists are usually served a 0.64-, 0.42-, or 0.32-cm wedge of the patty including the edges or a 1.27- or 2.54-cm square with the edges removed. When sausage-type products are evaluated for texture, a whole sausage may be served (Miller 1998). If only flavor is evaluated on a sausage, a 2.54-cm section with the end piece removed may be served. Temperature of the samples at all times must remain constant at 70°C (Miller 1998, Caine and others 2003). In addition, all samples must be coded with three-digit random numbers. Order presentation of the samples should be random (Miller 1998).

To conduct sensory evaluation of rib steaks, samples of 1.9 × 1.9 × 1.9-cm cubes are used (Caine and others 2003). To avoid difference in degree of doneness, slices of meat are cut in cubes of the same length. Cubes are cooked and the final internal temperature is monitored (Denoyelle and Lebihan 2003). Steaks are broiled to an internal temperature of 34°C, turned over and then broiled to a final temperature of 68°C. Steaks are cut into 1.25-cm cubes and served to the panelists. The containers used to serve the samples (beakers) are preheated to 76°C to help maintain the sample temperature (Li and others 2001).

Selection and Training of Panelists

The number of panelists depends on training criteria. However, a minimum of six panelists is recommended. Panelists should be trained in the proper techniques to conduct the evaluation, such as chewing a sample, expectorating a sample, rinsing of the mouth in between samples, and familiarization with the methodology. During this training, reference samples are presented and meat attributes must be incorporated in terms of descriptors such as juiciness, flavor intensity, off flavor, etc. (Miller 1998). To conduct time-intensity analysis, panelists take 12 one-hour training sessions to familiarize themselves with the use of a computer mouse to move along the 60-pixel line (Jimenez and others 2003a). In other instances, 14 one-hour sessions are recommended, divided into six sessions of initial training, two sessions to check for performance, four sessions of additional training, and two sessions for a second check for performance (Labbe and others 2003).

Methods

Sensory perceived texture has been defined as an attribute of a substance resulting from the combination of physical properties and perceived by the senses of touch, sight, and hearing. Evaluation of texture by sensory methods accounts for mechanisms of texture perception, physiological mechanisms related to food breakdown during mastication, and food structure (Wilkinson and others 2000). Difference testing is conducted to identify sensory differences samples. The most common difference tests are triangle tests, two-out-of-five tests, duo-trio tests, and triangle tests (Miller 1998, Rhee and others 2003). For processed meat products, the Spectrum Method, the Quantitative Descriptive Analysis (QDA®), or the Free-Choice Profiling Method is frequently used (Miller 1998). The Spectrum Method is a descriptive attribute method that uses a 0- to 15-point universal scale where 0 = none and 15 = extremely intense. The QDA® method is a method that was developed to determine differences in sensory attributes of food products. In this method, a 15-cm line scale is used to quantify each sensory attribute. The Free-Choice Profiling Method allows the freedom for panelists to develop their own sensory descriptors for a product (Miller 1998, Delahunty and others 1997).

To evaluate the sensory properties of irradiated ground beef, scorecards containing 15-cm lines with anchor words are used. The attributes and anchors used are flavor (weak and strong), texture (tough to tender), juiciness (dry to moist), and aftertaste (none to strong) (Murano and others 1998). To evaluate

Table 26.1. Attributes used in sensory evaluation to describe meat.

Descriptor	Definition	Scale	References
Hardness	The force required when compressing a substance between molar teeth	1 = very soft to 9 = very hard	Ruiz et al. 2003; Peachey et al. 2002; Szczesniak 2002
Toughness	The combination of cohesiveness, chewiness, softness, and juiciness		Monin 1998; Peachey et al. 2002
Springiness	The degree to which a product returns to its original shape once it has been compressed between the teeth	1 = non-elastic or plastic and 5 = extremely springy	Ruiz et al. 2003; Szczesniak 2002
Juiciness	How much juice is expressed as you bite down on the sample with back molars, after 5 chews, or simply after mastication	1 = dry and 5 = juicy or 0 = not juice to 10 = very juicy	Ruiz et al. 2003; Peachey et al. 2002; Zimoch and Gullet 1997
Cohesiveness	Degree to which a substance is compressed between the teeth before it breaks or how the sample breaks on the third chew		Peachey et al. 2002; Szczesniak 2002
Tenderness	The required force to chew a 1.2 cm cube of meat, measured after three chews	tough to tender	Swatland and Findlay 1997
Chewiness	The length of time required to masticate a sample at a constant rate of force, to reduce it to a consistency suitable for swallowing	not chewy and very chewy	Peachey et al. 2002; Szczesniak 2002; Swatland and Findlay 1997; Zimoch and Findlay 1998
Color		5 = bright red, 3 = slightly brownish red, and 1 = brown, or red, brown and green in pork	Jimenez et al. 2003a; Jimenez et al. 2003b; O'Sullivan et al. 2003
Discoloration	Expressed in percentage surface discoloration	7 = no discoloration (0%) 5 = small discoloration (20-39%) 3 = moderate discoloration (60-79%) 1 = total discoloration (96-100%)	Jimenez et al. 2003a
Odor		8 = extremely beef like, 5 = slightly beef like, and 1 = extremely non-beef like	Jimenez et al. 2003a
Off-odor		5 = no off odor and 1 = extreme off-odor	Jimenez et al. 2003a
Flavor	The amount of beef flavor perceived in the meat after eight chews	cooked beef brothy, serumy, cardboard	Zimoch and Findlay 1998; Spanier et al. 1997

color of ground beef, a 5-point color scale has been used where 5 = bright red and 1 = brown. To evaluate odor, an 8-point scale is used where 8 = extremely beef like and 1 = extreme non-beef like (Jimenez and others 2003c). To evaluate low-fat beef burgers, tenderness, juiciness, and meaty flavor are evaluated using an 8-point scale. Nonmeat flavor, overall flavor, overall texture and overall acceptability are evaluated using a 6-point scale (Troy and others 1999). To evaluate changes during storage of irradiated meat, a scorecard with 15-cm lines with anchor words is used. Another scorecard includes a 100-point scale to describe odor intensity, tenderness, juiciness, residue (amount of tissue perceived after swallowing), overall flavor intensity, flavor quality, and overall liking. A score of 1 designates low odor, low flavor intensity, tough, dry, little fiber and amount of residue, and disagreeable flavor and acceptability; while a score of 100 designates high odor and flavor intensity, tender, juicy, high fiber and amount of residue, and agreeable flavor and high acceptability (Villarroel and others 2003).

To measure texture of cured hams, descriptors such as hardness, pastiness, crumbliness, and adhesivity are used with a scale from 0 to 10, where 0 = absence and 10 = intense. References for each texture descriptor are used. For example, for pastiness and crumbliness, a 2-mm-thick slice of a mince *Longissimus lumborum* muscle containing 1 gram (g) of papain per kilogram (kg) of muscle is used (Gurrero and others 1999). To evaluate tenderness, perceptible connective tissue, juiciness, and flavor on rib steaks, a 9-point scale has been used, with 9 = extremely tender, no perceptible connective tissue, extremely juicy, and intense beef flavor, and 1 = extremely tough, abundant connective tissue, extremely dry, and bland beef flavor (Caine and others 2003).

To study beef broth flavor, panelists are trained to describe flavor descriptors such as oily, fatty, sour, bitter, metallic, bloody, umami, tasteless, gelatinous, warmed-over, burnt, astringent, beef tea, boiled, ammonia-like, and sulphurous. The intensity of each descriptor is recorded in terms of not detectable, weak, medium, and strong. To conduct this type of flavor panel, rank order tests and triangle tests are used (Cambero and others 2000).

The time intensity methodology differs from conventional methods because it measures temporal changes in meat texture perception that take place in the mouth during chewing (Butler and others 1996, Duizer and others 1996). Panelists are assigned to computers where they input their responses for toughness (vertical scale) and juiciness (horizontal scale) on a time-intensity line of 60 units in length. Each scale is labeled with the appropriate descriptors (Zimoch and Findlay 1998). The time-intensity parameters used are maximum intensity, time to maximum intensity, duration, and area under the curve (Duizer and others 1996).

Statistical Analysis

Data generated from sensory evaluation tests are produced by judgments of the human subjects. A task for the sensory scientist is to establish whether or not product difference is significant. The presentation of samples is crucial and must be standardized with regard to form, temperature, conditions of lighting, coding, etc. Presentation order must also be considered especially when samples with strong flavors or odors are to be evaluated. Replication in sensory evaluation differs since there is more than one measuring unit, that is, more than one panelist. True replication requires each panelist to perform a judgment two or more times on samples of the same product (Bower 1995).

The type of data generated by sensory methods depends on the type of evaluation conducted. For instance, difference tests produce nominal data, whereas ranking produces ordinal data. Therefore, testing data for normality is recommended that can be accomplished just by simple graphical techniques (Bower 1995). According to the data distribution, the sensory scientist must decide if a parametric or nonparametric method should be selected to analyze the data. For sensory discrimination tests, nonparametric methods are used. Common tests include the binomial test and the chi-square test (Bower 1996). Some of the statistical tests for comparing two groups of data include the sign test, Wilcoxon signed rank, Mann-Whitney U test, two-sample t-test, and paired t-test (Bower 1997a). For sensory tests that involve more than two sample groups, it is recommended to use a statistical design and conduct analysis of variance (ANOVA). Some of the common designs used in sensory evaluation are completely randomized design, randomized block design, Kruskal-Wallis, and Friedman's 2-way. The reason to use ANOVA is to partition the sources of

variation into its component parts to enable an assessment on the magnitude of effect on each source on the responses (Bower 1997b).

PHYSICAL-CHEMICAL ANALYSIS

pH

To measure pH of liver, or any other type of muscle, 1:10 ratio sample:distilled water is recommended (Hernandez and others 1999). Muscle has to be homogenized and the resulting slurry used to measure pH (Dainty 1996, Jeremiah and others 2003). The use of metallic probes is recommended to measure pH of carcasses. Metallic probes are more robust and less destructive than glass probes. To measure pH, the probe is inserted into the muscle at least 1 cm up to 5 cm deep (Byrne and others 2000, Young and others 2004). Testing for pH is required in every carcass, usually after 24 hours of slaughter. However, a simple measurement like this can be affected by premature measurement, miscalibration, contamination of electrodes or sensors, and temperature effects (Young and others 2004). Also, pH of muscle can be measured using a surface pH probe (Mustapha and others 2002). Reading with surface electrodes can be compared to pH readings taken with glass electrodes.

Water-holding Capacity

There are many variations for measuring water-holding capacity (WHC) in meat. Methodologies to estimate WHC have been divided based on the type of meat and the process the meat is subjected to. These methods include (1) drip loss in raw, whole meat, (2) water loss in cooked, whole meat, and (3) water loss in cooked, comminuted meat products (Hertog and others 1997, Lawrence and others 2003). Drip loss in raw, whole meat is based on the losses of water that occur when myofibrils shrink due to the changes in pH during rigor. To conduct this test, the meat sample is cut from the carcass, weighed, and immediately placed in the container where the test will be conducted. After 24 hours, samples are reweighed. The recommended sample size for this test is about 80 g. Calculations are related to the initial weight and expressed as percent drip loss. The second method to evaluate water losses in whole cooked samples is based on meat protein denaturation during cooking. The samples to be used in this test should be freshly cut. Individual samples are placed in polyethylene bags in the water bath. Heating is stopped when samples reach 55°C (rare), 65°C (medium), 80°C (well done), and 95°C (thoroughly cooked). After heating, samples are cooled for 30 minutes at about 15°C. Samples are blotted and weighed. The heating loss is expressed as gram/loss of initial weight or as percent heating loss. The last methodology is to estimate water losses in heated comminuted meat products. This test is based on the ability of meat proteins to form different types of gels and colloidal systems. To conduct this test, 10 g of sample is stuffed in a tube and closed tightly. The tubes are heated in a water bath. After heating, the tubes are cooled to about 40–45°C. Samples are centrifuged at 550 × g for 15 minutes. The amount of fat and aqueous phase is weighed (Honikel 1997, 1998). Variations on the speed to centrifuge, time, and temperature can be encountered, e.g., 27,000 × g for 30 minutes at 4°C (Lawrence and others 2003).

To determine WHC of cooked burgers, 10-g samples are weighed in glass jars and heated at 90°C for 10 minutes. After heating, samples are removed from the jars. Once the samples are cooled, they are wrapped in cotton cheesecloth and centrifuged for 10 minutes at 9000 × g at 4°C, and then the samples are reweighed (Troy and others 1999). In addition, video image analysis (VIA) has been used to predict water-holding capacity. For this, a sample of meat (400–600 milligram [mg]) is placed on a filter paper (7-cm diameter), weighed, sandwiched between translucent plastic plates, and pressed at 35 kg/square centimeter (cm^2) for 1 minute. The meat and liquid area on the filter paper are measured by a planimeter and by VIA. Results are expressed in cm^2 (Irie and others 1996). Another way to measure water-holding capacity is expressed as press juice (Maria and others 2003).

Drip Loss and Cooking Loss

Drip loss can be used to express water-holding capacity (Pedersen and others 2003). Meat samples are placed in plastic bags and heated for 120 minutes at 60°C in a water bath. To estimate drip loss or purge, samples are weighed before and after storage and the results expressed in percentage (Boles and Swan 2002). Cooking losses are determined by weighing samples before and after cooking (Ertbjerg and others 1999, Obuz and Dikeman 2003).

Color

Color may be defined as the impact of the wavelengths of light in the visual spectrum from 390 to 760 nanometers (nm) on the human retina (Brewer 1998, Francis 1995). The apparent color is affected by the amount of water in fresh meat. The color of red meat changes over time as the pigments bind oxygen and become oxidized changing to a brown or gray color. Color also changes as a result of microbial growth, cooking, and exposure to chemicals (acids, salts, etc.) (Brewer 1998). Color may be evaluated subjectively (visually) or objectively (using a colorimeter or spectrophotometer). The type of light in which an object is viewed can affect its appearance. The Commission Internationale de l'Eclairage (CIE) developed standardized illuminants for color judgment by human observers or measurement by instruments (HunterLab 1996a). The CIELAB color scale is a color scale organized in a cube form. The L* axis runs from top to bottom where the top maximum value is 100 (white) and the bottom minimum value is 0 (black). The a* and b* axes have no specific numerical limits. This scale goes from positive a* (red) to negative a* (green) and positive b* (yellow) to negative b* (blue) (HunterLab 1996b, 2000a).

There are five instrumental color evaluation systems: Munsell color solid, CIE color solid, reflectance spectroscopy, tristimulus colorimetry, and Hunter color solid. In the Munsell system, color is described in terms of hue and chroma and has five principal hues (red, yellow, green, blue, and purple). Chroma is used to describe the color intensity. The CIE system uses a chromaticity diagram. Reflectance spectroscopy uses wavelengths between 400 to 700 nm and transformed into CIE X, Y, and Z values. Tristimulus colorimetry employs filters to simulate the response to the human eye, light reflected at 45° passes through an X, Y, or Z filter. The Hunter system uses L, a*, and b* values and is based on Tristimulus colorimetry (AMS 1991). In meat, there are three sources of color variation: the content of pigment intrinsic to the muscle, the preslaughter period, and the slaughter process as related to the rate of pH decline and storage conditions (Barbut 2001, Bro and Jakobsen 2002). To measure color in meat, sampling should be conducted after at least 24 hours postmortem. If storage is to occur, the sample should be refrigerated at no higher than 4°C (Honikel 1997). Factors to be considered when evaluating color are listed under ASTM Method D1729 and include the light source (daylight, incandescent, or cool white fluorescent), photometric conditions (intensity of 75–175 foot candles), geometric conditions (0° light source overhead and 45° viewing angle), and background to samples being viewed (neutral gray and uncluttered). When reporting color measurements, the following information must be provided: the color scale, the illuminant, the observer, the instrument type, geometry and mode, the basis of the instrument calibration, the sample preparation method, and the sample presentation method (HunterLab 2000b). To measure flat opaque solids (meat), samples must be rotated to average several readings. The recommended scale is CIE L*a*b* with an illuminant/observer of D65/10° (Ruiz and others 2003; Boles and Swan 2002; HunterLab 2000b; Gasperlin and others 2000, 2001). In some cases D45/2° is used to measure color in meats (Jimenez and others 2003). In addition, it is recommended that a glass should be placed between the sample and the instrument to flatten the meat surface as much as possible and to avoid drippings on the lamp (Abril and others 2001). In some cases when samples are wrapped in high clarity film color, measurements can be taken directly (Farouk and others 2003). It is important to remember that to measure color of fresh meat cuts, samples are kept at 4°C for 4 hours to allow color development (bloom) and then color is measured (Lynch and others 2002, Van Oeckel and others 1999). In some cases, samples are allowed to bloom for only 30 minutes before color is measured (Van Moesecke and others 2001). When polyethylene films are used to pack cuts of meat, cuts are allowed to bloom for 1 day (Moore and others 2003).

The Japanese Color Standard System consists of six plastic disks with a meat-like appearance developed from objective colorimetry. For pork, the scores are 1 to 6, from pale gray to dark purple (Van Oeckel and others 1999). To choose the instrumental method to measure color it is necessary to consider what instrumental methodology to use (pigment extraction or reflectance), how to express the data, and how to use the data. There are variations in terms of how to measure color of fresh meat, cured meat, and cooked meat. For fresh meat, reflectance methods are recommended. Reflectance measurements are affected by muscle structure, surface moisture, fat

content, and pigment concentrations. For cured meats, the reflectance ratio of wavelengths 650/570 is recommended. Hunter Lab values are also used but they do not follow cured color changes. For cooked meat, color measurement of hue angle ($\tan^{-1}$ b/a) is recommended (AMS 1991, Berry and Abraham 1996). Color of expressed juices of cooked beef can be used to assess the doneness of the samples. However, color must be measured immediately after expression of the juice before oxidation of the pigments occurs (Senter and others 1997).

Near-infrared Spectroscopy

The techniques to determine meat quality parameters are time consuming, expensive, and often destructive. Near infrared (NIR) analyses are performed in reflectance and transmission modes. Reflectance is performed in the 833–2,500 nm range. Transmission analysis is conducted between 833–2,500 nm using 3-mm thick fresh samples (Leroy and others 2003). Others use 1,100–2,500 nm range at 4-nm intervals (Rodbotten and others 2000) or 400–2,498 nm range at 2-nm intervals (Liu and others 2003). NIR spectroscopy is used for the assessment of fat, moisture, and protein. Samples for NIR are cut to make the muscle fiber direction parallel to the measurement surface. Slices of 1.5-cm thickness are cut and cut again into a circular shape of 4–5 cm in diameter (Liu and others 2003). Fiber-optic spectroscopy is used to estimate bovine fat quality using wavelengths of 400 to 1,100 nm. Results are promising when the internal reflectance of the intermuscular fat values are associated with saturated fatty acids and with monounsaturated fatty acid content (Irie and others 2003). However, no conclusive evidence that NIR can predict tenderness of meat cuts has been published (Rodbotten and others 2000).

Because freezing affects the quality of meat due to the formation of ice crystals, several methods to discriminate between unfrozen and frozen meat have been studied. Chemical and biochemical methods are available but are time consuming. Therefore, NIR has been suggested to measure physical and chemical changes in meat and meat juice. For this, drip juices are analyzed with a technique called dry extract spectroscopy by infrared reflection. Measurements are performed at 400–2,500 nm range with scanning in the 100–2,500 nm region. This technique is very useful to screen beef into unfrozen or frozen and thawed (Thyholt and Isaksson 1997).

TEXTURE ANALYSIS

Texture is a sensory property, which can be described as a multiparameter attribute that is derived from the structure of the food and is detected by several senses. Textural characteristics can be grouped into mechanical characteristics (hardness, cohesiveness, viscosity, springiness, and adhesiveness); geometrical characteristics (particle size, shape, and orientation); and other characteristics (moisture content and fat content) (Szczesniak 2002). Whenever possible, assessments are to be performed immediately. If samples must be frozen, they should be vacuum packed, frozen quickly, and stored at −18°C or below (Honikel 1997). It is important to remember that understanding the chemical composition of muscle will help to develop methodologies to enhance palatability of individual muscles or muscle groups (Jimenez and others 2003b, Jeremiah and others 2003, Cheftel and Culioli 1997).

Meat texture evaluation methods can be grouped into sensory methods, instrumental methods, and indirect methods. The most common instrumental methods are compression, penetration, shear, and extension tests and texture profile analysis. Indirect methods include determination of collagen content, the myofibrillar fragmentation index, activity of proteases, and histological analyses. An infrequently used method to evaluate texture of meat is grinding (Kamdem and Hardy 1995). Meat texture can be evaluated by both sensory and instrumental methods. The types of forces that can be applied are compression, tension, or shear (Tornberg 1996).

There are some less traditional methods to investigate food texture. One of these methods involves the study of the masticatory process. This method is based on the recording of electromyographic activities of masticatory muscles and chewing rate since these two parameters are influenced by the physical properties of foods. To use this methodology, subjects are given chewing gum to become familiar with the instrument. Jaw movements are recorded, and each masticatory cycle is analyzed (Peyron and others 1996). The influence of texture on various aspects of the masticatory process and the effect of texture on salivary flow has also been reported. To investigate the relationship between meat structure

and swallowable textures after chewing, subjects are asked to chew cold meat samples without swallowing, and to spit out the bolus when swallowing would normally be triggered (Mioche and others 2002). The Meat Industry Research Industry of New Zealand (MIRINZ) has developed an instrument designed to predict the tenderness of meat. The instrument (MIRINZ tenderometer) has two sets of pins on which the meat samples are impaled. The torque required to rotate the inner pin is measured and correlated to taste, olfactory, and feeling factors measured by a trained panel. This instrument appears to be an alternative to the Warner-Bratzler (WB) shear measurement (Jeremiah and Phillips 2000, Peachey and others 2002). Also, a straight, not sharpened blade (7-cm wide by 0.2-cm thick) has been used to measure shear force in beef patties (Berry 1998).

Warner-Bratzler

The Warner-Bratzler (WB) is the most common shear test to evaluate texture in meat (Honikel 1997). The WB technique is the instrumental method that yields the best correlation with sensory panel scores for meat toughness (Monin 1998). Maximum load and toughness, expressed as the energy necessary to break the sample, can be assessed using a WB device. The texture of raw meat can be analyzed using a modified compression device that avoids transversal elongation of the sample (Campo and others 2000). Samples are cut perpendicular to the muscle fiber orientation producing 2.54-cm-thick steaks. Steaks are cooked at 70°C and then cooled at 4°C for at least 10 hours. Samples are trimmed of visible connective tissue and epimisium to expose the muscle fiber orientation (Belew and others 2003). An option used in pork is to place samples in plastic bags in a water bath at 75°C for 50 minutes and then cooled in cold tap water for 40 minutes. Cores of 1.25 cm parallel to the longitudinal orientation of the muscle fibers are obtained from the cooked samples and used to measure texture (Van Oeckel and others 1999).

The WB shear device is pushed at 50–100 mm/minute between side plates positioned to provide a minimum gap between blade and plates. Another alternative is to use a crosshead speed of 250 mm/min (Obuz and others 2003). The sample to evaluate should be cut from a block of cooked meat. Sample strips should be cut with 100 × 100 square mm cross-section and fiber parallel to a long dimension of at least 30 mm. The parameters to be measured are peak force and the total energy (Honikel 1997). To estimate texture in low fat burgers, a WB V-shaped blade (500 N) and multi-bladed Kramer shearing device (2 kN) can be used. The crosshead speed is 25 cm min^{-1}. Instrumental values obtained from the shear force test included peak force (N) and peak energy (J). For dry-cured ham, cubes of 1 cm^3 and a flat compression surface are suggested. Crosshead speed is 150 mm/minute and compressions of 70% achieved (Troy and others 1996). Others report speeds of 2.0 mm/second through a fixed distance of 30 mm (Ashie and others 2002).

Tensile Methods

The tensile test is used for structural investigations in beef and pork (Tornberg 1996). This test can be performed with either cooked or raw samples. The sample of meat should be sliced with a thin-bladed sharp knife to produce least damage. The standard thickness is 3.5 mm. Templates can be used in order to cut the meat samples. Width and thickness of the samples after cutting is measured with a caliper. Samples are subjected to extension at a strain rate of 2 minutes. The criterion for acceptable test results is that fracture occurs in the parallel-sided region of the sample. The results are expressed in Pascals (Honikel 1997).

Penetrometer

The penetrometer test resembles the process of mastication and is measured by the ease of the first bite between the teeth. A cylindrical flat-ended plunger (diameter 1.3 cm, area of 1 cm^2) is driven vertically 80% of the way through a 1-cm-thick meat sample cut. The plunger is driven (100 mm/minute) twice into the meat at each location and the work and force deformation curves recorded. Hardness, cohesiveness, and gumminess can then be calculated (Honikel 1997).

Texture Profile Analysis

Texture profile analysis (TPA) involves compressing a meat sample at least twice and quantifying the mechanical parameters from the force-deformation curves (Szczesniak 2002). Samples are usually 40

mm on the side and 20 mm in thickness and are compressed twice with a cylindrical probe of 30 mm diameter. Compression ratios of 25, 50, and 75% have been reported with crosshead of 5 cm/minute. With this methodology, hardness, cohesiveness, elasticity, and chewiness can be obtained (Kamdem and Hardy 1995).

To conduct TPA on steaks that have been frozen, samples are thawed at 2°C for about 24 hours. Steaks are grilled to an internal temperature of 40°C on one side then turned and cooked to an internal temperature of 72°C on the other side. Samples are placed in polyethylene bags and immersed in an ice bath to stop further cooking. Samples are stored at 2°C overnight. Steak strips are used to conduct TPA where samples undergo double compression cycles of 80%. Hardness, cohesiveness, springiness, resilience, adhesiveness, and chewiness are calculated (Caine and others 2003). Another alternative for semitendinosus muscles is to use a cork-bore to obtain cylindrical samples with 6-mm radius and approximately 13 mm in height (Palka and Daun 1999).

Computer Vision

The methods to measure meat quality (palatability) rely on subjective visual methodologies. Objective methods of beef quality have been a long-time desire of the industry (Tan 2004). Computer vision acquires images with a physical image sensor. The technology aims to duplicate the effect of human vision. A computer vision system consists of illumination, a camera, an image capture board, and computer hardware, and software (Brosnan and Sun 2004, Li and others 1999). Images are acquired under visible light and UV light. This increases the contrast between muscle fiber bundles and collagen or fat (Basset and others 2000). The use of a bit-map based image is not suitable due to the relatively large data size per image (Toraichi and others 2002). Techniques have been developed to determine skeletal maturity of beef carcasses. Maturity refers to the physiological age. There are five maturity levels, from A (young) to E (old). To estimate maturity, digital color images are taken under fluorescent lighting conditions. For "A" maturity, the cartilage in the thoracic vertebrate is free of ossification (Tan 2004).

Scoring marbling is determined by comparing the meat with standard images of each grade. A grading system based on image processing takes 12 standard images and compares them to the standards (Shiranita and others 2000). The process to obtain images includes filtering, background removal, segmentation of fat from muscles, isolation of the muscle, and segmentation of marbling from the muscle (Tan 2004). This technology allows the percentage of marbling, the number of marblings, the number of large and small marblings, and the amount of scattered marblings to be estimated. The system works with a monochromatic image. The captured image is binarized into what is called "fat-pixel pattern" and "muscle-pixel pattern." Next, a histogram is plotted with the pixels from the image, which shows different distribution for muscle and fat (Shiranita and others 2000). The marbling score is a measure of the distribution density of fat in the rib-eye region (Shiranita and others 1998).

FLAVOR ANALYSIS

The flavor of meat depends on a wide range of extrinsic and intrinsic factors related with the structure of meat and with postmortem processes. Raw meat has little aroma and a characteristic serum-like flavor. Meat develops its characteristic flavor and aroma after heat treatment in terms of cooking time and temperature (Cambero and others 2000). Meat flavor development relies on a complex set of reactions that occur during cooking. Intramuscular fat, peptides, and volatile compounds play a major role in flavor development (Monin 1998). During cooking a complex series of thermally induced reactions occur between nonvolatile components of lean and fatty tissues. The major precursors of meat flavor are divided into water-soluble and lipids. The main reactions during cooking are the Maillard reaction between amino acids and reducing sugars, and the thermal degradation of lipids (Mottram 1998).

Dietary composition of feed affects the composition of phospholipids and physical meat characteristics as well as pH, cooking loss, texture, and color (Scheeder and others 2000). In addition, in some cases, such as fermented meat products, bacteria play a major role in the production of flavor. The most common bacteria used are lactic acid bacteria. They cause proteolysis and act on carbohydrates and lipids to liberate free fatty acids (Montel and others 1998). Triglycerides from adipose tissue undergo intense lipolysis during salting. Triglycerides and phospholipids release free fatty acids by enzymatic

action, which can undergo oxidation to form peroxides, forming volatile aroma compounds with further reactions (Toldra and others 1997).

Fatty Acids

In ruminant muscle and adipose tissue, polyunsaturated free fatty acids (PUFA) are restricted almost exclusively to the phospholipids fraction, for example 18:2 n-6 linoleic or 18:3 n-3 α-linoleic. Red muscles have a higher proportion of phospholipids than "white" muscles (Wood and others 2003). PUFA perform vital functions in biological membranes. Some PUFA are essential because they cannot be synthesized by humans (Raes and others 2003). Measuring fatty acids in meat is important due to the effect on meat flavor from the production of volatile, odorous, lipid oxidation products during cooking and the involvement of these with Maillard reaction products forming other volatile compounds (Wood and others 2003).

Samples for chemical analyses like PUFA can be frozen and stored at −20°C until lipid extraction. Fat is extracted with chloroform/methanol by homogenization at room temperature. The extraction mixture is stored at 5°C for 18 hours and washed with 0.02% aqueous calcium chloride ($CaCl_2$). The organic phase is dried with sodium sulphate (Na_2SO_4) and potassium carbonate (K_2CO_3) and the solvent removed under nitrogen. Fatty acid ethyl esters (FAME) are prepared using the extracted lipid fraction. FAME are redissolved in hexane and stored at −20°C until gas chromatography (GC) analysis is performed (108). The temperature program for the GC is 150°C during 2 minutes followed by an increase of 1.5°C/minute to 175°C, followed by an increase of 5°C (Raes and others 2003).

Oxidative Quality

The development of oxidative off flavors (rancidity) has long been recognized in meat products. The propensity of meats and meat products to undergo oxidation depends on several factors including preslaughter conditions (Gray and others 1996). Thiobarbituric reagent substances (TBARS) test with or without modification is used to determine rancidity. The modified method involves the homogenization of the sample with cold phosphate buffer; next, trichloroacetic acetic acid is added and the mixture is centrifuged for 5 minutes and filtered through Whatman No. 4 filter paper. An aliquot of 2 milliliter is transferred to a clear glass tube and 0.02 M of 2-thiobarbturic acid reagent is added, boiled for 20 minutes and cooled immediately. Absorbance is measured using a spectrophotometer at 533 nm. The absorbance is then multiplied by a factor of 12.21 to obtain the TBARS value (mg of malonaldehyde per kg of meat) (Chung and others 2002, Connolly and Decker 2004) or μmoles of malonaldehyde/kg meat (Jakobsen and Bertelsen 2000).

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27
Shelf Life of Meats

Roberto S. Chamul

Shelf Life
Spoilage Microorganisms
Pathogenic Microorganisms
Detection and Enumeration Methods
Preservation Technologies
- Chilling
- Freezing
- Vacuum-packaging
- Modified Atmosphere Packaging
- Irradiation
- Ultra-high Pressure
- Antimicrobials

References

SHELF LIFE

Food security measurements in the meat industry include veterinary inspection prior to slaughter, laboratory screening for unusual levels of compounds in animal tissues, use of metal detectors, carcass treatment with hot water or steam, postpackaging treatment, and microbiological testing (Hueston 2002). Skeletal muscle from healthy animals has been considered sterile prior to slaughter (Huffman 2002). However, there are intrinsic factors such as pH, acidity, buffering power, water activity, redox potential, presence of antimicrobials, identity, and distribution of natural microflora that affect the shelf life of meat. Additionally, there are extrinsic factors such as temperature, relative humidity, light intensity and wavelength, atmospheric gas composition and ratio, packaging characteristics, processing characteristics, storage, distribution, and display considerations that will also affect shelf life of meat (McDonald and Sun 1999). Extrinsic factors are by far the greatest contributor to carcass and meat contamination (Huffman 2002). Intrinsic factors like pH and the concentration of L-lactate and fat affect the shelf life (rate of spoilage) of vacuum-packed pork and beef (Blixt and Borch 2002).

To extend shelf life, several postharvest techniques are used to decontaminate carcasses and fresh meat. These techniques include chemical dehairing, hot water rinse, steam pasteurization, steam vacuum, chemical rinses, and use of lactoferrin. Chemical dehairing involves applying sodium sulfide, using hydrogen peroxide, and rinsing with lactic acid. The use of hot water (>74°C) produces a sanitizing effect in the reduction of aerobic plate counts, total coliforms, *Salmonella typhimurium*, and *E. coli* O157:H7. Steam pasteurization is an extension of the use of hot water. This technique reduces total aerobic plate counts and *E. coli*. However, steam pasteurization can lead to meat surface discoloration. Steam vacuum involves steam pasteurization or hot water followed by vacuuming. This technique reduces aerobic plate counts and total coliform counts. Also, organic acids are used for chemical rinses. Acetic acid and citric acid in concentrations of 1.5–2.5% are recommended. Commercial compounds such as $Safe_2O^{TM}$ are available for chemical rinsing. Other compounds classified as microbial blocking agents are used to decontaminate carcasses. Among these compounds is lactoferrin, an iron-binding protein that is effective against *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* (Huffman 2002).

In addition to microbial deterioration, lipid oxidation is one of the primary mechanisms of quality deterioration in meat products. Lipid oxidation in meat changes quality in color, flavor, texture, and nutritive value. Lipid oxidation affects shelf life, which can be extended through dietary vitamin E supplementation

(Gray and others 1996). Ruminant meats are relatively a good source of n-3 polyunsaturated fatty acids (PUFA) due to the presence of 18:3 in grass. These compounds affect flavor producing a "grass fed" taste and oxidation due to points of unsaturation (Wood and others 2003).

Color fading in displayed meat is another quality issue that affects the shelf life of meat. Color fading of beef steaks displayed in refrigerated cabinets is caused by the combination of oxygen tension, surface microbial growth, temperature, and type of lighting. Light plays a role in pigment photooxidation. Studies suggest that lighting without ultraviolet (UV) radiation lead to a delay of meat spoilage as assessed by surface color, lipid oxidation, bacterial counts, and sensory evaluation (discoloration and color). Shelf life of meat can be extended when low UV lamps or fluorescent lamps with UV filter are used (Djenane and others 2001). When beef is pasteurized using hot water (85°C) for 60 seconds, vacuum packed and stored at 2°C, the color of pasteurized meat is rated paler as compared to nonpasteurized meat. Odors are perceived similarly (Gill and Badoni 2002).

SPOILAGE MICROORGANISMS

Meat is described as spoiled if organoleptic changes make it unacceptable to the consumer. The most common changes are appearance, off odors, and slime formation (Ellis and Goodacre 2001). Growth of spoilage bacteria results in the reduced shelf life of meat causing major economic losses (Venkitanarayanan and others 1997). The colonization and growth of microorganisms on meat surfaces occurs gradually. First, the bacterial cells need to attach to the meat surface, which may be a reversible stage. The next step is an irreversible attachment in which the bacterium produces an adhesive extracellular polysaccharide (Ellis and Goodacre 2001). Recontamination with pathogens during cutting or packaging may result in higher growth on decontaminated cuts than on untreated meat due to the lack of competing nonpathogenic microorganisms (Nissen and others 2001). On the other hand, there is a phenomenon called sticky aging, which is an abacterial enzymatic process that occurs in beef when maintained at body temperature during the first 14–18 hours postmortem. During this process, butyric acid is produced from the meat (Stephan and others 1997).

Off odors and off flavors are a common result of microbial and chemical spoilage. Indole is attributed to growth of enterobacteriaceae in aerobic and vacuum-packed stored bovine tripe. Gluconic and 2-oxogluconic acids are produced as glucose is oxidized by *Pseudomonas* during aerobic storage (Dainty 1996). Biogenic amine is considered a freshness marker. Amines are promoted by enzymatic reactions, microbial activity, or endogenous tissue activities. The most common amines produced in meat are tryptamine, putrescine, cadaverine, serotonin, tyramine, spermidine, and spermine. In red meat the level of biogenic amines are low during the first 9 days of storage (≤30 milligrams per kilogram [mg/kg]), but after 36 days the levels of biogenic amines, especially cadaverine and tyramine, become high (≥120 mg/kg) (Vinci and Antonelli 2002). Concentration of biogenic amines such as spermine increases after 76 days of storage in vacuum-packaged meat at 0°C (Lee and Yoon 2001).

During deboning, meat undergoes extensive handling and is exposed to the environment, which makes it susceptible to contamination. After deboning, the cuts are trimmed, vacuum-packaged, and boxed. Microbial contamination may be overcome with vacuum packaging; however, spoilage bacteria such as *Pseudomonas* spp. (Nel and others 2004), *Acinetobacter*, *Aeromonas*, and *Lactobacillus* (Huffman 2002) can contaminate the meat surface before packing. Other possible spoilage bacteria include *Moraxella* and *Psychrobacter* (Ellis and Goodacre 2001), enterobacteriaceae, *Psychrobacter*, *Shewanella*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Brochothrix*, and *Kurthia* spp. (Pin and Baranyi 1998). *Leuconostoc mesenteroides*, *Lactobacillus sake,* and *L. curvatus* are common components of the microflora of meat subjected to vacuum packaging (Dykes and others 1995). At levels of 10^7 colony-forming unit (cfu)/square centimeters (cm^2), off odors become evident and once the meat surface reaches 10^8 cfu/cm^2, recognizable off odors are produced. The off odor produced is recognized as dairy, buttery, fatty, and cheesy to a sickly sweet, fruity aroma, and finally putrid odor at 10^9 cfu/cm^2 (Ellis and Goodacre 2001). The type of bacteria is more important than the numbers when it comes to lipid oxidation in meat. Fresh raw beef has a weak lactic acid or bloodlike odor. This odor changes to an old, stale, oxidized, putrid, and rancid odor. Bacteria

such as *Pseudomonas, Acinetobacter, Moraxella, and Lactobacillus* are most common on chilled meat (Chung and others 2002). Lactic acid bacteria can interfere with spoilage and pathogenic bacteria by producing bacteriocins that extend shelf life or inhibit food-borne pathogens (Hugas 1998). For example, cooked ham is a highly perishable meat product. When *Lactobacillus alimentarius* or *Staphylococcus xylosus* are used as protective cultures, ham produced with the *L. alimentarius* is acceptable for up to 28 days compared with ham without the lactic culture with a shelf life of 21 days. These protective cultures reduce total aerobic bacteria, micrococci, and staphylococci (Kotzekidou and Bloukas 1996). Enterococci form part of the lactic acid bacteria group. They are present in unprocessed red meats and can spoil processed meats (Evans and others 2003, Franz and others 2003).

In fresh ground beef stored aerobically, the dominant spoilage organism is *Pseudomonas* spp. This genus undergoes a phenomenon called quorum sensing, where microorganisms produce certain small molecules that pass outside of producing cells and accumulate in the environment. When a critical level (a quorum) is reached, the inducer substances induce pigment production and slime formation. In Gram-negative bacteria, the inducer substances are acylhomoserine lactones (Jay and others 2003).

PATHOGENIC MICROORGANISMS

The USDA Food Safety Inspection Service (FSIS) has proposed that all slaughter establishments should apply at least one antimicrobial treatment or other approved intervention procedure to livestock carcasses (Shimoni and Labuza 2000) in order to avoid potential pathogens such as *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli,* and *Salmonella* spp. (Huffman 2002, Lee and Yoon 2001). Other pathogenic bacteria of concern are *Aeromonas* spp., *Arcobacter* spp., and *Campylobacter* spp. (Borch and Arinder 2002). USDA requires mandatory testing by the manufacturer for generic *E. coli* and random testing for specific pathogens (Shimoni and Labuza 2000). *Yersinia enterocolitica* grows slower under anaerobic conditions (Huffman 2002). *C. perfringens* is also found in meat. The spores of this organism are heat resistant surviving normal cooking or thermal processing conditions (Huang 2003).

L. monocytogenes is found in soil, water, and the digestive system of warm-blooded animals. It is commonly found in unprocessed foods of animal origin like raw milk, meat, poultry, and fish (Doyle and others 2003, Sofos and others 2003). FSIS and the Food and Drug Administration (FDA) have set "zero tolerance" for *L. monocytogenes* (Doyle and others 2003). *L. monocytogenes* is a Gram-positive facultative anaerobe bacterium (Huffman 2002, Novak and Juneja 2003). The versatility of *Listeria* to grow in a broad temperature range (2–45°C) limits the effectiveness of restrictive measures to control this pathogen. Refrigerated and frozen storage of heat-adapted *L. monocytogenes* in ground beef does not decrease the potential of this pathogen to survive additional low temperature cooking of food (Novak and Juneja 2003).

E. coli O157:H7 is an uncommon contaminant in beef and other foods. Cattle may carry this pathogen in their intestines but exhibit no signs of illness (Doyle and Sofos 2002). *E. coli* O157:H7 was first identified as a human pathogen in 1982. It is found in the feces of healthy cattle, and it is transmitted to humans through contaminated food, water, and direct contact with infected people or animals. This pathogen causes bloody diarrhea or hemolytic uremic syndrome (Mead and Griffin 1998). *E. coli* can survive in acidic conditions (Mustapha and others 2002). Mathematical models for exposure assessment have been developed specifically for *E. coli* O157:H7. These mathematical models consider five factors: (1) processing and grinding (mass of package, mass of trimmings, cross contamination, reduction due to spray washing, and growth during processing); (2) storage conditions (time in storage, maximum temperature experience during storage, maximum population density); (3) cooking (cooking preference, final internal temperature); (4) consumption (mass consumed/age dependent); and (5) dose-response (host susceptibility, probability of infection) (Cassin and others 1998).

DETECTION AND ENUMERATION METHODS

To estimate spoilage bacterial load on meat, a fluorescein diacetate hydrolysis test has been proposed. This test involves the swabbing of a 25-cm^2 meat surface with cotton swabs and then washing in a tube with 9 milliliters (mL) of peptonated water.

Resazurin reagent (1 mL) is added to 8 mL of the test solution where the swab was washed. The mixture is incubated at 25°C, and the time to change color from violet to pink is monitored. Results indicate that reazurin reduction time and fluorescein diacetate hydrolysis can be used as rapid methods to estimate spoilage bacterial load in aerobically stored meat (Venkitanarayanan and others 1997).

For enumeration of aerobic plate counts, plate count agar plates are incubated at 25°C for 72 hours or 30°C for 48 hours (Hinton and others 1998). To enumerate enterobacteriaceae (coliforms) the overlaid technique is used with Violet Red Bile agar plates incubated for 24–48 hours at 32°C (Lee and Yoon 2001) or 24 hours at 37°C (Hinton and others 1998). To quantify *Bacillus cereus*, selective agar plates are used. Plates are incubated at 30°C for 18–24 hours. Typical colonies are blue, with egg yolk precipitation (Lee and Yoon 2001). To cultivate and enumerate *Staphylococcus aureus*, Baird-Parker agar plates are incubated for 24–26 hours at 35°C. Typical colonies are black with white margins surrounded by clear zones (Lee and Yoon 2001, Cassin and others 1998). Cetrimide agar plates are used to cultivate and enumerate *Pseudomonas* spp. Plates are incubated at 25°C for 24 hours. Colonies are fluorescent under UV light (Lee and Yoon 2001).

To detect *Listeria monocytogenes*, a preenrichment step is necessary. After preenrichment, Oxford agar is inoculated and plates are incubated at 37°C for 48 hours (Lee and Yoon 2001). A rapid method to detect *Listeria* and next-day confirmation of *L. monocytogenes* has been developed. This methodology consists of 6-hour preenrichment, followed by overnight incubation in selective broth at 35°C. Changes in light transmittance in the selective broth are registered by an optical sensor. *Listeria* hydrolyze esculin resulting in black coloration and a drop in light transmittance (Peng and Shelef 2000).

Violet Red Bile plates with 4-methylumbelliferone glucuronide (MUG) are used to enumerate *Escherichia coli* at 37°C for 18–24 hours. Typical colonies are fluorescent blue under UV light (Lee and Yoon 2001). Also immunomagnetic separation can be used to enumerate *E. coli* O157:H7 (35). To isolate *E. coli* O157:H7, Sorbitol McConkey agar is used. Plates are incubated at 37°C for 24 hours (Dykes and Moorhead 2001). A simple method for detecting presumptive *E. coli* on fresh retail beef has been developed. The method is based on the use of double-strength lauryl sulfate tryptone broth plus 5-bromo-4 chloro-3-indolyl β-D-glucuronide and a Durham tube (Bolling and others 2002). To estimate *Salmonella*, Xylose Lysine Desoxychocolate agar is used. Plates are incubated at 37°C for 24 hours (Dykes and Moorhead 2001).

Current rapid enumeration methods for microorganisms are based on microscopy, adenosine-5′-triphosphate (ATP) bioluminescence, or the measurement of electrical phenomena. Microscopy techniques are based on the use of fluorescent dyes. ATP bioluminescence acts by measuring ATP levels in bacterial cells in culture. Current detection methods are based on immunological or nucleic acid-base procedures. The most common form of these methods is the enzyme-linked immunosorbent assays (ELISA). Nucleic acid-based procedures use probes that are small segments of single-stranded complementary nucleic acid that are used to detect either DNA or RNA sequences to identify a specific microorganism. The most common nucleic acid detection method used is the polymerase chain reaction (PCR) (Ellis and Goodacre 2001).

Rapid and accurate pathogen testing is vital in the prevention of outbreaks. A combination of enzyme immunoassays (EIA) and culture methods are used in testing for *Salmonella*; this technique gives reliable results within 48 hours. However, a presumptive positive EIA result must be confirmed requiring an additional 72 hours. On the other hand, PCR tests for *Salmonella* are used, but some tests can take up to several days. An alternative for this is the use of real-time PCR with fluorescent hybridization probes. This methodology is ideal for rapid detection of *Salmonella* in raw and ready-to-eat meat products and gives results in less than 12 hours (Ellis and Goodacre 2001). Real-time PCR is also used for quantitative meat species testing (Ellingson and others 2004) and includes incubation for 6 hours at 35–37°C of the sample homogenate in peptone water with novobiocin. An aliquot of the sample is transferred to a tube to centrifuge at 2,500 × gravity (*g*) for 10 minutes. The pellets are extracted with the QIAGEN QIAmp DNA mini kit. Oligonucleotides used to detect *Salmonella* amplify the 251 base pairs product spanning from base 2,305 to base 2,555. Extracted DNA is used as template for PCR amplification. The conditions to amplify DNA are 2 minutes at 95°C and 45 heating/cooling cycles. DNA is denatured using hybridization probes (Ellis and Goodacre 2001).

Recent concerns about prion diseases has led scientists to develop rapid methods to detect infectious agents responsible for transmitting bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob Disease (vCJD). A sensitive and semiquantitative TaqMan™ real-time PCR system has been developed to detect prions. This methodology relies on three basic steps: DNA extraction, use of oligonucleotide primers and probes, and the PCR conditions to amplify DNA (Sawyer and others 2003).

PRESERVATION TECHNOLOGIES

Preservative packaging for raw meats delays appearance of deterioration and retards the onset of bacterial spoilage. Browning is slowed in atmospheres poor in oxygen, and persistent browning is prevented entirely when meat is packed under oxygen-depleted atmospheres. To control bacterial spoilage, temperature is also a major factor that requires control (Brodman and Moor 2003). Modified atmosphere (MA) is used to extend the shelf life of meat products. The two major concerns for MA-packed products is microbiological and appearance shelf life. Gas mixtures of 60–70% carbon dioxide (CO_2), 30–40% nitrogen (N_2), 0.3–0.5% carbon monoxide (CO) have been used. However, temperature plays a major role. High concentrations of CO_2 and 4°C prolong the shelf life of ground beef. *Yersinia enterocolitica* is almost inhibited at 4 and 10°C using high CO_2 concentrations. The same conditions cause *L. monocytogenes* to grow slower but do not affect *Salmonella* in ground beef at 10°C (Gill 1996).

Multilayered antimicrobial polyethylene (PE) films have been used to package ground beef using a grapefruit seed extract as an antimicrobial agent. This antimicrobial film has shown activity against *E. coli*, *S. aureus*, and *Bacillus subtilis*. In addition, these films contribute to a reduction of the growth rates of aerobic and coliform bacteria when compared to plain PE film (Nissen and others 2000). Antimicrobial films are designed to slowly release bacterial inhibitors. These are used in combination with vacuum packaging under refrigerated conditions. Acetic and propionic acid do not have any effect on lactic acid bacteria but delay the growth of enterobacteriaceae and *Serratia liquefaciens* (Ha and others 2001). Nisin, an antimicrobial peptide, is used as an inhibitor when incorporated into PE-based plastic film used to wrap meat cuts. They have proven to control indicator bacteria such as *Lactobacillus helveticus* and *Brochothrix thermosphacta* as compared with meat cuts vacuum packaged without nisin and held under the same storage conditions (Ouattara and others 2000).

Microbial contamination of carcasses results from processing to conditioning. Processing influences not only the quantity of microorganisms but also the type. Meat stored under aerobic conditions is rapidly spoiled by bacteria that cause discoloration and off odors. The most common bacterium responsible for these changes is *Pseudomonas*. Additionally, other factors that influence bacterial growth and therefore shelf life are oxygen and water activity. Under refrigerated conditions, *Brochothrix thermosphacta* is a common spoilage microorganism. Vacuum or MA also affects microorganisms. In MA several lactic acid bacteria are present, with the most common species being *Lactobacillus sakei* and *L. curvatus* (Siragusa and others 1999).

CHILLING

To extend the shelf life of meat, chilled storage of fresh beef under vacuum or carbon dioxide is used commercially. Meat packed under vacuum or carbon dioxide kept at low temperatures and inoculated with *E. coli* and *Salmonella* does not support the growth of these two pathogens (Dykes and Moorhead 2001). The objectives of postslaughter carcass and product handling are to minimize contamination and to restrict subsequent proliferation. Temperature is one of the most important factors influencing the growth of microorganisms. When hot boned meats cool in vacuum packs or in bulk-packed cartons, they cool slowly, therefore, increasing the chances of microbial proliferation. However, compared to cooling/boning regimes, where temperature of carcasses is reduced to 7°C or less, studies indicated that both processes are comparable and that microbiologically they are equivalent (Labadie 1999).

The rate of heat removal and the resulting rate of temperature reduction of the carcass have a substantial influence on storage life and eating quality of meat. All meat temperatures within the carcass must be below 7°C before the carcass is further processed. After chilling, carcass meat is stored for a

period of hours and up to 2 weeks. Low temperatures, minimal air movement, and high relative humidity should be maintained to maximize storage life (Bell and others 1998). Once the initial microbial population increases in refrigerated beef packed with high gaseous permeability film (polyethylene) or low-gaseous permeability films (ethyl vinyl acetate, SARAN, polyvinylidene chloride copolymer), the adaptation period of bacterial growth decreases regardless of the packaging film (James 1996).

Freezing

The main reason batches of frozen foods have different microbial counts and profiles are variations in the initial load and the type of bacteria. In addition, the preparatory processes, freezing conditions, and storage temperature affect the fluctuations of microbial counts during freezing (Gianuzzi and others 1998). Freezing initiates several physical and physicochemical changes that lead to deterioration of meat. Manufacturing beef from exporting countries is still packed in cartons and frozen to −18°C. Studies conducted on the rate of freezing show that this rate alone does not affect protein solubility or meat color. Slowly frozen semimembranosus have more drip than fast frozen muscles. In addition, storage at a temperature below −18°C affects sarcoplasmic protein solubility and myofibrillar protein solubility. Current practice of blast freezing and storage at −18 to −20°C is sufficient to maintain quality of manufacturing beef (Nussinovitch and Peleg 2000).

Vacuum Packaging

Discoloration or browning results from oxidation of deoxymyoglobin or oxymyoglobin to metmyoglobin. The principal function of preservative packaging is to delay microbial spoilage by restricting the growth of spoilage organisms. Growth of aerobic organisms is inhibited when atmosphere inside a package is modified. Meat surfaces exposed to oxygen-containing atmospheres sustained accelerated bacterial growth and discoloration due to oxidation of myoglobin. Films with minimum oxygen permeability (<100 mL/[m^2-24 hour] atmosphere at 23°C and 0% relative humidity [rh]) can generally be used for vacuum or modified atmosphere. Storage life can be increased 10–15% by using film with a permeability of <2 m/(m^2-24 hour) atmosphere at 23°C and 0% rh. Most multilayer meat packaging films are of multilayer construction since gas transmission rate is a function of polymer type, film thickness, temperature, and humidity (Farouk and others 2003).

The following microflora has been identified in vacuum-packaged beef under chilling storage: *Brochothrix thermosphacta*, *Carnobacterium divergens*, *Carnobacterium piscicola*, *Lactobacillus algidus*, *Lactobacillus* spp., *Lactococcus piscium*, *Leuconostoc gelidum*, *Acinetobacter*, *Aeromonas*, *Bacillus*, *Corynebacterium*, *Enterobacteriaceae*, *Pseudomonas*, and *Psychrobacter* (Jeremiah 2001). Slicing of meat and repacking influence shelf life (Sakala and others 2002). Cuts of meat that are vacuum packaged and stored from 52 days at 0°C exceed total aerobic plate counts (10^7 cfu/cm^2) but enterobacteriaceae and *Pseudomonas* show retardation (Lee and Yoon 2001).

Evacuation of air from the package is clearly beneficial. Meat can be vacuum packaged in a heat shrinkable plastic bag, with low permeability, and sealed. The effectiveness of vacuum packaging depends upon close contact between a packaging film of low gas permeability and all product surfaces. Vacuum packaging achieves its preservative effect by maintaining an oxygen-depleted atmosphere. It also reduces off odor development and moisture losses. Beef processed and vacuum packaged under appropriate conditions has a shelf life of 70 days, and vacuum packaged beef stored at 1°C has a storage life of 11 weeks. However, off odors and discoloration can be perceived after 11 weeks (Farouk and others 2003).

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is a food preservation method important in the meat industry. This technique involves modifying an atmosphere by permitting air to be enclosed or by injecting a desired initial gas mixture (Holley and McKellar 1996). An indicator of freshness for meat is the bright red color. Concentration of CO affects the color of meat cuts. For instance, ground beef, and loineye, inside round and tenderloin steaks, stored for up to 35 days at 2°C results in typical bloomed color (Skandamis and Nychas 2002). Even when using MAP, oxygen is not always completely removed or may permeate through the packaging material. The

presence of small amounts of oxygen in cooked MAP products accelerates oxidation. If MAP products contain high levels of oxygen (>1.2%), this results in quality deterioration and reduced shelf life (Hunt and others 2004).

Temperature, CO_2, and water activity are factors that influence the growth of spoilage microorganisms in cooked meat subjected to MAP (Smiddy and others 2002). All atmospheres containing CO_2 reduced total aerobic population numbers; however, lactic acid bacteria were not affected. Concentrations of 0.5–0.75% of CO_2 are able to extend shelf life by 5–10 days at 1±1°C (Devlieghere and others 1999). A 100% CO_2 concentration and low storage temperature inhibit bacterial growth, especially *Pseudomonas* and *Brochothrix thermosphacta*. Lactic acid bacteria (10^5–10^6) also inhibit growth of *Pseudomonas* and B. *thermosphacta* (Luño and others 1998). Lactic acid bacteria increase to a maximum of 5.5 log cfu/cm^2 after 8 weeks in beef rib eyes kept under 100% CO_2 at 2°C. After 6 weeks, the flavor of samples drops to an inappropriate level. Flavor descriptors include "off barny" aromatic and "off barny" aftertaste, suggesting that grow rates of these organisms are related to the organoleptic changes in meat under MA and refrigerated storage conditions (Nissen and others 1996). Meat cuts undergoing hot deboning and that are chilled at 7°C, electrically stimulated, and MA-packed produce meat of acceptable tenderness within 8 days of slaughter. Steaks after 24 hours show dull dark lean tissue and gray to green discoloration of the fat. Aroma also deteriorates as an indication of spoilage (Nattress and Jeremiah 2000).

Utilization of spices, spice mixtures, or spice extracts such as marjoram, caraway, sage, basil, thyme, and ginger in semiprepared products intended to be frozen for up to 6 months is recommended to prolong the shelf life of the food and to inhibit lipid peroxidation during heat treatment and chilling storage (Bell and others 1996). The use of antioxidant mixtures like rosemary and vitamin C together with the absence of UV radiation reduced the rate of metmyoglobin formation, lipid oxidation, and microbial growth under a modified atmosphere (70% O_2, 20% CO_2, and 10% N_2). These conditions extended the shelf life from about 10 to 20 days (El-Alim and others 1999).

Sodium lactate is a compound that can be used to extend the shelf life of meat products packed under MA and low refrigeration temperatures. There is a synergistic effect between sodium lactate and CO_2 that extends the meat products' shelf life; this can be explained by the reduction of pH in the medium. In addition, CO_2 changes the permeability and rigidity of the microbial cell membrane and decreases the activity of membrane bound proteins (Djenane and others 2003). Meat pH values under MA remain between 5.3 and 5.6. For beef cuts under MA, L* color value increases with storage time while a* color value decreases. Shelf life (measured as color and odor degradation) can be as short as 5 to 15 days depending on the breed and mostly due to lipid oxidation when samples are kept at 2±1°C with 90–95% relative humidity (Devlieghere and others 2000).

Irradiation

Food irradiation can extend the shelf life of perishable foods like beef and eliminate food-borne pathogens. High dose sterilization (10–50 kilogray [kGy]) is used to sterilize foods and eliminates food-borne viruses. Medium dose pasteurization (1–10 kGy) reduces spoilage microorganisms and also can be used to treat raw or frozen meat. Both doses can be applied to meat (Insausti and others 2001). Doses of 5.0 kGy reduce bacteria counts by 2–3 log cycles, and when samples of meat are heated in a microwave oven, bacterial counts are reduced by 1 log cycle in 2 seconds and by 2 log cycles in 30-second exposure (Crawford and Ruff 1996). After treatment with 5 kGy to ground beef, lipids suffer oxidative damage, especially ground beef with high content of fat (30%) (Aziz and others 2002, Al-Bachir and Mehio 2001). Irradiation reduces the counts of microorganisms and increases the shelf life of luncheon meat from 10 to 14 weeks (Aziz and others 2002). A dose of 2 kGy causes ground beef patties to develop more off odors and off colors, and lower International Commission on Illumination (CIE) L*, a*, and b* after 4 days of storage at 0±1°C (Poon and others 2003). Incorporation of antioxidants to minced beef irradiated with 4 kGy results in better retention of color. Irradiation increases Hunter "a" values up to 4 days when α-tocopheryl acetate is used and up to 6 days when rosemary extracts are added. Thiobarbi-turic reacting substances (TBARS) values increase with increasing irradiation dose; however, supplemented samples with antioxidants have lower TBARS (Montgomery and others 2003).

To produce ready-to-use shelf life stable meat products, it is common to use a combination of technologies like irradiation, reduced water activity, and vacuum packing. These hurdles are used to prevent the growth of *Clostridium sporogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. Radiation treatments of 2.5 kGy result in complete elimination of inoculated *S. aureus* and *B. cereus*. Water activity of 0.85 combined with vacuum packing prevents the growth of these three microorganisms. In general, these three technologies used in combination, result in microbiologically safe and shelf-stable meat products (Formanek and others 2003).

Ultra-high Pressure

High pressure can modify the enzymatic system, texture ultrastructure, and myofibrillar proteins of meat. High pressure has an effect on color. The increase in L* values begins at 200 mega Pascal (MPa). These color changes can be explained as a result of pigment denaturation and increase drip losses or damage of the porphyric ring and protein coagulation. Pressure intensity is more significant than holding time for redness, total color difference, and metmyoglobin content. Pressures higher than 300 MPa induce modification of meat color. Pressures of 520 MPa with contact times of 260 seconds decrease the total flora (Chawla and Chander 2003).

Antimicrobials

Multiple intervention technology involves the use of different barriers or hurdles such as pH changes, oxidizing environments, or other environmental changes to cause disruption of microbial cells or cellular metabolism, to either destroy or retard cell growth. Treatments using combinations of acetic acid and cetylpyridinium chloride, chlorine dioxide and cetylpyridinium chloride, and cetylpyridinium chloride and trisodium phosphate reduce *E. coli*, *Salmonella*, coliforms, and aerobic plate counts; however, cetylpyridinium chloride and trisodium phosphate have been shown to preserve the color of meat surfaces better than the other treatments (Jung and others 2003). Active packaging is one that changes to extend shelf life or improve safety or sensory properties. Antimicrobial packaging is an active packaging concept. Antimicrobial compounds have been tested in active packaging; some of these compounds are organic acids and their salts, enzymes, bacteriocins, and compounds such as triclosan, silver zeolites, and fungicides (Pohlman and others 2002).

One of the problems in the meat industry is *E. coli* O157:H7. To reduce the incidence of food-borne pathogens, two types of interventions are commonly used, one dealing with the whole carcass, the other dealing with the use of antimicrobial agents. However, there is no single intervention that will result in 100% pathogen-free meat. Bacterial attachment occurs the first minute of bacterial contact with tissue surfaces. Therefore, physical removal of contamination is the primary means for controlling *E. coli*. The most common methodologies to accomplish this include trimming and washing before slaughter (Quintavalla and Vicini 2002) and low intensity ultrasound (Brown 2003). The use of antimicrobial agents include lactoferrin, peroxyacids, acidified sodium chloride and ozone (Quintavalla and Vicini 2002), acetic acid, lactic acid, trisodium phosphate, citric acid, formic acid, and gluconic acid (Brown 2003).

Chemical treatments commonly used in the meat industry are peroxyacetic acid (0.02%), acidified sodium chloride (0.16%), and lactic acid (2 and 4%). There are widely used alternatives to decontaminate carcasses. Peroxyacetic acid at 0.02% and acidified sodium chlorite solutions have little effect on aerobes and coliforms. Lactic acid (4%) results in bacterial reductions higher than 1.5 log cycles. Treatment with 2% lactic acid is similar to lactic acid at 4%. The major concern with these acid treatments is injured bacteria that may recover during storage and that become resistant to these types of acid treatments (Pohlman and McElyea 2003, Gill and Landers 2003, Gill and Badoni 2004).

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Packaging and Freezing of Beef as Related to Sensory Properties

Robert W. Rogers

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References

INTRODUCTION

When evaluating the sensory properties of beef, various preslaughter factors such as the animal's diet, vitamin E supplementation, age, breed, sex, health status, etc., should be considered. Likewise, there are various postslaughter factors that may play a role in the sensory characteristics of beef. These factors may include (a) electrical stimulation of the carcass; (b) the length of aging of the carcass prior to cutting the meat and evaluating the sensory properties; (c) whether the meat was removed from the bone in a prerigor state or after the completion of rigor; (d) chilling rate of the carcass; (e) amount of external fat covering on the carcass; (f) the amount of internal fat (marbling) in the meat; (g) the degree of muscling of the carcass; (h) the particular cut or muscle being evaluated; (i) the packaging material used to package the product; (j) the length of time of storage of the packaged product; (k) the state of the product while in storage (fresh, cured, refrigerated, frozen); (l) the method of cooking (broiling, roasting, frying, microwave cooking, etc.); (m) distance of the meat from the heat source; (n) the presence or absence of forced air movement during cooking; (o) cooking temperature; (p) the length of time from cooking to evaluation; (q) the matter of serving the product immediately upon cooking or waiting and reheating the product before serving; (r) the presence or absence of salt and/or seasoning on or in the product; (s) the presence or absence of visual fat and connective tissue in the sample; (t) whether or not the meat has been subjected to irradiation and if so, how much; (u) the microbiological condition of the fresh meat before and after cooking, etc.

When evaluating beef for its sensory properties, all of the above factors and other possible conditions should be considered. The emphasis of this chapter is to discuss the major relevant factors that affect the sensory properties of beef as they relate to the packaging and freezing prior to preparing and presenting it for sensory evaluation by a trained or expert panel, a consumer panel, or the public at large. In order to make valid comparisons and sound conclusions about the sensory properties of beef, there are several factors that should be considered. Some of these factors include controlling the raw materials, the overall packaging, handling of the food during preparation and presentation, to name a few. These factors must be standardized as much as possible in order to eliminate unexplained variations so the main effects of the study can be properly evaluated.

The material presented in this chapter is primarily what is considered to be well-accepted information and is not a review of the scientific literature on the subjects discussed. However, a list of several good references is provided so that those interested in more detail of the many scientific principles involved will be able to access that information with relative ease.

PACKAGING

The main purpose of the primary package of beef, as for any food, is for the protection of the food during handling, storage, shipping, and sales of the product. In addition to the primary package, there may also be secondary and tertiary packaging materials and systems involved. Consumer packaging of meats began with the development of the supermarket industry. However, since this material covers matters only relating to the sensory properties of beef, the functions of the other levels of packaging, above the primary package, will not be discussed in any detail. The primary package is the one that actually comes in direct contact with the food product and is generally considered to be the single most important component of the packing process. However, for the purpose of the sensory evaluation of beef products, the proper identification of the meat is also essential, so one might say the graphics identifying the product properly are also extremely important.

The packaging material chosen should be appropriate for the type of storage to which the product is to be exposed. If the product is to be used as a fresh (not frozen) item, then the appearance of the package in the retail counter is of extreme importance. The type of packaging material used will determine the visual appearance, a sensory trait of the beef. Consumers expect beef to be a "bright cherry red" color in order to indicate freshness and to present the appearance to which they have become accustomed. Therefore, a packaging film that allows the passage of some oxygen is essential if the color is to be as expected. The packaging film companies that supply packaging materials for fresh beef offer different types and thickness of film, but they all possess the ability to allow for the passage of enough oxygen, at atmospheric pressure, to keep the "bloom" or "bright cherry red" color for a short period of time, usually up to 48 to 72 hours, or maybe slightly longer. After prolonged exposure to oxygen, the pigment "oxymyoglobin" will become oxidized to "metmyoglobin," which produces an undesirable brown color that is formed when the iron atom in the myoglobin pigment becomes oxidized, loses an electron, and changes from the Fe 2+ (ferrous) to the Fe 3+ (ferric) state.

The time that the product will remain in the desirable state can vary considerably, depending on the microbiological condition of the product, the temperature, and the light intensity under which the product is stored and if the animals had been subjected to vitamin E supplementation in their diets prior to harvesting, as this can significantly improve the color of beef over a greater period of time. Beef products offered for typical retail sale that have been kept very clean, in low temperature storage (0°C to 3°C) and in the dark or under very low light intensity will have a much longer shelf life, being defined here as the time that the visual color is acceptable, than will identical products where the microbiological numbers are high and the temperature and light intensity are high. Of course, it is understood that the proper packaging film has been used to display these products as fresh items. The packaging materials commonly available for the packaging of all meats are often composed of different layers of materials that perform different functions such as retard water movement; regulate air movement; add strength; provide heat sealing capability, thermoformability, sparkle, etc.; and are designed to perform the functions desired in the packaging process.

It should be noted, however, that the packaging materials used for fresh meat display should not be used to package meat that is to be frozen, as the material will allow the passage of oxygen, which will cause excessive oxidation of the product and produce undesirable sensory characteristics of the products when cooked and served.

The packaging materials for beef that is to be frozen should have essentially the same physical characteristics as the material for fresh beef, except they should have a very good oxygen barrier, a very good sealing capacity, and be "tougher" so as not to crack or break under freezer conditions. These products are what is commonly called "barrier bags" or "barrier film" and are commonly used for vacuum packaging. In order to preserve the native sensory properties of the frozen meat, the oxygen must be removed and kept out during storage. If this is accomplished, the shelf life of beef can easily be from 9 to 12 months without any significant loss of desirable organoleptic traits. However, if the seal breaks or the package is punctured, the shelf life is minimal, as the oxygen will cause oxidation and the presence of undesirable sensory properties as well as the occurrence of dehydration of the surface, known as freezer burn. Likewise, leaving loose

packaging material around the meat allows for "frosting" inside the package, which produces a very unattractive package and presents a situation for possible loss of desirable sensory properties of the meat.

Beef may also be packaged in a modified atmosphere package or controlled atmosphere package normally called MAP or CAP packaging. The principle involved here is that the oxygen is removed and replaced with other gases, normally carbon dioxide and nitrogen. These packages may have a fluffed or pillow appearance and leave a lot of headspace where frosting can occur, thus this type of packaging is generally only used for storage and display of nonfrozen products.

If beef is to be frozen for the purpose of accumulating samples in research projects, for example, the proper identification of the material is essential. Likewise, if commercially packaged beef products are to be evaluated for their sensory properties, having the graphics to identify the cut of beef and any additives present is essential in order to properly evaluate the sensory properties as they might well be influenced by the cut of meat, the presence of additives, etc.

In summary, the packaging material used, the storage conditions used, and the package graphics are the three most important factors that will affect the sensory properties and data analysis as related to the packaging of beef.

FREEZING

The freezing of beef for storage, or as some call it, preservation, has been in existence for many years. Factors relating to the quality of frozen beef were mentioned even in the earliest textbooks used to teach about processing and storage of beef at colleges and universities in the early 1900s. Many refer to freezing as a method of preservation of meat, but others only consider freezing to be a processing method. The fact that freezing under commercial or household conditions requires a continual input of energy does not meet the test of keeping the product in an acceptable fashion under whatever conditions to which it might be exposed. When considering freezing as a preservation method, one should compare freezing to dehydration or canning where there is no demand for continued energy input in order to keep the products in an acceptable fashion, microbiologically or physically. The reader will have to decide whether or not to consider freezing a preservation or a processing method. In either case, freezing and subsequent freezer storage is a good method to extend the sensory properties of beef if the process is properly performed.

Freezing is a chilling procedure that results in the formation of ice crystals in the meat. Due to the presence of solutes in meat, it generally is considered to be frozen only when it reaches a temperature of $-2°C$ or lower. The exact amount of water remaining in solution, or as free water, depends on the exact temperature of the product. At $-5°C$, approximately 85% of the water is in the form of ice, whereas at $-30°C$, almost 100% of the water is frozen.

The purpose of freezing is to keep the original characteristics of the product, over an extended period of time, as close as possible to those of the fresh product. The freezing process is not expected to improve any of the characteristics of the beef, so the ultimate quality of the frozen product depends heavily upon the state or condition of the product prior to freezing. Frozen meat used to carry a poor image by many because it was the routine practice to freeze meats only at the end of their natural shelf life as fresh meats. This practice led to producing frozen products that had been exposed to conditions that allowed for microbiological growth, oxidation, drying, etc., and were of minimal quality at best. However, if done properly, frozen beef products can be routinely produced that will keep the basic characteristics of fresh products for extended periods of time (i.e., 9 to 12 months). It should be kept in mind that in addition to the original meat quality characteristics, the packaging, the actual freezing process, and the storage conditions can all have significant effects on the sensory and nutritional properties of frozen beef. For example, ground beef will have a shorter shelf life than intact cuts due to the greater surface area that is exposed which increases the potential for oxidation (rancidity). Likewise, beef from animals fed on high forage diets tend to have a shorter shelf life, due to a greater amount of unsaturated fats being deposited as compared to meat from animals fed high concentrate (grain) diets. It is the areas of the double bonds in the fatty acids that allow for the oxidation of the fat, thus causing rancidity. It should be noted here that the freezing and storage of precooked beef will produce products with relatively short shelf lives as these

products will normally display "warmed-over flavor" upon re-heating, due to oxidation of the double bonds during the cooking process. It should also be noted that meat that contains salt, for example, will become rancid much sooner due to the oxidizing ability of the salt. The oxidation of frozen meats appears to be related to low water activity. Fresh meat has a high water activity level (0.97 to 0.99) whereas frozen meat has water activity levels of around 0.6 to 0.8. At these low levels of water activity, the heme pigments appear to initiate autooxidation.

The freezing of beef, or any meat, is to primarily stop the growth or replication of microorganisms. Freezing simply "ties up" the water so that it is unavailable for the microorganisms to use for their life processes. Some organisms may be killed during this process, but freezing is not a reliable method to destroy microorganisms, except for parasites. Freezing slows chemical reactions but does not stop them, so it should be kept in mind that there is also some loss of vitamins during long term storage of beef, as well as for all foods.

The speed at which beef is frozen is an important factor to consider when evaluating the quality of the product upon thawing and cooking. This is especially true at and near the point of freezing (−1°C to −4°C). Water allowed to stay in this range significantly affects the size of ice crystals formed. If frozen slowly, the ice crystals formed are large crystals (140 to 150 μmeter [m] in diameter) and damage the muscle cells, thus allowing a greater amount of "drip" or "purge" upon thawing of the product. However, when the critical temperature of −1 to −4°C is passed within a short period of time (1.5 to 2.0 hours), the water in the cells freeze before substantial diffusion of the water to the outside of the cell can occur. Today it is common in industry to freeze beef by cryogenic methods using either liquid nitrogen or liquid carbon dioxide (CO_2). These materials produce very low temperatures (i.e., −73°C to −129°C in liquid nitrogen tunnels and about −62°C in CO_2 tunnels) and allow for ideal conditions for freezing beef. However, if there are significant temperature fluctuations during storage, small ice crystals can thaw and recrystallize, which can also lead to extensive damage to the cell membranes and cause excessive drip or purge as well as increase rancidity. The storage of frozen beef for extended periods of time in so called "frost-free" refrigerator units, which defrost themselves often, can magnify the problem related to temperature fluctuations and the shelf life of frozen beef.

The time that has elapsed between the harvesting of the animal and placing the beef in the freezer is also a very important factor. For optimum quality, beef should be frozen only after the completion of rigor mortis, which is the stiffening of the body after death. If beef is frozen prior to the completion of rigor, the muscles will contract an abnormal amount and upon thawing demonstrate "thaw rigor," and this will in turn produce meat with a higher level of contraction and greater toughness. Likewise, the rapid chilling or freezing of a carcass after harvesting causes what is known as "cold shortening," which also produces increased toughness of the meat. This toughness is due to the method of handling alone and does not reflect other true differences in the tenderness of the meat had the imposed toughness due to freezing or rapid chilling not been present. In research projects, it is common to store beef from animals harvested over a particular period of time. The length of time from harvesting to freezing must be kept constant, as different times of aging under refrigeration can be a significant factor in the ultimate level of tenderness of the meat. Most meat scientists generally accept that a fixed time of 7 to 14 days from harvesting to freezing should elapse. During this time it is customary to leave the muscles intact in the carcass or wholesale cut, whereupon the cuts are obtained, packaged, identified, frozen rapidly, and stored in the freezer at about −17°C until evaluated.

Freezing of beef also causes the pigment, myoglobin, to use greater amounts of oxygen, thus showing the product to be dark in color rather than bright red. This dark color has caused many problems in trying to merchandise frozen cuts of beef, as consumers generally do not accept this dark color as an indicator of fresh or high quality beef. However, the color of the meat returns to normal upon cooking as the myoglobin turns to denatured metmyoglobin, the pigment responsible for the formation of the brown color of cooked beef.

When using frozen beef, the product should always be thawed under refrigerated conditions (1 to 4°C) in order to maintain the optimum quality and safety of the product. Likewise, it is generally accepted that once meat has been thawed it should not be refrozen. Each time the product is frozen, the ice crystals can cause additional damage to the cells and

allow for a greater amount of water loss (purge), and thus reduce the juiciness of the product. However, if the product has been thawed under refrigeration there is generally no concern for the safety of the meat, just a loss of quality.

In summary, the freezing of beef is an acceptable method to extend the shelf life of the product provided it has been properly packaged, quickly frozen, stored at the proper temperature, and the packaging material has not been damaged so as to allow the entrance of oxygen or the loss of moisture. However, for optimum quality, the length of the storage should not exceed 9 to 12 months. Otherwise significant loss of the desirable sensory properties related to odor and taste can occur. The length of storage time should not normally affect the degree of tenderness or juiciness of beef.

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Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

Part V
Pork Quality

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29
Fresh and Frozen Pork Color

Donald H. Kropf

Pork Color
Pork Color Basics
- Oxygenation or Blooming
- Discoloration
- Other Color Effects
- Influence of Rate of pH Decline
- Factors Affecting Pork Color Stability
- Live Animal Selection
- Nutritional Effects
- Environmental Impacts
- Transport and Handling
- Holding Conditions (Lairage)
- Stun, Bleed, and Scald Influence
- Dressing and Chilling
- Interactions and Compensatory Effects of Previously Mentioned Factors
- Fabrication/Enhancement
- Freezing
- Packaging
- Lighting Effects on Color
- Consumer Expectations
- Pork Color Evaluation

References

PORK COLOR

The color of pork, or any other object, is determined by (1) the light source under which the pork or other object is displayed, (2) the light reflecting or absorbing properties of the pork sample, and finally by (3) the light detector, which could be the rods and cones of the human eye coupled to appropriate brain cells or the wavelength or color sensor on a color measuring instrument such as a reflectance spectrophotometer or colorimeter (Figure 29.1).

Visible light is a form of energy that travels in waves. The mix of various wavelengths (energy) detected by the eye and interpreted by the brain determines what color we perceive. Only the visible energy wavelengths contribute to a particular color or shade of color or mix of colors to the naked eye. The visible wavelengths in the order of the color they portray from the shortest to the longest wavelengths are violet, indigo, blue, green, yellow, orange, and red. Visible violet is preceded in wavelength by invisible ultraviolet (UV), and red is followed by invisible near-infrared and infrared wavelengths. Color is almost always a mixture of various visible wavelengths in differing quantities.

Light sources vary in their spectral energy distribution (the mix and quantity of emission of different wavelengths). A light emitting a high proportion of blue wavelengths will make objects under them appear more blue, and a light with a high proportion of red emission will make objects appear more red.

Lighting can have a profound effect on appearance of pork meat (Kropf 1980). Some lights enhance the bright pink color and may even mislead the customer. These would be on the warm end of the lighting scale and have color temperatures of less than 2,700° Kelvin. Lights with a cool color rendition might be 4,100° Kelvin or higher and result in a bluish and less pink appearance of the meat. Many markets currently use lights of approximately 3,000° Kelvin color temperature.

For instrumental color measurement, an illuminant should be specified. This results in a computer adjustment for certain color reflectance measurements, thus simulates visual color evaluation under different lighting systems. Some illuminants will be described later in the discussion of pork color measurement.

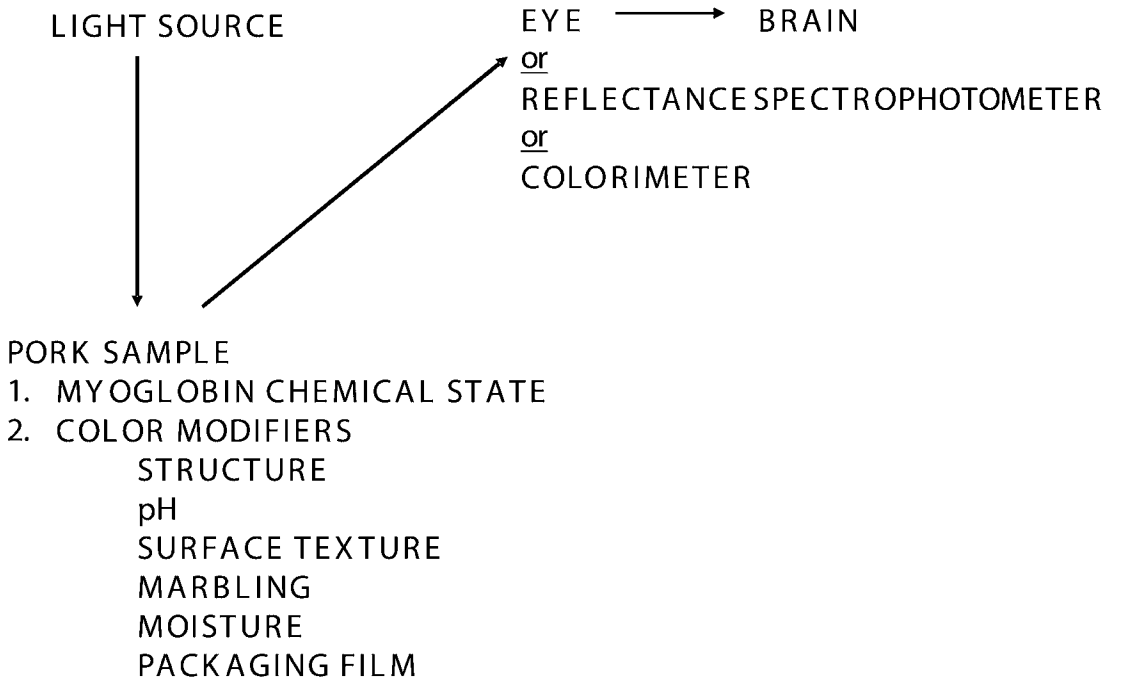

Figure 29.1. Light pork color, detection—interpretation.

The color of different pork cuts or pork products is greatly influenced by their ability to reflect or absorb various wavelengths of visible light. A high proportion of reflected red wavelengths result in a redder color. Total light reflectance will influence the lightness or darkness of an object. More total light reflectance will result in a lighter color whereas more total light absorbance will result in a darker color.

Pork muscle color is often described, very simply, as either a bright, pale or dark pink to reddish tone, which can deteriorate to a faded, brown, tan, greenish, or almost colorless appearance. Color, whether determined by visual perception or by instrumental readings, is influenced by intrinsic characteristics (Figure 29.1). Pork color can be influenced by the genetics of the live animal, the nutritional program administered, antemortem conditions and handling (those events and conditions prior to harvest), the various processes associated with harvesting the animal (stunning, dressing, and chilling), and by processing, packaging, distribution, and marketing conditions. These will be discussed later in this chapter.

Vision is a complex phenomenon that deals not only with the physical aspects of energy from the electromagnetic spectrum, but color perception is also a psychological phenomenon that is affected by the observer and their experience with color (MacDougall 1994). Visual appraisal is frequently set up to assess how close color is to an arbitrary point of consumer rejection and to quantitate color changes up to and beyond this point.

Instrumental color measurement is an effort to measure color in a manner that relates closely to visual evaluation. Details and protocol of both visual and instrumental color evaluation will be discussed later in this chapter.

PORK COLOR BASICS

Understanding the meat color triangle (Figure 29.2) is very helpful as the proportion and location of deoxy-, oxy- and metmyoglobin, the chemical forms of myoglobin that have the major influence on meat color, are important. Myoglobin is the major meat pigment that influences meat color, but hemoglobin

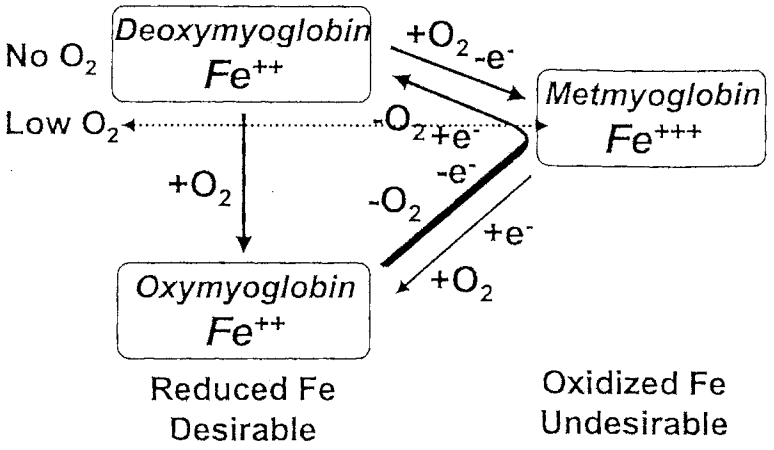

Figure 29.2. Fresh meat color (myoglobin) triangle.

and cytochrome oxidase also have a role. The interconversion of these chemical forms of myoglobin is influenced by extrinsic factors such as temperature, light exposure, microbial load, and relative pressures of oxygen and other gases that can be controlled by the packaging system (O'Keefe and Hood 1982, Faustman and Cassens 1990, Zhu and Brewer 1998). Intrinsic traits such as pH, muscle fiber type, oxygen consumption rate, and metmyoglobin-reducing capacity are also influencing factors.

Deoxymyoglobin contains iron in the reduced or ferrous state (Fe^{++}) and has a darker purple-pink color. Its formation is favored at extremely low partial oxygen pressures (1.4 mm mercury [Hg] or less; Figure 29.3) and when exposed to air or another source of oxygen, it combines very quickly with oxygen to form the bright pink color of oxymyoglobin, which also has iron in the ferrous (Fe^{++}) state, but has oxygen attached at the sixth ligand position. Oxymyoglobin is stable at high partial pressures of oxygen, but if relatively low oxygen partial pressure develops, it is vulnerable to oxidation with conversion to brown metmyoglobin and resultant discoloration.

Oxygenation or Blooming

Because of the anaerobic condition of muscle before cutting, the fresh cut surface of pork will briefly present the purplish pink color of deoxymyoglobin. Immediately after cutting, myoglobin at the newly cut surface will begin to react with oxygen of the air to form oxymyoglobin. This reaction is called oxygenation or blooming and after 20 to 30 minutes, the meat may be considered bright colored enough to place in display. This surface layer of oxymyoglobin gradually becomes thicker, thus deeper from the surface. This change occurs more rapidly at colder chilled temperatures. While oxygen diffusion into muscle should theoretically be faster at warmer temperatures, such as 4.4°C (40°F) versus 0°C (32°F), meat enzyme competition for oxygen is greater at the higher temperature and this works against oxygen penetration at the warmer temperature. Thus oxygen penetration is greater at the colder temperature. Both low temperature and low pH increase oxygen solubility and discourage the enzyme activity that uses up oxygen, thus both cold temperature and low pH encourage more rapid oxygenation (Ledward 1992, Zhu and others 2001).

For intact muscles, oxygen penetrates more deeply from the surface with longer time after exposure to air and this may continue for several days. A deeper more rapidly developing layer of pink oxymyoglobin will be encouraged by exposing the meat to higher oxygen pressure such as high oxygen modified atmosphere packaging (MAP) or hyperbaric conditions (Taylor 1982).

Relatively fresh muscle with a good supply of metmyoglobin reducing ability will appear to have two pigment layers—oxymyoglobin toward the surface and deoxymyoglobin at deeper locations. The

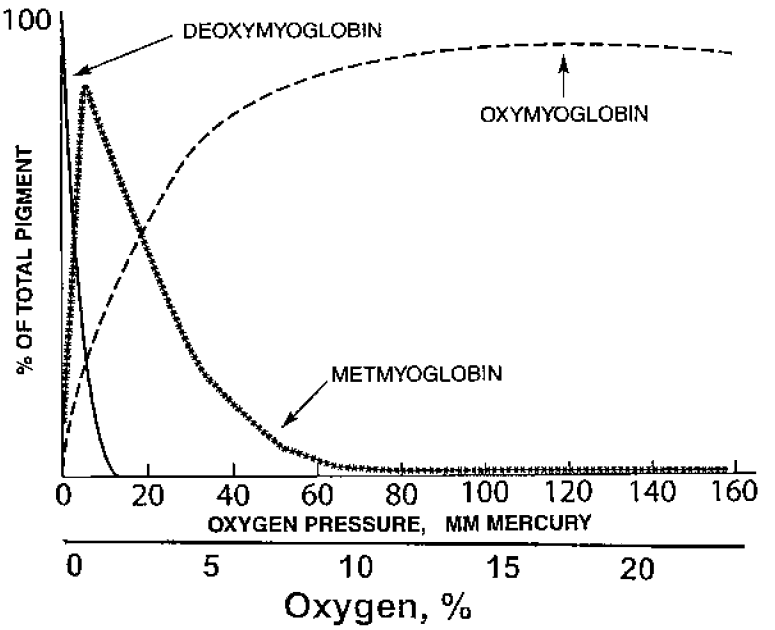

Figure 29.3. Relationship of partial oxygen and percent oxygen to deoxy-, met-, and oxy-myoglobin formation.

layer description has been frequently used (Kropf 2000, Ledward 1992), but a sharp boundary does not occur but rather a gradient from one pigment chemical form to another.

Even though the deoxy form is less stable, color stability is more achievable if myoglobin remains as deoxymyoglobin until bright red or bright pink is required to satisfy meat purchasers. Unnecessary and uncontrolled pigment changes are costly in terms of losing ability to return to deoxymyoglobin and these shorten color life.

Discoloration

The onset of meat discoloration is delayed by low temperature storage and display (Faustman and Cassens 1990). When reducing mechanisms of the muscle are approaching depletion and oxygen supply neither favors deoxymyoglobin or oxymyoglobin, a third layer of pigment, brown metmyoglobin begins to form. This brown layer becomes thicker with time and also moves toward the muscle surface. This brown layer is frequently not uniform in its closeness to the surface, thus spotty, nonuniform discoloration may be viewed at the surface. At some point, both the human eye and reflectance instruments begin to "see" the brown discoloration. MacDougall (1994) mentions optical infinity, the meat sample thickness that obliterates color of nonmeat background material so sample reflectance is due only to the sample with its layers of myoglobin chemical forms. Optical infinity is assured with a 10- to 15-millimeter (mm) thick sample and usually with only 6 mm. His statement suggests that subsurface metmyoglobin at a greater depth would not influence meat surface color measurement. The perception of surface color is not only due to the chemical form of myoglobin immediately at the surface, but also partially influenced by the subsurface chemical state of myoglobin. Pork with a high degree of light scatter or refraction by myofilament lattice (Swatland 2003), such as pale, soft, exudative allows less subsurface contribution to visual perception or instrumental readings than dark, firm, and dry muscle that demonstrates fewer of these effects.

Deoxymyoglobin and oxymyoglobin, which are both in the reduced state, can oxidize to metmyoglobin (Figure 29.2), which has a dull brown color associated

with deterioration of quality. Metmyoglobin can be slowly converted to deoxymyoglobin by enzyme-mediated reactions termed metmyoglobin-reducing activity. Muscle tissue that is deficient in the enzymes that mediate metmyoglobin reduction or in reduced cofactors necessary for reduction is unable to reconvert metmyoglobin, which then persists.

Meat that has been ground, flaked, or made into small particles presents a more complicated case of multiple loci of different chemical forms of myoglobin, because multiple surfaces have been exposed to oxygen. In the extreme case of ground meat, all particles are susceptible to oxygenation during any exposure to oxygen such as grinding, but when they are packed into a solid mass for packaging, the chemical form of myoglobin at any place is dependent upon the continuing pattern of oxygen level, which is affected by muscle metabolism, consumption of oxygen, and also the availability or depletion of reducing capability. Often the center of a relatively fresh ground meat mass, given sufficient time, will revert to deoxymyoglobin while the surface continues in an oxymyoglobin state. Since so much of meat mass has been exposed to oxygen because of the small particle size and the enormous surface area, the process of converting oxymyoglobin to metmyoglobin to deoxymyoglobin may use up enzymatic resources of reducing capability. Therefore, this quickly reaches a condition where the metmyoglobin reducing ability is depleted and some or all of the ground meat will revert to the oxidized brown metmyoglobin state.

Other Color Effects

Green discoloration in fresh meat has been attributed to altered heme structure, possibly to choleglobin or sulfmyoglobin forms of myoglobin (Ledward 1992). Choleglobin results from the reaction of myoglobin with hydrogen peroxide, which may have resulted from microbial metabolism or chemical reactions. Sulfmyoglobin results from the reaction between hydrogen sulfide and myoglobin. Hydrogen sulfide can result from microbial metabolism or sometimes from chemical residue of certain treatments of carcasses or meat cuts. Fox (1966) also presents further myoglobin degradation that influences color.

An additional color problem is the appearance of iridescence, a rainbow-colored effect, sometimes called mother-of-pearl color, which shows itself as green, yellow, orange, and/or weird red over part of the cut surface of fresh, cooked, or cured pork (Lawrence and others 2002). This is caused by light diffraction (bending of light rays), which is influenced by the distance between microstructural units in muscle. The distance between these structural units may be increased by adding water and other ingredients, or decreased by varying degrees of cooking shrink. Blade tenderizing pork cuts before slicing or cutting a fresh surface may help reduce both the intensity of iridescence and the amount of the surface affected. Iridescence is harmless to consumers, but products exhibiting iridescence may be rejected by the consumer because of the mistaken impression that the iridescence is caused by microbial action or is an indicator of an inferior product. Iridescence is most apt to occur when muscle is cut perpendicular to the muscle fiber direction.

Bertelsen and others (2000) present an excellent discussion of oxidative balance of meat, which can have a large influence on color and shelf life. Intrinsic factors involved include meat content, concentrations, and reactivity of oxidation vulnerable substances, oxidation catalysts (prooxidants), and antioxidants. Oxidation substrates include lipids (phospholipids are especially vulnerable), proteins, and pigments. Prooxidants include transition metals, especially copper and iron, iron-containing proteins such as heme pigments, and enzymes. Endogenous antioxidants include lipid soluble carotenoids, ubiquinon, and ascorbic acid; cytosolic originated carnosine/anserine, glutathione, polyamines, uric acid, and superoxide dismutase; and enzymic catalase and glutathion peroxidase.

Influence of Rate of pH Decline

Pork color is strongly influenced by the rate and extent of postmortem pH decline. The pH of living muscle is approximately neutral. The pH decline during postmortem metabolism, or the conversion of muscle to meat, results from anaerobic glycolysis with the resultant production of lactic acid. The faster that glycogen is broken down (glycolysis), the faster lactic acid levels build up in the muscle and consequently, the more rapid the decline in pH. This becomes critical when the pH decline is too rapid in relation to chill rate (temperature decline) of the muscle. A very rapid decline in pH can lead to pork

that is pale, soft, and watery. The soft and watery traits result from muscle pH approaching the isoelectic pH of muscle proteins so that water-holding capacity of the muscle is minimal and much of intracellular water is released to intercellular space resulting in the soft, exudative (watery) condition. This change can increase the light-scattering effect of muscle structure plus an effect on myofilament structure (Swatland 2003), contributing to the pale color.

Pigs that have low stores of muscle glycogen produce pork that is classified as dark, firm, and dry (DFD). Low initial stores of muscle glycogen can be found in animals that are exhausted at the time of harvest, or sometimes in pigs that have been held for 6 hours or longer after unloading before harvest. Therefore, the insufficient level of glycogen in the muscle results in insufficient lactic acid to lower the pH significantly. The end result is fresh pork products with a high pH, a high water-holding capacity, and a translucent nature, which results in a darker color. They also are more susceptible to microbial growth and/or spoilage as many of the spoilage bacteria grow more rapidly at higher pH.

Briskey (1964) reported six patterns of postmortem pH decline, which were described as follows: (1) a slow, gradual decrease to an ultimate pH of 6.0 to 6.5 or higher (dark muscle); (2) a slow gradual decrease to an ultimate pH of 5.7 to 6.0 (slightly dark); (3) a gradual decrease to a pH of approximately 5.7 in 8 hours, with an ultimate pH of 5.3 to 5.7 (normal muscle); (4) a relatively rapid decrease to approximately 5.5 in 3 hours, with an ultimate pH of 5.3 to 5.6 (slightly pale, soft, exudative [PSE]); (5) a rapid to a slightly gradual but extremely extensive decrease to an ultimate pH of about 5.0 (slightly dark to extremely pale, but in all cases extremely exudative); and (6) a rapid decrease to a pH of 5.1 at 1.5 hours and a slight subsequent elevation to 5.3 to 5.6 (extremely PSE). These six patterns of pH decline help describe pork color and related quality traits. Each carcass may exhibit a unique pattern, but generally the pattern will fit closely to one of those described above. Some pork has also been reported to show regular color (bright pink), but have a soft texture and an exudative or watery surface pale, soft, and exudative (PSE).

Individual muscles within a pork carcass or pork cut often exhibit different colors or shades of pink. This can be due to a number of reasons, but one reason is that muscles with more intense pink have a higher myoglobin content. Some muscles also tend to be more glycolytic in postmortem metabolism than others due to a higher proportion of alpha white (type II b) muscle fibers.

Factors Affecting Pork Color Stability

A very thorough and concise review of factors impacting color and related quality traits is presented in *A System for Assuring Pork Quality* (Meisinger 2002).

Live Animal Selection

Animal genetics is a major factor influencing muscle color, since some pigs are genetically predisposed to be more stress susceptible and demonstrate marked responses to preslaughter stressors such as improper handling, warm temperatures (or large fluctuations of ambient temperatures over a short period of time), mixing of pig strangers (pigs that were previously housed in separate pens), and harvesting pigs without sufficient resting after they were unloaded from the transport vehicle and prior to stunning. The occurrence of one or more of these stressors may result in a more rapid postmortem (after harvest) muscle pH decline, which can lead to the development of PSE muscle, particularly for those animals that were genetically more susceptible to the effects of stress.

Doumit and others (2002) and Meisinger (2002) summarized research dealing with biochemical characterization that is related to pork quality. Much work has focused on PSE pork associated with the halothane or stress gene (calcium-release channel defect), also the Napole gene (RN-) which results in pigs with abnormally high storage of glycogen that consequently produce more lactate and hydrogen ions during postmortem glycolysis. These conditions have been brought on by selecting for rapid gain and leaner carcasses and by failure to select against these quality defects. Their data suggest that breed differences in meat color and water-holding capacity are not explained by differences in total heme pigment concentration, glycolytic enzyme capacity, or buffering capacity. However, a slightly lower glycolytic potential in loin muscle of Berkshire pigs was related to a slightly higher ultimate pH in this muscle in Berkshire pigs compared with

Yorkshire pigs. The Berkshire muscle demonstrated a more gradual pH decline in the first 180 minutes postmortem, which led to a more desirable color.

Nutritional Effects

Some feedstuff components and supplementary feed additives can influence pork quality. Much interest has been focused on those with antioxidant potential (Bertelsen and others 2000, Buckley and others 1995) such as α-tocopherol (vitamin E), which has improved pork color and muscle membrane integrity during long storage, presumably by preventing oxidation of membranal phospholipids. This effect seems dose dependant as 200 milligrams (mg) α-tocopherol acetate per kilogram (kg) of feed was effective in one study, but a 100-mg level in another study did not affect color. Carotenoid antioxidants have the ability to inactivate singlet oxygen (Decker and Xu 1998), but other mechanisms are still under study. β-carotene acted as an antioxidant at 15 parts per million (ppm), but as a prooxidant at 50 ppm, thus would not have the same influence on color at all levels of use.

Meisinger (2002) summarizes the effect of nutritional input on pork color and related quality factors. L-carnitine at high levels reduced pork paleness. Magnesium supplementation in various forms was effective in reducing PSE pork. Its addition for 5 days before slaughter was recommended, as was adding 5 mg daily of the amino acid tryptophan. Magnesium reduces plasma cortisol and catecholamine concentrations, thus may reduce an animal's glycolytic response to preslaughter stress. Some studies noted improved muscle color with feeding 55,000 or 175,000 IU vitamin D-3 per kg for 10 days before slaughter.

Holding off feed before slaughter had mixed results on color, as a 36- or 60-hour feed withdrawal improved color for animals with low, but not those with high, glycolytic potential when pig groups were mixed during withdrawal time.

Very little research has dealt with pork fat color. Ringkob (2003) compared pork fat color from a study feeding a primarily barley diet and a corn diet by use of a yellow split of a CMY format and comparing to readings from Japanese fat block models. Fat from the barley diet qualified pigs for requirements of the Japanese market, that from the corn diet pigs or store samples each had about half of samples not qualifying.

Environmental Impacts

Some studies have noted that environmental temperature can influence pork quality. A year-long study by Forrest and others (1963) noted that cool temperature in early spring followed by a sudden warming resulted in an increase in PSE hams. Cold weather increased the proportion of carcasses with DFD hams.

Transport and Handling

Since pigs are stress vulnerable to hot temperature, crowding, and mixing of animal groups, such practices as loading and hauling at night, providing minimum stress during loading (no electric prod use), and keeping intact pig groups that were reared together and not mixing pig groups are all important in minimizing negative effects on pork color. Conditions that distract or startle animals and present new social situations cause stress (Faustman and Cassens 1990) and impact pork color. Flat floor trailers enable less stressful loading and unloading than possum belly semi-trailers (Meisinger 2002).

Holding Conditions (Lairage)

Pigs that are rested for 2 to 4 hours after unloading and before moving to the stunning area will present the least effect on pork muscle color. Sufficient room so all pigs have access to water and can move freely are essential as are clean floors with nonslick surfaces. Too long holding, such as 6 hours or more, will increase incidence of dark pork. Driving alleys should be wide with rounded corners, no obstructions, and no shadows or noise to cause pigs to balk. Except during cold conditions, using misting or water sprays will be beneficial (Long and Tarrant 1990) and should reduce incidence of pale pork color.

Stun, Bleed, and Scald Influence

The two most widely used humane systems for stunning pigs to render them insensible to pain are electrical stunning and carbon dioxide immobilization. Color affecting problems associated with electrical stunning include broken vertebrae and scapulas,

blood spots, and bruising, all of which can create unsightly local effects. Berghaus and Troeger (1998) stated that too many Coulombs (amperes × seconds applied) are related to a quality decrease of pork. Combining a head-applied high frequency stunning with a low frequency cardiac arrest is very promising for minimal influence on quality.

Carbon dioxide stunning has reduced blood splash, bone fractures, and PSE pork. Velarde and others (2001) reported reducing PSE from 35.6% incidence with electrical stunning to 4.5% with carbon dioxide with strongly notable reduction in both pinpoint and large hemorrhages. These effects are less pronounced in stress susceptible pigs, and may be more likely in halothane positive pigs.

Shorter intervals from stun to bleed minimize blood splash and reduce PSE, with a ≤10 second interval being optimal. This short interval is achievable with horizontal bleeding done before shackling and vertical suspension. Longer time from bleeding to scalding also had some negative effects on color of some muscles (Lonergan and others 2002).

Dressing and Chilling

The shortest time possible from stun/bleed to placing carcasses in chill is beneficial to improving pork color. An ideal plant might achieve this in 25 minutes, but this time frequently ranges from 30 to 60 minutes.

Several studies (Honickel 1986; Ohene-Adjei and others 2002, 2003) suggested that rapid chilling cannot compensate for the rapid pH decline and quality loss of pigs that are stress prone, especially for ham muscles more deeply located from the surface. Accelerated chill can improve color and reduce drip loss from *Longissimus* muscle and others located closer to the surface.

Interactions and Compensatory Effects of Previously Mentioned Factors

Preslaughter inputs (genetics, nutrition, transport, and handling) set the stage for response to the slaughter process as suggested by Sosnicki and others (1998) and as stated by Lonergan and others (2002). They further stated that harvest and processing steps cannot improve the quality defined by the preslaughter inputs, but may either sustain this quality level or allow it to diminish.

Fabrication/Enhancement

Color will be improved and color stability lengthened by quickly and systematically moving pork cuts from fabrication to enhancement (when used) to packaging with minimum exposure to air and lighting. This is especially important for retail cuts that will go into case-ready packages. Careless placement or stacking of cuts in a large container increases drip loss with a further negative impact on color stability. Partial overlapping of adjacent cuts can lead to color variation within single cuts because of the varied exposure to air.

Enhancement is widely used for case-ready fresh pork cuts. Those that may improve color stability (Miller 1998) include sodium, potassium, or calcium lactate, ascorbic acid or sodium isoascorbate, carnosine, anserine, phenolic antioxidants, rosemary and its extractives, and other plant source materials. Those that may diminish color stability are alkaline phosphates, salt, water contaminants, ascorbic acid or sodium isoascorbate, organic acids, and heavy metals. Several are included in both lists, since their effect may depend on the concentration used.

Prooxidant and antioxidant components of muscle (Bertelsen and others 2002) were discussed earlier and can impact color. When considering ingredients for enhancement, sodium or potassium lactate may create a darker colored meat originally, but the color will be stable for a reasonably long display life. Some others, such as rosemary, create a brighter color initially but this color may not be as stable. Current enhancement of fresh pork frequently creates a product that is very glossy, shiny, wet on the surface, creating an appearance that may be detrimental to some customers. Color is a major factor but not the only one contributing to product appearance. Traits of wetness, dryness, and freshness may influence product appearance and acceptance beyond their influence on color.

Freezing

Much research on effects of freezing variables on meat color has been done on beef, likely because of its more intense color and higher myoglobin concentrations, but these findings should have some application to pork. Freezing at −12.2°C versus −34.4°C resulted in a darker color (Ramsbottom and Koonz 1941) and beef steaks frozen at −6.7,

−28.8, and −78.8°C resulted in darker than fresh, similar to fresh, and lighter colored than fresh, respectively (Ramsbottom and others 1949). However, Stoier and Borup (1995) reported that reducing freezing "rate" from 36 to 12 hours did not have much influence on the color of raw pork loin muscle. With most packaging systems, especially those with an oxygen-barrier film, cuts to be frozen should be immediately frozen after packaging or serious color darkening will occur. Longer freezer storage increased protein denaturation and water (ice) recrystallization (Calvelo 1981) with increasing oxidation and freezer burn also resulting. Desiccation on the surface of meat cuts sometimes produces a color that progressively changes from whitish to grayish-yellow to brown and is accompanied by tissue alteration to a spongy or corky condition with honeycomb-like air pockets, known as freezer burn. It is more apt to occur with voids between meat and film (skin tight film is best) and is also accelerated with fluctuating freezer temperature. MacDougall (1994) has stated that frozen meat is more susceptible to photochemical oxidation than to other deterioration mechanisms that are slowed at frozen temperatures, especially those caused by microorganisms.

Ultra chill of pork carcasses or freezing pork cuts can lead to a black bone discoloration in the porous regions of such bones as vertebrae. Autoxidation rate constants for porcine myoglobin are greater than those for bovine and ovine, accounting for color vulnerability of pork (Zachariah and Satterlee 1973). This rate constant showed a dramatic peak for pork at −11 to −12°C, with a marked decrease at −18 to −25°C. This shows the necessity of rapid freezing to minimize time at these most vulnerable temperatures. During thawing, rapid temperature change also is needed to minimize myoglobin autoxidation with its resultant color deterioration.

Product problems that occur with freezing, frozen display or storage, and subsequent thawing can include such appearance problems as dark red or brown discoloration, freezer burn and/or dehydration, color splotching (unevenness of color), frost accumulation in packages, and product bleaching or whitening (Kropf 1982). Color unevenness may result from variation in the degree of myoglobin oxidation or reduction on a muscle due to variations in partial oxygen pressure in skintight packages or from local dehydration and dehydration-induced oxidation. Frost accumulation in the package results from freezer display or storage temperature fluctuation and from packaging film that fits too loosely on a given cut. Bleaching or whitening results from too rapid freezing so the countless tiny ice crystals cause intense light scattering.

Packaging

Several packaging options are currently used for fresh retail pork cuts. Traditional overwrap has a fresh pork cut placed on a soaker pad on a Styrofoam tray that is overwrapped with highly oxygen permeable polyvinyl chloride (PVC) film in the USA or polyethylene in some other countries. For this system, usually final retail cuts are prepared from wholesale cuts at individual stores. This method depends on a rapid bloom (oxygenation) but has a limited display life of 2 to 3 days for whole muscle cuts and likely 1 to 1½ days for ground product. Therefore is not suggested for case-ready pork prepared at a central location and distributed to stores. Wholesale loins and other fresh pork cuts are sometimes distributed in vacuum or a gas pack system to provide more flexibility in the days before these cuts must be retail packaged and sold.

One large study done under commercial cutting and packaging conditions used a single gas flush of 0.61 liter of carbon dioxide per kilogram of meat and studied effects of storage for up to 19 days before cutting pork loin chops and overwrapping them for display (Warren and others 1992). The initial carbon dioxide concentration of 78.5% of the within-bag atmosphere, decreased to 55.1% by day 3 and 41% by day 19, partially due to carbon dioxide absorption into the meat. Oxygen, at 3.1% initially, increased to 7.1% during the storage. The increase of oxygen is partially due to oxygen that had been absorbed by the meat before packaging and later coming out of the meat into the within-package atmosphere and partially due to ingress of oxygen through the large area of the bag. While microbial counts increased from day 3 to day 19, even then they were less than 5 log colony-forming units (cfu) per cm^2 surface area. With longer storage, loin weight loss and discoloration increased. At day 19, while off odor was acceptable, discoloration at some locations was the limiting factor in shelf life. Loin eye (longissimus) color for chops cut and overwrapped in polyvinyl chloride and placed on a soaker pad on a Styrofoam tray was acceptable

through 3 days of display after the 19-day storage time. The psoas major (tenderloin) still had acceptable visual color for 2 display days when it had been stored in the gas-pack for up to 10 days. Muscles from the sirloin end of the loin had acceptable color for 2 days of display if the loins had been stored in gas-pack for up to 11 days. The color stability of the psoas major was the limiting factor of pork chops in this study.

Another pork loin study compared gas mixes of 100% carbon dioxide; 75% carbon dioxide and 25% nitrogen; 50% each of carbon dioxide and nitrogen; 25% carbon dioxide, 65% nitrogen, and 10% oxygen; and another stored in a vacuum package (Sörheim and others 1996). Gas packages were triple flushed using a Corr-Vac mark III packager (M-TEK Inc., Elgin, IL). Loin sections were stored at 34°F for either 14 or 22 days, after which chops were cut, placed on soaker pads on polystyrene trays, and overwrapped with polyvinyl chloride film and displayed at 37°F under 100 foot-candles (1076 lux) of display lighting with twice a day defrost of display cases. Oxygen concentration in bags flushed with carbon dioxide and nitrogen was 0.1 to 0.4%. The gas mix containing 10% oxygen resulted in chops with more graying and greening, stronger off odors, and higher psychrotropic microbial counts by over 1 log. Drip loss from loins stored in the 100% carbon dioxide was higher than from other treatments. Display life of chops was similar for all treatments, except shorter for those stored in the gas mix containing 10% oxygen. Another study found no advantage for triple-flush over single-flush procedure for gas-pack of pork loins regarding display life of overwrapped chops. This study also compared gas to meat ratios of 0.7, 1.3, and 3.0 to 1 for gas-pack pork loins that were cut into chops and displayed, and found no display life advantage for any one.

Vacuum packaged pork retail cuts, in the author's opinion, can have a very acceptable color. Although the myoglobin reverts to deoxymyoglobin with a purplish-pink color, the lower myoglobin content of pork creates a situation where the color is not unacceptably dark. A negative aspect is that the pressure of the vacuuming process results in more purge and the purge may take on a greenish color. With inadequate evacuation of air, brown metmyoglobin may be formed on meat surface. This oxygen is available for both muscle and microbial metabolism and if oxygen is returned to a sufficiently low level, this color may change to purplish-pink deoxymyoglobin.

The rapid expansion of case-ready marketing of fresh, chilled pork cuts has resulted in a dramatic increase in use of modified atmosphere packaging (MAP), primarily high oxygen MAP. This system may use an in-package gas composition of up to 80% oxygen to ensure a longer color life and of at least 20% carbon dioxide to control microbial problems. Pork loin chops in high oxygen MAP had an acceptable color saturation index and visual color for 8 to 12 days. Odor and rancid taste may be the limiting factor rather than discoloration. However, enhancement ingredients can control these problems.

Ultra-low oxygen MAP depends on a nitrogen and carbon dioxide gas mixture to maintain myoglobin in the deoxy chemical state during storage and distribution. Very low oxygen levels conserve the ability of such meat to bloom when exposed to air shortly before the retailer places these cuts in display. For ultra-low oxygen MAP, a slight amount of residual oxygen frequently occurs due to small pockets of air not removed initially by evacuation or flushing. These cause major discoloration, lessened ability to bloom, and shortened display life. Initial oxygen concentrations >1.0% seriously compromised color stability of pork. Oxygen scavengers are needed to get residual oxygen low enough and fast enough to save pork muscle ability to bloom and have an adequate display life.

Carbon monoxide at a low level in package gases had the ability to form a stable reddish-pink carboxy myoglobin (Sörheim and others 2001). Such use has been approved by the Food and Drug Administration in the USA when used at 0.4% in a nitrogen-carbon dioxide gas in a master pack. The individual retail packages are overwrapped with a gas-permeable film. Its color reflectance spectrum is very similar to oxymyoglobin, but it is more resistant to oxidation. This acceptable color appears to stay only during "microbial shelf life" of the product. Its use overcame bone blackening in pork chops.

Lighting Effects on Color

A number of studies indicate that exposure of meat to lighting causes more rapid and detrimental color deterioration, although not all studies agree with this finding (Kropf 1980).

Perceived and real lighting effects could result from the following: (1) temperature elevation at the meat surface (less from deluxe fluorescent or lamps with dichroic filters than from incandescent lighting); (2) photochemical or photooxidation effect possibly through singlet oxygen as a responsible agent; and (3) difference in color rendition due to different spectral energy emission patterns (Kropf 1980, Faustman and Cassens 1990).

Archer and Bandfield (1950) suggested "great destruction" of heme pigments was due to strong absorption at certain wavelengths. In the visible spectrum, myoglobin is characterized by high absorbancies at certain wavelengths called Soret bands or peaks. Iverson (1985) stated that if the energy spikes at certain wavelengths of lighting emissions matched those wavelengths, even faster pigment oxidation and discoloration could result. Lighting is also characterized by nonvisible UV emission. Bertelsen and Skibsted (1987) reported on photooxidation of oxymyoglobin by specific UV wavelengths with the most destructive oxidation at shortest wavelengths. Andersen and others (1988) stated that combined action of light and oxygen cause color fade, and a further study (Andersen and others 1990) reported that modified packaging designed to lessen UV exposure protected against photodegradation. Gould (1963) disagreed with some other studies and reported that pork discoloration under lighting was primarily due to warmer product surface temperature and that light intensity that did not raise the surface temperature did not affect discoloration.

Color rendition is related to how closely spectral energy distribution matches the light reflectance of visible wavelengths. Barbut (2001) reported that incandescent lighting made meat appear redder than under fluorescent or metal halide lighting when used at a light intensity of 760 lux. This effect was less pronounced for pork than for beef because of pork's lower myoglobin concentration.

Light sources are characterized by several systems as to their color rendering properties. Color rendering index (CRI) is based on emissions at eight specific wavelengths and is a widely accepted system. Since some of the wavelengths do not relate well to meat color, this system has limited value in describing appropriate meat display lighting, even though the Lighting Handbook (IESNA 2001) suggests use of fluorescent lamps with high CRI plus a "strong content of red wavelengths." The author's opinion is that color temperature, in degrees Kelvin, is a more meaningful indicator of recommended display lighting.

Studies of effects of lighting on fresh pork are few in number. One project studied 1-inch-thick fresh pork chops from each of four pork loins of normal color displayed at 34°F and 200 foot-candles for 18 hours before evaluation of color. The chops were placed on foam white trays and wrapped with an oxygen-permeable film and displayed under deluxe cool white fluorescent light, cool white Surlyn coated fluorescent lights, warm white fluorescent lights, or cool flood incandescent light. Panelists evaluated the loin eye muscle of the chops for color desirability. Chops under deluxe cool white were rated most desirable, followed by cool flood incandescent. The least desirable color rating resulted for the other two types of lights (Calkins et al. 1986).

A second phase of the study used 40 frozen pork loin chops. All of the chops were in retail display under the same four light sources for 5 days, but were subdivided into 12- or 24-hour-per-day light exposure. Chops under the cool flood incandescent lights had the most rapid increase in metmyoglobin, resulting in a more undesirable color. This type of light also generated enough heat to elevate the surface temperature of the chops from 3.6 to 14.4°F. This temperature increase, which was not observed for the other light sources, could be responsible for the accelerated rate of metmyoglobin formation. Under the cool flood incandescent lights, the percentage of oxymyoglobin, the desirably colored pigment on the surface of the chops, decreased from 63% at 12 hours to 40% after 5 days. This decrease was compared to a change from 73% initially to 63% for chops under the other lights at 12 hours display time.

Another research study used loin eye samples from seven pork loins to determine display color stability when packaged in oxygen-permeable PVC film or when packaged under vacuum in an oxygen-barrier film. All chops were visually evaluated by four experienced evaluators under their assigned display light, which included GE Natural, Sylvania Grolux Wide Spectrum, NAFA, Sylvania Incandescent Fluorescent, GE Deluxe Warm White, GE Deluxe Cool White, or GE Cool White. All chops were also evaluated under one common light source,

GE Deluxe Warm White. The first five light sources listed match the color reflectance of the pork muscle quite well. Deluxe cool white is a slightly cooler, bluer light. Cool white, a light widely used for general store lighting, is very cool and blue for pork display. No visual differences were noted among lights when samples were evaluated under the common light source or by reflectance measurements. This means the lights did not differ in photochemical effect, but the different color scores were due to color rendition. Similar results were found when vacuum-packaged pork chop muscles were evaluated (Kropf et al. 1987).

Lamp technology is changing rapidly so that consultation with lamp manufacturers is advocated. Efficiency of converting electric power into light energy is very important and becomes more so as energy costs rise or electrical shortages occur. Trade-offs between good color rendition and efficient lighting should be guided by the perceived requirements for marketing meat products. Light sources continue to be modified.

Barbut (2001) reported meat display lighting in five surveyed meat counters to range from about 60 to 100 foot-candles, but some other surveys found up to 350 foot-candles (3,766 lux; Kropf 1980). More intense lighting is likely used now, partly because of the use of multishelf meat display cases with lights at all levels.

Consumer Expectations

Research generally indicates that color is an important fresh pork trait considered when customers choose meat purchases. Color is often indicative of other pork quality traits, thus a major concern to the pork industry (Warner and others 1993). Wachholz and others (1978) used pork chops from 96 loins representing PSE, normal, and DFD quality, all at equal prices in a supermarket display. Over one-half (146) of purchases were of normal quality, while DFD (72) and PSE (62) purchases were less. They suggested that some customers were unaware of DFD and PSE quality defects or may have actually preferred them. In the eyes of some of the consumers, fresh pork is expected to have a homogeneous reddish pink color (Van Oeckel and others 1999). Pink color, wet/dry condition, overall acceptability, and purchase intent were rated by 556 consumers for pork loin slices displayed in five retail supermarkets (Brewer and McKeith 1999). All three other factors were related to purchase intent.

Pork Color Evaluation

Pork color can be evaluated both visually and instrumentally. Visual color assessment closely approximates evaluation by the customer, thus it is important. However, this is subjective and people's judgment can be influenced by personal preference, visual deficiencies, appearance factors other than color, and personal happenings to panelists, thus are not repeatable. Using a visual panel requires panelist training with samples that embrace the variation that will occur in the actual study.

A number of color standards have been developed over the years and many are listed in the "Guidelines for Meat Color Evaluation" (Hunt and others 1991). The guidelines also contain much useful information about sample preparation, and both visual and instrumental color evaluation. Study of this information should be a requirement before planning an experiment with color evaluation.

Some of the color standards are useful for assessing the effects of pig selection and breeding, nutritional variables, other antemortem factors, and handling-processing factors through carcass chilling on color and related color traits. Such color differences may affect value of pork cuts and carcasses. The first widely used pictorial standard was "Pork Quality Standards" (Special bulletin 9, Wisconsin Experiment Station and Extension Service 1963). This pictorial standard was developed separately for loins and hams, and illustrations combined color, structure, and firmness on a 5-point scale. Other early scales also combined these quality traits. The National Pork Producers Council developed pictures for quality assessment in 1973. These pictures were revised in 1983, 1988, 1991, 1994, and again in 1999. These standards separated color from firmness-wateriness, resulting in a separate scale for both attributes. Some muscles with normal color could still be classified as having soft, watery structure. Most recently they have adopted the Japanese 6-point color scale. These were three-dimensional plastic colored chips based on equal spacing of HunterLab L*, a*, and b* values (defined later in this section) between adjacent color chips (Nakai and others 1975).

The National Pork Producers Council Pork Composition and Quality Assessment Procedures

(NPPC 2000) with color scores and associated Minolta L* values are as follows: 1 = Pale grayish pink to white, L* approximately 61; 2 = Grayish pink, L* approximately 55; 3 = Reddish pink, L* approximately 49; 4 = Dark reddish pink, L* approximately 43; 5 = Purplish red, L* approximately 37; and 6 = Dark purplish red, L* approximately 31. National Pork Producers Council (NPPC 1998) pork quality targets for color score were given as 3.0 to 5.0, using this 6-point scale. Meeting this target value would facilitate providing pork that would meet quality standards of customers, including the stringent standards of export customers.

Color evaluation has also been used to determine the effects of meat cut enhancement, packaging systems, storage and transportation, and display conditions. Some color standards in the guidelines are designed for these purposes. These also cover the colors produced by vacuum and by modified atmosphere packaging.

Instrumental color measurements, often determined by reflectance, are useful to substantiate visual panel data. These are not influenced by the "people" effects, thus have the benefit of control of conditions that may influence color measurements. The Guidelines (Hunt and others 1991) give important considerations about methodology, calculation of estimated percentage of myoglobin forms, and interpretation of color measurement results. They also describe a number of color evaluation systems.

Brewer and others (2001) explored the effect of the amount of bloom time before taking color measurements on stabilization of these values. In their study, L* value was unaffected by bloom time, hue angle stabilized after 5 minutes, a* and b* stabilized at 10 minutes, and chroma (saturation index) stabilized at 20 minutes postcutting. The L* values were most strongly related to visual color in their study.

Meisinger (1999) indicated that a system or process needs to be developed that can objectively measure fresh pork color/quality at line speed; such a system may include vision-based technology or fiber optic probes or some other type of measuring device that can quantify some factor that has a meaningful relationship with pork color and/or quality differences that affect consumer acceptance and that influence product value.

Pork color and quality differences affect product value. Therefore, a system that can rapidly evaluate these quality levels so price incentives or discounts can be used to encourage production of pork carcasses with highly acceptable quality. In attempting to establish a meaningful muscle location at which to appraise carcass quality, it was noted that different anatomical locations in the pork loin have differing quality (Waylan and others 1999). These researchers noted that the palest visual color and highest L* value was found at the most posterior location of the loin (toward the ham). At the most anterior portion of the loin, the visual color was more desirable with lower L* values and higher a* values (redness). Color values were reasonably uniform through most of the rest of the loin. This indicates that if a system were developed to measure color at a single place along the loin as an indicator of quality, it may over- or underestimate the quality of the remaining regions of the loin.

Much research has been directed toward the use of sensors or techniques to measure quality differences as summarized by Brondum (1998), Forrest (1998), and Berg (2000). Van Oeckel and others (1999) studied the extent to which instrumental color determinations by FOPu (light scattering), Göfo (reflectance), and Labscan II (CIE L*, CIE a* and CIE b*, hue and chroma) were related to the Japanese color grades. Additionally, four online methods: pH1, FOP1, PQM1 (conductivity), and Double Density Light Transmission (DDLT) were evaluated for their ability to predict color. One hundred twenty samples of *M. longissimus thoracis et lumborum* from animals of different genotypes were analyzed. Of the instrumental color determinations, CIE L* ($r = -0.82$), FOPu ($r = -0.70$), and Göfo ($r = 0.70$) were most strongly correlated with the Japanese color scores. Prediction of Japanese color scores by the four online methods produced determination coefficients between 15 and 28%. FoPu, Göfo, and CIE L* were better estimated by DDLT than the other online instruments. DDLT was originally designed to estimate lean meat percentage with a probe evaluation between the third and fourth most posterior rib location of the *longissimus thoracis*, 7 cm from the carcass midline, but the reflection curve of the muscle additionally provides a quality assessment. The DDLT uses an 880-nanometer (nm) energy wavelength with an 80-nm bandwidth. Because of the number of erroneous quality assessments, the authors state that the color estimates, while useful for a population of pigs, are not appropriate for individual pigs. They conclude

Table 29.1. Initial and final L*, a*, and b* values for pork longissimus of light, normal, and dark color (Venkat and others 1998).

	Lighter colored chops		Normal colored chops		Darker colored chops	
	Initial	Final	Initial	Final	Initial	Final
L* values	59.4	57.4	56.6	57.9	49.5	50.5
a* values	7.1	4.0	7.4	4.2	10.3	7.4
b* values	21.2	19.4	22.2	20.3	21.0	19.9

that online techniques when used early postmortem are not suitable to determine pork quality for single carcasses.

Morgan and others (1997) led a discussion on objective color standards for pork that raised some useful questions about such methodology. Some points follow: are color coordinates for Japanese color standards the middle or one end of each color score, how many color points are needed in a color standard, and can human vision discriminate between a 1 and a 2 as between a 3 and a 4? There was some advocacy for a red, green, blue (RGB) color system instead of CIE L*, a*, b*.

Extending display life of pork is always of interest, because minimizing loss from product discount or disposal is important to all of the partners in the pork production, processing, and marketing chain. With this in mind, additional color scales have been developed to evaluate color changes over display periods, and to more adequately determine display life of pork cuts and products. A number of these are presented in Hunt and others (1991). These are determined by observing color deterioration changes during display and by putting the observed changes in meaningful word descriptions and more recently, into picture standards (Venkat and others 1998). This study also recognized that color deterioration changes are quite different for PSE, normal, and DFD pork, therefore, different picture standards were developed for these quality variables for assessment of color deterioration. Chops that had lighter than normal pigmentation developed tan/brown discoloration faster than darker colored chops. Their initial and final L*, a*, and b* values are reported in Table 29.1, and final values represented those at the greatest color deterioration.

For instrumental measurement of color reflectance, details of the procedure that must be given are illuminant, viewing angle, and aperture size, but sometimes these are omitted and their impact is not recognized. MacDougall (1994) presented data on the effect of such variables on measured color. Sterrenburg (1989) reported that smaller aperture sizes do not give the same reflectance spectra as larger ones, because red reflectance is lessened by use of smaller aperture. This could be a problem if use of 8-mm and 50-mm apertures, which are commonly used with the Minolta instrument, were assumed to give comparable readings. Stephens (2004) explored the use of 1.75-, 1.0-, 0.5-, and 0.25-inch aperture diameter on color readings taken centered on the same location of uniform colored pork longissimus muscle samples, and when using illuminant A, C, or D_{65}. a* values, also hue angle were lessened, especially for illuminants C and D_{65}, by use of smaller apertures.

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30
Microbiological and Sensory Properties of Fresh and Frozen Pork Products

Lisa McKee

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INTRODUCTION

Pork has been used as human food for centuries. Evidence indicates hogs were domesticated for food in the Middle East about 7000 BC and arrived in the region that became the U.S. with Hernando de Soto in 1539 (USDA-FSIS 2003). During the nineteenth century, the addition of salt as a preservative resulted in the popular product salt pork. The tradition of "salt pork" remains today in the form of cured products such as ham and lunchmeat, which account for about 60% of pork consumption at lunch. Fresh pork products, however, particularly in the form of chops, are more popular at dinner, accounting for about 35% of pork consumption at that meal (NPD 2003).

An increase in consumer knowledge about food safety has been attributed to greater media coverage of food-borne illness outbreaks (Woodburn and Raab 1997). Forty percent of respondents, however, either thought food could not be made safe to eat or could not cite a way to make food safe. One-third of the respondents in a survey by Altekruse and others (1996) reported unsafe food preparation practices, including not washing their hands and cross-contaminating raw meat and other foods. Improper food handling has frequently been reported to be a leading cause of food-borne illness worldwide (Scott 1996, Jay and others 1999). The concerns about consumer food handling point to the continuing need to produce meat products that meet high standards for microbiological as well as sensory quality.

GENERAL MICROBIAL TYPES AND LOADS ON PORK

Contamination of pork carcasses with fecal and ingested materials during processing is unavoidable. The Pathogen Reduction, Hazard Analysis and Critical Control Point Systems (PR-HACCP) rule, published by the United States Department of Agriculture Food Safety and Inspection Service in 1996, requires pork processors to create and implement HACCP plans and standard procedures for sanitation operations. Pork production plants must also evaluate products for generic *Escherichia coli* as a measure of process control adequacy and meet *Salmonella* standards set in the rule (USDA-FSIS 2000).

A variety of microorganisms, including pathogens such as *Salmonella*, *Campylobacter*, *Escherichia coli*, *Yersinia* and *Listeria*, and spoilage organisms such as *Lactobacillus* and *Pseudomonas*, are commonly detected on pork and other meats. *Trichinella spiralis*, a parasitic roundworm, has also been associated with pork products. Rates of contamination vary widely and depend on a number of factors including area of the carcass evaluated, method of evaluation, and where in the process the evaluation is made. Hides, intestinal contents, personnel, and equipment are only a few of the numerous potential sources of contamination during pork processing.

Common Pathogens on Pork

Trichinella spiralis is a parasitic roundworm that invades the tissues of both carnivorous and omnivorous animals. Humans become infected with the organism through consumption of inadequately cooked meat containing *Trichinella* larval cysts. Once in the gastrointestinal tract, the larvae invade the bowel wall, producing symptoms such as abdominal pain, vomiting, diarrhea or constipation, and fever. As the larvae migrate into muscle tissues, symptoms such as edema, headaches, rashes, and coughing may develop.

Trichinella has historically been associated with pork as a result of hogs fed garbage containing animal waste materials (Roy and others 2003). Rates of trichinellosis have declined substantially over time due to laws such as the Federal Swine Health Protection Act, which prohibits the use of potentially contaminated garbage as swine feed. The *Trichinae* Herd Certification Program, a joint effort of the USDA, the National Pork Producers Council, and the pork processing industry, is a preharvest documentation system that allows production facilities that follow the program stipulations to be certified as *Trichinella*-free (National Pork Board 2000). Despite such programs, pork was reported to be the source of trichinellosis in 22 of 72 cases in the U.S. between 1997 and 2001 (Roy and others 2003).

Although *Salmonella* is more often associated with poultry products, the pathogen is a hazard in all meats. Provisions in the PR-HACCP rule require all meat processing plants to meet a standard for *Salmonella* rates. For pork that performance standard is 8.7% or no more than 6 *Salmonella*-positive samples in a 55-sample set (USDA-FSIS 2000). Analysis of 1,532 sponge samples collected during 1998 and 1999 from swine carcasses at 33 large processing plants indicated a *Salmonella*-positive rate of 6.5% (USDA-FSIS 2000). Duffy and others (2001), however, detected *Salmonella* on 9.6% of retail pork samples. Epling and others (1993) reported 29% of 225 swine carcasses were *Salmonella* positive. Data generally indicate *Salmonella* is most often isolated prior to fabrication and/or refrigerated storage and decreases in *Salmonella* levels are frequently noted as carcasses proceed through processing (Epling and others 1993, Saide-Alboronoz and others 1995).

Yersinia is a gram negative, psychrotrophic, facultative anaerobe. Although one species, *Yersinia pestis*, is responsible for plague, the most common species associated with food-borne illness is *Yersinia enterocolitica*. Yersiniosis results in acute gastroenteritis, fever, bloody diarrhea, and vomiting and has been associated with pseudoappendicitis, reactive arthritis, and other severe conditions.

Yersinia enterocolitica was isolated from 5 of 69 processed and 63 of 128 raw retail pork products by Schiemann (1980). Myers and others (1982) recovered *Yersinia enterocolitica* from 6% of vacuum-packaged pork samples, including both frozen and refrigerated samples. Duffy and others (2001) reported *Yersinia enterocolitica* in 19.8% of whole muscle, store-packaged pork samples and in 11.5% of store ground pork samples. Outbreaks of yersiniosis due to chitterlings (pork intestines) have been reported (MMWR 1990, 2003).

Campylobacter is another pathogen frequently isolated from meats. Although *Campylobacter jejuni* is the most commonly isolated species in meats such as beef, the presence of *Campylobacter coli* is more frequently reported in pork. Epling and others (1993) isolated *Campylobacter coli* from 23 of 225 pork carcasses while Bracewell and others (1985) reported a 12.5% isolation rate of the same organism from 112 freshly slaughtered pork carcasses. *Campylobacter coli* was isolated from 5% of pork chop samples and 4.2% of pork sausage samples in a cooperative study by nine U.S. laboratories (Stern and others 1985). Duffy and others (2001) detected *Campylobacter jejuni/coli* in 1.3% of retail and 6.7% of processing plant pork samples. Although *Campylobacter* has been detected in pork products, many of the processing steps in pork production, including singeing, scalding, and chilling, are detrimental to *Campylobacter* survival

(Oosterom and others 1983, Bracewell and others 1985).

Increased handling is often associated with increases in microbial loads in pork and other meats. Duffy and others (2001) collected 384 retail pork samples from 24 stores in 6 cities, including store packaged whole muscle and ground products, prepackaged ground pork, and marinated whole muscle products. The highest aerobic plate and coliform counts were in store ground samples. Pork samples from slaughter and fabrication plants, however, had higher aerobic plate and total coliform counts compared to pork from hot-boning and further processing plants.

CONTROLLING MICROORGANISMS ON PORK PRODUCTS AND THE EFFECT ON SENSORY PROPERTIES

Temperature

Although the effect of both hot and cold temperatures on microbial levels in pork products has been investigated, cold temperatures are by far the most frequent method of controlling microbial loads in meats. Chilling has been cited as a critical control point in pork processing although microbial loads have been reported to both increase (Bolton and others 2002) and decrease (Gill and Bryant 1992, Carr and others 1998) during chilling. The effect of methods of chilling as well as temperature of storage on microbial loads and sensory properties of pork have been investigated.

Freezing can be an effective means of reducing microbial loads/infectivity on foods. Kotula and others (1990) predicted the thermal death time of *Trichinella spiralis* in pork to be 8 minutes at −20°C with an upper confidence level of 48 minutes. Greer and Murray (1991), however, noted a greater lag time for psychrotrophs, pseudomonads, *Brochothrix thermosphacta,* and enteric bacteria in fresh pork compared to vacuum-packaged pork frozen for 90 days at −30°C and then thawed at 1.7°C for 2 days. Bacterial generation time was not different between fresh and frozen/thawed samples. Although freezing was associated with only a slight darkening and loss of brightness, an interaction between muscle quality and freezing/thawing on appearance case-life of the pork was noted. While freezing/thawing significantly reduced the appearance case-life of the pork regardless of muscle quality, odor was not affected by freezing/thawing treatments.

Cryogenic chilling is another method of cooling that can affect both microbial loads and quality attributes of pork. Neither chilling in liquid nitrogen for 1 or 3 minutes nor chilling at 1°C for 24 hours were effective in reducing mesophilic bacterial loads in pork and no differences in protein solubility or sarcomere length were noted (Jones and others 1991). Liquid nitrogen was most effective at reducing *Pseudomonas* spp.; reductions in *Brochothrix thermosphacta* B2, *Escherichia coli* ATCC 11775, and *Salmonella typhimurium* ATCC 14028 due to liquid nitrogen treatments were also noted. Although color of liquid nitrogen-treated muscle was slightly darker and muscle shear values were higher for carcasses treated with liquid nitrogen for 1 minute, few significant differences in objective pork quality characteristics were found. Subjective evaluation of palatability characteristics such as initial tenderness, juiciness, and flavor desirability by a six-member sensory panel indicated little difference in all characteristics except sour off flavors that were higher in liquid nitrogen-treated carcasses from pigs with high glycolytic rates.

Lower storage temperatures are typically associated with lower microbial growth. Lee and others (1985) found pork samples stored above 0°C had shelf lives of 14 to 28 days while those stored at −4°C had minimal changes in microbial and quality characteristics through 49 days. Pork stored at 7°C was consistently rated lower for overall appearance and had increased exudate, green discoloration, and off odors compared to samples stored at −4, 0, or 3°C.

Washing and Sanitizing

Washing and sanitizing are some of the most commonly used methods for reducing microbial loads on pork and other meats. Water was compared to several combination treatments as a decontamination method on lean and fat-covered pork tissue (Castelo and others 2001). Reductions in coliform levels of 1.46 $\log_{10}$ colony-forming units (cfu)/square centimeters (cm^2) (15 seconds) to 2.52 $\log_{10}$ cfu/cm^2 (180 seconds) were noted when 15°C water was used to wash lean tissue contaminated with feces. Water plus lactic acid and three treatments using

combinations of water, hot water, hot air, and lactic acid were more effective than water alone at reducing levels of aerobic bacteria, coliforms, *Escherichia coli*, lactic acid bacteria, and psychrotrophic bacteria. Combination treatments were associated with a decrease in redness and an increase in yellowness. Ground pork samples treated with water plus lactic acid were reported to be dark red with a mushy appearance. Brown cooked spots were noted in samples treated with any combination method.

The use of acids as decontaminating agents has also been investigated by others. Prasai and others (1992) sprayed 1% lactic acid at 55°C onto pork carcasses immediately after dehairing, after evisceration, and at both points in the processing. Mean total aerobic counts were always lower in acid-treated carcasses, but the decreases were not statistically significant when compared to control carcass results. Overall appearance, lean color, fat color, surface discoloration, and off odor as evaluated by a four-member trained panel also did not differ between treated and untreated samples. Van Netten and others (1995), however, reported undesirable color changes in pork carcasses decontaminated with either 2 or 5% lactic acid. Hot (55°C) lactic acid solutions were more consistently effective in reducing *Salmonella typhimurium* contamination on pork carcasses than were cold solutions in that study. Mendonca and others (1989) dipped pork chops into 1% acetic acid, 1% acetic acid/1% lactic acid, 1.5% acetic acid/1.5% sodium acetate, 3% acetic acid/3% sodium ascorbate, 3% acetic acid/2% sodium chloride (NaCl) or sterile, distilled water. Chops were subsequently vacuum packaged and stored at 2 to 4°C for 6 weeks. All samples except those containing 3% acetic acid had microbial loads of 10^7 to 10^8 cfu/cm^2 after 3 weeks storage and were considered spoiled. Samples treated with solutions containing 3% acetic acid had lower mesophilic and psychrotrophic counts than all other samples throughout the evaluation period. All treatments containing acetic acid, however, were effective at reducing *Enterobacteriaceae* levels. Treated samples were darker and tended to be less red and yellow than controls, but the presence of sodium ascorbate minimized these detrimental effects on pork color. Fu and others (1994) studied the microbial and quality characteristics of pork cuts from carcasses treated with 1.5% acetic, citric, or lactic acids. Acetic and citric acids were associated with a decrease in total aerobic plate and coliform counts during the first 14 days of storage at 2 to 4°C and all acid-treated samples had lower total aerobic plate counts compared to controls at 42 days storage. Off odors were detected by sensory panelists at day 5 for citric acid, day 7 for acetic acid, and day 14 for lactic acid, and all samples exhibited undesirable off odors by day 35. Surface color of chops changed from red 1 day after cutting to gray-pink 8 to 12 days after cutting. Color, however, as well as other sensory characteristics were not different between treatments. Acetic acid was also found to be the most effective antimicrobial agent on pork loin chops by Lin and Chuang (2001), but the low pH of the chops was associated with an undesirable pale and watery appearance.

Added Substances

Pork longissimus muscle marinated with a combination of citric acid and sodium acid pyrophosphate had lower total aerobic plate counts at 14 and 21 days when stored at 4°C compared to those marinated with either sodium tripolyphosphate or sodium acid pyrophosphate (Cannon and others 1993). The lower pH in pork from the citric acid treatment, however, was associated with lower juiciness, tenderness, and palatability compared with muscle from the higher pH treatments. Another phosphate compound, trisodium phosphate (TSP), has been reported to be an effective antimicrobial agent in beef and poultry, but results have been less consistent in pork. No improvements in mean aerobic plate counts were found when pork carcasses and loins were dipped in, sprayed with, or scalded with TSP solutions in a study by Morris and others (1997). Trained sensory panelists found few significant differences in discoloration, color uniformity, color, texture, or off odor. Lin and Chuang (2001), however, reported TSP-treated pork chops had lower total aerobic and psychrotrophic counts compared to control chops after 6 days of refrigerated storage. The TSP-treated chops were also reported to have an unacceptable dark, dry surface.

The addition of 2 to 3% sodium or potassium lactate has been reported to reduce total aerobic plate counts and extend refrigerated shelf life of pork products by 12 to 14 days (Brewer and others 1991, Lamkey and others 1991, O'Connor and others 1993, Lin and Chuang 2001, Tan and Shelef 2002). The delay in microbial deterioration was associated

with a delay in sour and off-flavor development (Brewer and others 1991). Protection of red colors and enhancement of juiciness and pork flavors has been consistently reported. Protection of fat color (white) during chub storage (Lamkey and others 1991) and fat stability (Tan and Shelef 2002) with the addition of lactates have also been reported. Brewer and others (1991) reported enhanced salty flavors in fresh pork sausage containing sodium lactate, but O'Connor and others (1993) found the salty flavor of sodium was less intense from sodium lactate than from sodium chloride.

Irradiation

Irradiation treatment of pork and other meats as a means of microbial reduction was found safe by the USDA in 1997 (USDA-FSIS 1999). The final rule approving irradiation of meats was issued in 2000. The PR-HACCP rules require irradiation be done in accordance with an established HACCP plan with irradiation being designated as a critical control point. Although not currently used extensively in the United States, the effects of irradiation on microbial loads and sensory properties of pork have been studied.

An absorbed dose of 1.91 kilogray (kGy) or higher was identified by Thayer and others (1993) as necessary to reduce microbial loads in vacuum-packaged fresh pork to undetectable levels. The levels of mesophiles, psychrotrophs, and anaerobes/facultative anaerobes were reduced by a 1-kGy irradiation treatment (Mattison and others 1986, Ehioba and others 1987), but a sublethal effect was noted by Ehioba and others (1987) in vacuum-packaged pork treated with that irradiation level. An irradiation dose of 1.75 kGy reduced microbial loads of 10^6 cells/gram (g) by 1 to 5 logs depending on the microbial species (Grant and Patterson 1991a). Of the pathogens studied, *Clostridium perfringens* was the most resistant and *Yersinia enterocolitica* the least resistant to irradiation. Gram negative organisms, particularly *Pseudomonas* and *Enterobacter*, accounted for 96% of isolates in fresh ground pork initially but only 76% of isolates in nonirradiated vacuum-packaged pork after 9 days at 5°C (Ehioba and others 1988). Microflora in irradiated samples was primarily gram positive immediately after treatment. *Lactobacillus* and coryneforms predominated after 9 and 12 days of storage at 5°C. *Lactobacillus* was also the predominant organism detected in irradiated pork stored under 25 or 50% carbon dioxide (CO_2) by Grant and Patterson (1991b) and Lebepe and others (1990) noted lactobacilli seemed to be the microbial type least affected by irradiation. Thayer and others (1993) detected *Staphylococcus*, *Micrococcus,* and yeasts in fresh pork irradiated with 0.57 kGy.

In contrast to studies with poultry, only limited effects on sensory properties of pork due to irradiation have been reported. Mattison and others (1986), using triangle tests, reported short-term irradiation affects on pork sensory properties 24 hours after treatment, but those effects disappeared after 2 weeks of storage at 4°C. Grant and Patterson (1991b), however, reported a distinct "irradiation odor" in pork chops. Although it did not change in intensity over storage time, the odor was described as more "sour" or "dairy" as storage progressed. Lebepe and others (1990) noted a higher red value in irradiated pork chops stored at 5°C under fluorescent light but inconsistent differences in lightness and yellowness. A trained descriptive panel was able to detect differences in some characteristics of irradiated chilled (bitterness, sour, browned/roasted, pork identity, and sweet flavors) or frozen (browned/roasted flavors, juiciness, and toughness) pork chops, but results for many of the flavor characteristics evaluated were inconsistent (Luchsinger and others 1996). Dose level, irradiation source, and packaging type did not influence fat-like flavor, juiciness, metallic flavor, toughness, or raw and cooked pork aromas in chilled pork chops nor bitterness, fat-like, pork identity, sour or sweet flavors, or raw and cooked pork aromas in frozen chops. No differences in overall acceptance, meatiness, freshness, tenderness, or juiciness were detected by consumer panelists in chilled, vacuum-packaged pork chops irradiated at 2.5 kGy compared to nonirradiated controls (Luchsinger and others 1996).

High-pressure Processing

High-pressure processing has received increased attention in recent years as a food preservation method. The application of high pressure causes volume changes in the food or its constituent parts, resulting in protein denaturation, textural alterations, gelation, greater flavor and color retention, and enzyme modifications (Knorr 1993, Hugas and others 2002). High-pressure processing can also

effectively reduce microbial loads, but the results depend on many factors including amount of pressure applied, type of microorganism, presence of spores, temperature, and water activity.

Although hydrodynamic pressure was associated with a decrease in numbers of *Trichinella spiralis* recovered from pork, infectivity of the larvae was not eliminated by the treatment (Gamble and others 1998). D-values of 2.17 minutes at 414 mega Pascal (MPa) and 25°C for *Listeria monocytogenes* and 1.48 minutes at 414 MPa and 2°C for *Salmonella typhimurium* were reported by Ananth and others (1998). Psychrotrophs reached 5.71 $\log_{10}$ cfu/g after 33 days storage at 4°C in pork loin treated with pressure at 25°C compared to 7.0 $\log_{10}$ cfu/g in untreated loins. Mesophiles exceeded 6.0 $\log_{10}$ cfu/g in both untreated and pressure-treated samples after 3 days when stored aerobically at 25°C and all samples were considered spoiled after 5 days storage at 25°C. Murano and others (1999) found a 10 $\log_{10}$ reduction in a resistant strain of *Listeria monocytogenes* in ground pork patties treated with 414 MPa at 50°C for 6 minutes. The addition of the mild heat to the pressure treatment reduced the D value ranges from 1.89 to 4.17 minutes to 0.37 to 0.63 minutes. Treated samples had a shelf life of 28 days compared to 5 days for control patties when stored at 4°C.

The use of high-pressure processing to reduce microbial loads may also result in changes in pork quality characteristics. Sensory panelists, however, could not distinguish between high-pressure plus heat-treated pork patties and controls after grilling (Murano and others 1999). Ananth and others (1998) also reported no differences in flavor, juiciness, firmness, color, texture, water-holding capacity, and moisture between pressure treated (25°C) and control samples when the pork loins were cooked and evaluated by panelists using descriptive analysis.

Packaging Materials and Environments

Packaging materials and environments can have significant affects on both microbiological and sensory qualities of pork products. Weakley and others (1986) compared the effects of parchment wrapping versus vacuum packaging on quality characteristics of fresh pork loins that had been either hot or conventionally processed. Few differences in processing/packaging treatments were noted during storage at 2°C until day 14 when vacuum-packaged loins exhibited both lower total plate counts and lower off odor scores. Vacuum-packaged pork loins had less surface discoloration than parchment-wrapped loins at days 7 and 14. Higher surface discoloration was also associated with parchment wrapping in a study by Vrana and others (1985). Fresh pork loins were first stored unwrapped, wrapped in parchment, or vacuum packaged. Chops cut from each type of stored loin were then displayed wrapped in either oxygen-permeable or high-oxygen barrier film. Lean color scores differed little, but overall appearance was lower in chops from parchment-wrapped and vacuum-packaged loins compared to unwrapped loins. Chops from unwrapped loins also had less off odors and cooked off odors. The high off odor scores for chops from vacuum-packaged loins were the result of microbial counts of 10^8 cfu/cm^2 or higher at 6 to 10 days of storage. Cooked off odors were described as putrid in unwrapped chops displayed in oxygen-permeable wrap, gaseous hydrogen sulfide in parchment-wrapped/high-oxygen barrier packaged chops, and sour-acidic in vacuum packaged/high-oxygen barrier packaged chops. Microflora of chops displayed in high-oxygen barrier wrap was primarily *Lactobacillus* and *Micrococcus* spp. while *Pseudomonas* spp. predominated in chops displayed in oxygen-permeable wrap. Although microbial loads for chops from unwrapped loins were also 10^7 to 10^8 cfu/cm^2 at 6 to 10 days storage, lower off odor scores were apparently due to a shorter exposure time to high microbial loads.

Modification of the atmosphere within packaged pork products has also been investigated. One of the most common gases used in such modifications is CO_2. Greer and others (1993) detected only lactic acid bacteria in pork loin stored under CO_2 at -1.5°C through 24 weeks, with a maximum of 10^7 cfu/cm^2 at 9 weeks. Lactic acid bacteria and pseudomonads were the primary spoilage organisms detected during retail display of the loins. When a five-member sensory panel evaluated odor of displayed pork on a 7-point hedonic scale and appearance on a 5-point acceptability scale, scores for both characteristics decreased as storage time in CO_2 increased. Linear regression indicated a case-life reduction of about 1 day for each 6 weeks of storage in CO_2. Although lactic acid bacteria dominated the microflora of both vacuum-packaged pork and pork stored under CO_2 at 3°C, spoilage was attributed to a

substantial increase in *Brochothrix thermosphacta* by Gill and Harrison (1989). Color and texture were stable throughout the storage period for all samples. Sour-aromatic odors from *Brochothrix thermosphacta* and acid-dairy odors from lactic acid bacteria were unacceptably strong after 11 days storage in vacuum packaged samples and 39 days in CO_2 stored pork and were associated with microbial levels of 10^8 organisms/cm^2. Similar trends were noted for pork samples stored at −1.5°C although storage life increased to 10 weeks or more.

Scholtz and others (1992a) compared laboratory and commercial bulk packaging systems for pork using a 100% CO_2 atmosphere followed by aerobic retail display. Initial total microbial loads at day 0 of retail display were about 4 $\log_{10}$ cfu/cm^2 for all CO_2 bulk storage times. Counts increased as display time increased, reaching an unacceptable level of greater than 7 $\log_{10}$ cfu/cm^2 after 5 days of retail display. Anaerobic, lactic acid bacteria, and pseudomonad counts followed similar trends. Color of all samples as evaluated by a 10-member trained panel was pale to normal throughout bulk storage and retail display. The panel detected a fresh meat odor in all samples after 14 days storage under CO_2. Odor became increasingly unacceptable, however, as retail display progressed. The microbiological and sensory properties of pork stored under 100% CO_2 were not different from chops stored in PVC, modified atmosphere (25% CO_2/75% O_2), or vacuum packages through 4 days storage in a second study by Scholtz and others (1992b). Interactions between packaging type and color, odor, overall acceptability, and microbial loads were noted, however, during longer storage times. Storage life of the 100% CO_2 chops was superior overall.

The presence of environmental oxygen can have significant effects on both microbiological and quality characteristics of pork. Presence of residual oxygen in the packaging atmosphere has generally been associated with lighter, less red, and more yellow pork compared to oxygen-free atmospheres (Sørheim and others 1997, Lambert and others 1992a). Increased discoloration was also detected by sensory panelists as the oxygen level increased in the storage atmosphere. Although strong off odors were associated with the presence of oxygen immediately after treatment, those odors weakened during storage (Lambert and others 1992a). Microflora after modified atmosphere storage is often lactic acid bacteria, and few differences are generally reported regardless of whether or not oxygen is present (Lambert and others 1992b, Sørheim and others 1997).

Controlled atmospheres using combinations of CO_2, O_2, and N_2 have also been studied. The presence of O_2 has been associated with a greater increase in psychrotrophs compared to environments containing CO_2 or CO_2 and N_2 at storage temperatures of both 2 and 5°C (Spahl and others 1981). *Pseudomonas* accounted for as much as 60% of microbial loads in the samples. Rejection of chops by a sensory panel was associated with objectionable odors before appearance became a determining factor. Although the 100% CO_2 environment resulted in the greatest extension based on sensory results, combinations containing mixtures of CO_2 and N_2 were reported to be best for extending overall shelf life of the pork chops. Holley and others (1994) found pork stored in atmospheres of 100% CO_2 and 50% CO_2/50% N_2 had lower psychrotroph growth at both −1 and 4°C compared to 100% N_2. Storage environment had no effect on L, a, or b values or expressible juice and little effect on tenderness.

CONCLUSION

Reducing the microbial contamination of pork and other meats continues to be a challenge for the food industry. The presence of pathogens on pork can be a significant safety risk, particularly in light of the many reports of improper handling of meat by consumers. Growth of both pathogens and spoilage organisms on meat can also have a significant impact on organoleptic properties of pork and other meats and contribute to food waste. Methods used to reduce microbial loads on meats can also have significant impacts on texture, juiciness, appearance, odor, and other sensory properties. Research into newer technologies such as high-pressure processing may provide improved ways to produce pork products that are microbiologically stable and organoleptically acceptable.

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31
Pork Taint

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INTRODUCTION

Tainting in pork is normally thought of in two senses: the off flavor present when meat from intact males is consumed versus males that have been castrated and the off flavor that normally develops as the larger portions or cuts, such as the hams, are dry-cured improperly. Although both taints are undesirable and vary by production method and geographic location, there are methods by which each can be overcome. This section will attempt to discuss both causes of tainting; however, the major emphasis will be placed on tainting that is found predominantly in intact males from European sources.

BONE TAINT

Perhaps the most important cause of spoilage in dry-cured hams, amounting to losses of almost 5% annually is bone taint (Blanco and others 1997). This phenomenon, which occurs in larger muscle cuts with greater depth thereby slowing the internal chilling process, is the result of anaerobic bacterial growth. These bacteria (*C. perfringens*, Enterobacteriaceae) predominately create an off odor and off flavor deep within the muscle next to the bone that is often referred to as "bone taint" or "bone-sour" (Giolitti and others 1971). The first indication of such a problem is normally noticed as a result of the foul-smelling odor emitted from the muscle mass adjacent to the bone (Garcia and others 2000). Often, the unpleasant odor found in spoiled hams is related to a higher dimethyldisulphide level (Ames and Macleod 1985). These odors possibly originate from the microbial metabolism to higher levels of ketones, alcohols, and esters (Garcia and others 2000).

Although this problem is normally noticed in fully aged or ripened hams, the water activity of the tissue at that time (0.85) suggests that the actual spoilage occurs much earlier in the curing stage of the process (Cordoba and others 1994). As a safeguard against this issue, many country ham processors in the United States probe hams with small metal rods around the center of the deepest part of the ham to try to detect any off odor development. In fact, most official competition judges of intact dry-cured hams will commonly probe the hams because off odor constitutes a large portion of the final product score. Even though this problem is rather small in relation to the entire industry at only 5% occurrence in Europe and at similar or lower levels in the United States (Mikel, Unpublished Industry Data), bone tainting continues to create a substantial economic loss to the dry-cured industry.

BOAR TAINT

"Boar taint" is an issue very seldom noted in the United States due to the mainstream production practices of male castration of swine at an early age. In fact, usually the off odor occurrence is minimal in the U.S. except in the cases where old boars are salvaged once they are past a useful breeding age. However, typically, the meat from these animals is included in some type of highly seasoned further

processed item for the purpose of overshadowing any detectable off odor and flavor. In addition, the use of such meat is in such small quantities that the offending odor and pursuant flavor is diluted.

However, the problem with sex odor from boar carcasses has been well known for many decades according to Lerche (1936) as cited in Weiler and others (2000). European countries on the other hand, often produce their swine without castration methods being employed for many reasons—efficiency, animal welfare, consumer perception, etc. Therefore, quite an effort has been made to determine factors involved in boar tainting and how best to minimize the occurrence and subsequent effects.

Androstenone and skatole were first reported by Paterson (1968) as the two compounds responsible for boar taint in intact males. This taint is most strongly associated with odor from the fat but also has an effect, although to a lesser extent, on the flavor of the product as well (Malmfors and Lundstrom 1983). In addition, Paterson (1968) also reported that the androstenone, a testicular steroid, exhibited a "urine-like" odor. Skatole, on the other hand, exhibits more of a "fecal" or "manure-like" odor according to research by Vold (1970) as cited in Bonneau and others (2000). Dijksterhuis and others (2000) also reported the distinct difference in odors with androstenone having the urine odor and skatole the manure odor with a somewhat lesser naphthalene odor. In their research, they utilized trained panels from seven European countries to evaluate intact male pork in the form of loin chops. Fat samples were analyzed for variations in the content of the two compounds present and assigned to panelists accordingly. Any panelist insensitive to 0.5 parts per million (ppm) of androstenone (presented in a lard sample) was removed from the study. They used the following sensory attributes shown in Table 31.1 to assess taint both in odor and flavor. Use of these qualifiers dramatically enhanced sensory results. They found that all the attributes listed were beneficial in determining either androstenone or skatole content except for "sweat" and "sweet." They also found differences in sensitivity among countries, however, this is to be expected as different cultures have varying acceptance with respect to eating and liking fresh and processed pork from pigs produced under various production schemes.

Walstra and others (1997) reported that although there are no seasonal trends in androstenone and skatole levels, the contents did increase with increased carcass weight and decreased with increasing percentage of lean meat indicating a concentration of the compounds in the fat. Andersson and others (1997) also reported that the level of androstenone increased as sexual maturation advanced in the intact male with age. Bonneau and others (2000) reported that in the same European study, sensory panelists could distinguish three groups; boars with high skatole contents were easily separated from gilts and boars with low skatole contents and an intermediate group of boars with a high androstenone levels. They also determined that skatole was easier to perceive than androstenone and is the major contributor to off odors in boar meat.

Table 31.1. Sensory attributes used to describe boar taint from intact male swine.

	Odor	Flavor
1	pig	pig
2	urine	urine
3	manure/stable	manure/stable
4	naftaline/mothballs	naftaline/mothballs
5	rancid	rancid
6	sweet	sweet
7	sweat	sweat
8	abnormal odor	abnormal flavor

Source: Dijksterhuis and others 2000.

In a consumer study spanning seven European countries, Mathews and others (2000) found a significant difference in acceptability between consumers from different countries. Overall, 22% of the intact male samples were disliked for their flavor and 34% for their odor. In addition, as both skatole and androstenone levels increased in concentration, dissatisfaction levels increased for both odor and flavor. However, skatole concentrations had a much greater impact than androstenone levels on odor with similar effects on flavor scores.

Weiler and others (2000) also compared consumer acceptance as affected by country of origin. Consumers compared the acceptability of intact male loin samples segregated into nine categories based on androstenone (A) and skatole (S) concentrations (low A/low S, low A/medium S, low A/high S, medium A/low S, medium A/medium S, medium A/high S, high A/low S, high A/medium S, high A/high S).

Table 31.2. Distribution of consumers (%) in the different androstenone sensitivity classes (all consumers/country = 100%).

	Highly Sensitive (score 5–7)	Mildly Sensitive (score 4)	Insensitive (score 1–3)
Germany			
Total (n=472)	17.6	14.7	67.7
Men (n=217)	15.6	14.7	69.7
Women (n=255)	19.3	14.7	65.9
Spain			
Total (n=480)	30.9	15.5	53.6
Men (n=230)	23.7	16.6	59.7
Women (n=250)	37.3	14.6	48.1

Odor and Flavor Scores[a] in Cooked Pork Loin of German and Spanish Consumers by Androstenone Sensitivity and Skatole and Androstenone Level[b]

	Androstenone Sensitivity		Skatole Level			Androstenone Level			Interaction[c]		
	High	Mild or Insensitive	Low	Medium	High	Low	Medium	High	RSD	Skat And	And Sensitivity
Germany											
N	405	1,899	796	818	690	1,266	415	623			
Odor	4.47a	3.99b	3.94c	4.19b	4.55a	4.18	4.15	4.35	1.49	NS	NS
Flavor	3.58	3.32	3.36b	3.37b	3.63a	3.29	3.48	3.59	1.32	NS	*
Spain											
N	726	1,637	806	847	710	1,459	353	549			
Odor	4.12	3.84	3.75	3.91	4.28	3.87	4	4.07	1.22	**	**
Flavor	3.57	3.55	3.5	3.54	3.64	3.58	3.48	3.62	1.18	*	NS

[a]Odor and Flavor scores from 1 (Like Very Much) to 7 (Dislike Very Much); [b]Different Letters within Main Effects Indicate Significant Differences (P< 0.05)
[c]**: P<0.01, *: P<0.05, NS: Not Significant

Interaction Between Androstenone Sensitivity and Androstenone Level in Cooked Pork for the Odor Scores of the German and Spanish Consumers

	Androstenone Sensitivity						Significance of The Effect of Sensitivity[c]
	Highly Sensitive			Mildly Sensitive/Insensitive			
Androstenone Level[a]	n	LSM[b]	SE	n	LSM	SE	
Germany							
Low	231	4.36	0.1	1,035	4.00	0.05	*
Medium	63	4.43	0.19	352	3.88	0.08	*
High	111	4.63	0.14	512	4.06	0.07	*
Spain							
Low	455	3.90b	0.06	1,006	3.85	0.04	NS
Medium	97	4.22a	0.12	256	3.78	0.08	**
High	174	4.25a	0.09	375	3.88	0.06	***

[a]Significance of Difference Between Androstenone Levels; [b]LSM within the Same Column and Country with Different Letters are Significantly (P, 0.05) different.
[c] ***: P<0.001, **: P<0.01, *: P<0.05 NS: Not Significant. Source: Weiler and others 2000.

Table 31.3 Intensity of perception of the four substances.

	Intensity of Perception				
Substance	Very Strong	Strong	Intermediate	Weak	No Perception
Skatole	21.3%	36.9%	30.3%	10.7%	0.8%
Indole	1.7%	3.3%	17.4%	50.4%	27.2%
3α-ole	0.0%	1.7%	16.7%	52.5%	29.1%
A-one	4.1%	17.4%	21.5%	33.9%	23.1%

Influence of sex on the sensitivity of perception for the four substances

Substance	Sex	Sensitivity to Perception		% Anosmic
		Very strong to intermediate	Weak to no perception	(No perception)
Skatole	Males	88.4%	11.6%	0.0%
	Females	88.6%	11.4%	1.3%
Indole	Males	23.3%	76.7%	33.3%
	Females	22.8%	77.2%	24.1%
3α-ole	Males	18.6%	81.4%	32.6%
	Females	20.3%	79.7%	27.3%
A-one	Males	37.2%	62.8%	30.2%
	Females	46.9%	53.1%	19.2%

Influence of sensitivity of perception on pleasantness of the different scents (participants with "no perception" for a substance are excluded for the evaluation of that particular substance)

		Ranking		
Substance	Intensity of Perception	Pleasant	Neutral	Unpleasant
Skatole	Weak Perception	7.1%	35.7%	57.2%
	Very Strong to Intermediate Perception	0.9%	0.9%	98.2%
	All (except No Perception)	1.6%	4.9%	93.5%
Indole	Weak Perception	22.2%	65.1%	12.7%
	Very Strong to Intermediate Perception	11.1%	51.9%	37.0%
	All (except No Perception)	18.9%	61.1%	20.0%
3α-ole	Weak Perception	33.3%	48.5%	18.2%
	Very Strong to Intermediate Perception	34.8%	21.7%	43.5%
	All (except No Perception)	33.7%	41.5%	24.8%
A-one	Weak Perception	17.1%	63.4%	19.5%
	Very Strong to Intermediate Perception	0.0%	3.7%	96.3%
	All (except No Perception)	7.4%	29.5%	63.1%

Sensitivity of perception for androstenone in the test panel of the consumer survey

Sex (n)	Sensitive (very strong to intermediate)	Insensitive (weak to no perception)
Males (223)	29.60%	70.40%
Females (257)	33.90%	66.10%

Source: Weiler and others 2000.

Table 31.4.

Weighted Least Square (WLS) means[1] for the various attributes of ham

Attribute	Female	Low	High	Attribute	Female	Low	High
Overall Odor	6.7a	6.6a	6.9a	**Boar Odor**	1.6ab	1.5a	2.0b
Overall Flavor	7.1ab	7.1a	7.6b	**Boar Flavor**	1.8a	1.4a	2.9b

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.

Weighted Least Square (WLS) Means[1] for the Various Attributes of Bacon

Attribute	Female	Low	High	Attribute	Female	Low	High
Overall Odor	6.6a	6.4a	8.8b	**Boar Odor**	1.4a	1.5a	5.8b
Overall Flavor	7.8a	7.8a	9.3b	**Boar Flavor**	1.1a	1.4a	3.8b

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.

Weighted Least Square (WLS) Means[1] for the Various Attributes of Salami

Attribute	Female	Low	High	Attribute	Female	Low	High
Overall Odor	7.6a	7.5a	7.7a	**Boar Odor**	1.5b	0.8a	1.5b
Overall Flavor	7.3a	7.6a	7.3a	**Boar Flavor**	1.8b	1.1a	2.5c

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.

Weighted Least Square (WLS) Means[1] for the Various Attributes of Dry, Oven-Roasted Pork

Attribute	Female	Low	High	Attribute	Female	Low	High
Overall Odor	6.1a	6.2a	7.9b	**Boar Odor**	2.5a	3.0a	5.6b
Overall Flavor	6.4a	6.3a	7.9b	**Boar Flavor**	2.4a	2.7a	5.0b

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.

Weighted Least Square (WLS) Means[1] for the Various Attributes of Stewed, Oven Cooked Pork

Attribute	Female	Low	High	Attribute	Female	Low	High
Overall Odor	7.8a	8.1a	9.7b	**Boar Odor**	4.9a	5.4a	8.6b
Overall Flavor	6.5a	6.8a	8.0b	**Boar Flavor**	2.7a	3.6b	5.9c

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.

Weighted Least Square (WLS) Means[1] for the Various Attributes of Marinated, Oven Cooked Pork

Attributes	Female	Low	High	Attribute	Female	Low	High
Overall Odor	8.0a	7.7a	9.0b	**Boar Odor**	1.0a	1.1a	3.6b
Overall Flavor	7.2 a	7.1a	7.7b	**Boar Flavor**	0.9a	1.0a	3.2b

[1]Means on the same line not followed by a common letter are significantly different at the 5% level.
Source: McCauley and others 1997.

Eighteen percent of German and 31% of Spanish consumers were highly sensitive to androstenone with a larger portion being women, 19 versus 16% in Germany and 37 versus 24% in Spain (Table 31.2). For highly sensitive participants, androstenone was the major discriminant, while skatole had a greater affect in insensitive and mildly sensitive consumers (Table 31.3). This agreed with Griffith and Patterson (1970), who stated that some consumers were not capable of perceiving androstenone even at high concentrations although their ability to smell other substances is normally developed.

Table 31.5.

Scores for acceptability of pork according to Skatole/Androstenone classes (Mean ± SD).

	Classes			
	Gilts	LS/LA	LS/HA	HS/HA
Oven Procedure				
F=15.57 P<0.001	3.1 ± 1.2a	2.7 ± 1.5b	2.5 ± 1.5b	1.9 ± 1.5c
Hot Plate Procedure				
F=3.49 P=0.016	3.6 ± 1.2a	3.5 ± 1.3ab	3.3 ± 1.3ab	3.1 ± 1.3b

Scores for Cooking Odor Attributes in the French Sensory Study, According to Skatole/Androstenone Classes (mean ± SD)

			Classes			
Attributes	F	Probability Level	Gilts	LS/LA	LS/HA	HS/HA
Oven Procedure						
Pig	13.92	<0.001	3.5 ± 2.2 a	3.7 ± 2.1 a	3.2 ± 2.1 a	1.7 ± 1.6 b
Urine	11.96	<0.001	2.7 ± 2.5 a	2.2 ± 2.4 a	3.8 ± 2.7 b	4.7 ± 3.0 c
Manure	19.35	<0.001	2.7 ± 2.9 ab	2.3 ± 2.0 a	3.5 ± 2.2 b	5.5 ± 2.4 c
Naphthalene	10.14	<0.001	1.8 ± 2.2 a	1.5 ± 2.1 a	2.1 ± 2.5 a	3.3 ± 2.7 b
Rancid	1.42	0.24	1.0 ± 1.8	0.8 ± 1.7	1.1 ± 1.0	1.3 ± 2.3
Sweet	6.27	<0.001	2.2 ± 2.0 ab	2.7 ± 2.1 a	1.7 ± 1.9 bc	1.3 ± 1.8 c
Sweat	12.62	<0.001	2.0 ± 2.2 a	1.9 ± 1.9 a	2.7 ± 2.0 a	3.9 ± 2.5 b
Abnormal	15.15	<0.001	4.7 ± 2.9 ab	4.3 ± 2.7 a	5.5 ± 2.2 b	7.1 ± 2.2 c
Hot Plate Procedure						
Pig	4.77	0.003	3.1 ± 2.1 ab	3.1 ± 1.9 ab	3.7 ± 2.1 a	2.4 ± 1.8 b
Urine	5.37	0.002	3.0 ± 2.5 ab	3.0 ± 2.7 ab	2.2 ± 2.0 a	4.1 ± 2.5 b
Manure	7.59	<0.001	3.1 ± 2.9 a	2.9 ± 2.9 a	2.2 ± 2.2 a	4.2 ± 2.9 b
Naphthalene	2.52	0.06	1.1 ± 1.8	1.0 ± 1.6	0.6 ± 1.1	1.3 ± 1.7
Rancid	0.56	0.643	0.7 ± 1.3	0.6 ± 1.1	0.5 ± 1.0	0.5 ± 0.8
Sweet	1.40	0.245	1.8 ± 1.9	1.7 ± 1.9	1.7 ± 1.9	1.2 ± 1.5
Sweat	5.19	0.002	2.3 ± 1.8 a	2.3 ± 1.9 a	1.9 ± 1.7 a	3.3 ± 2.4 b
Abnormal	9.34	<0.001	5.1 ± 2.7 b	4.8 ± 2.4 b	3.8 ± 2.4 a	6.2 ± 2.2 c

Source: Siret and others 1997.

In a study by de Kock and others (2001), significant differences in sensitivity were noted among consumers from different ethnic groups in South Africa (black, white, and colored). White females were more negative toward boar odor than white males and blacks with a higher percentage of coloreds responding negatively to boar odors. In addition, black males were more critical than black females toward samples with detectable skatole. However, they stated that it was possible that the ethnic differences in this study could be compounded by age, socioeconomic status, and other culture-linked differences.

Cookery also has been shown to have an impact on the acceptability of products from intact males (Agerhem and Tornberg 1995). Skatole contribution to boar odor is greatest when products are cooked to an internal temperature of 68°C while androstenone contributes most to off odor when cooked to 80°C. Since the temperature levels fall into the range where most pork is cooked for sensory analysis, it is to be expected that both compounds would have the opportunity to contribute to off odors. McCauley and others (1997) reported that although cookery and/or serving temperatures may influence intact male pork acceptability, the method of cookery nor

Table 31.6. Androstenone and Skatole fat content and boar taint scores for samples used in sensory evaluation (mean ± SD).

		Backfat		Processed Meat			
				Cooked		Dry Cured	
	N	Androstenone	Skatole	Odor	Flavor	Odor	Flavor
Castrates	24	0.256 ± 0.097	0.029 ± 0.018	1.47 ± 0.36 a	1.15 ± 0.21 a	2.41 ± 0.63 a	1.80 ± 0.52 a
Entires	24						
LL	4	0.328 ± 0.129	0.024 ± 0.016	2.23 ± 0.21 a	1.83 ± 0.23 ab	3.01 ± 0.20 ab	2.20 ± 0.37 ab
LM	4	0.251 ± 0.069	0.123 ± 0.054	3.27 ± 0.23 b	1.27 ± 0.23 ab	3.75 ± 0.30 ab	2.04 ± 0.37 ab
ML	4	0.694 ± 0.116	0.033 ± 0.018	3.47 ± 0.12 b	2.53 ±0.12 cd	2.79 ± 0.57 ab	2.46 ± 0.43 ab
MM	4	0.569 ± 0.072	0.106 ± 0.029	3.66 ± 0.33 b	2.06 ± 0.24 bc	3.25 ± 0.20 ab	2.47 ± 0.27 ab
HL	4	1.551 ± 0.562	0.043 ± 0.027	4.60 ± 0.40 c	3.53 ± 0.90 d	3.40 ± 0.30 ab	2.48 ± 0.03 ab
HM	4	1.987 ± 0.614	0.131 ±0.055	4.93 ± 0.04 c	3.53 ± 0.50 d	4.26 ± 0.09 b	3.84 ± 0.04 b

Fat Samples for Entire Males: H = High; M = Medium; L = Low Levels of AN/SK. Means with Different Letters are Significantly Different at $P \leq 0.05$. AN/SK Content expressed in $\mu g/g^{-1}$. Scoring scale: 1 = minimum; 5 = maximum.

Source: Banon and others 2003

Table 31.7. Trained sensory panel scores for Loin Chops and Sausages from entire male and female pigs (24 Point Scale, 1 = very low intensity, 24 = very high intensity for attribute).

	Entire Male	Female	Significance	LSD
Lean				
Juiciness	15.6	15.7	ns	0.82
Tenderness	14	14	ns	0.82
Pork Flavor	13.8	13.7	ns	0.60
Abnormal Flavor	4.9	4.4	ns	0.62
Fat				
Pork Flavor	11.3	11.4	ns	0.56
Boar Flavor	2.8	2.4	*	0.37
Abnormal Flavor	4	4	ns	0.60
Pork Odor	10.7	11.3	*	0.60
Boar Odor	4.2	2.8	***	0.68
Abnormal Odor	4.3	4	ns	0.62
Overall Acceptability	13.7	13.9	ns	0.76
Tenderness	18.3	18.6	ns	0.40
Texture (Particle Size)	12.8	12.5	ns	0.54
Firmness	13	13	ns	0.64
Juiciness	12.5	12.8	ns	0.46
Greasiness	8.9	9	ns	0.42
Breadiness	11.9	11.8	ns	0.52
Springiness	12.2	11.8	ns	0.60
Graininess	10.5	10.2	ns	0.42
Meatiness	15.9	16	ns	0.42
Pork Flavor	15.1	15.4	ns	0.45
Sweetness	8.6	8.5	ns	0.46
Spiciness	10.7	10.7	ns	0.54
Saltiness	10.8	11.3	*	0.50
Herbiness	6	5.8	ns	0.39
Off Flavor	3.2	2.7	ns	0.46
Overall Acceptability	15.7	16.2	*	0.46

Source: Mathews and others 1997.

methods of processing except in the case of salami and hams, decreased perception of boar taint by trained professionals (Table 31.4).

Siret and others (1997) also evaluated cookery methods' affects on boar odor from intact male products. They prepared products at commercial stabilization temperatures (250°C for 10 minutes) and by pan frying for 6 minutes. All products were served to consumers or trained French and German panelists. Table 31.5 indicates the results of the consumer test with the pan frying method being the less discriminating method. Although products with high levels of boar taint were equally rejected by panelists regardless of cookery method, products with lower concentrations of either steroid were less detectable with the pan frying method. In addition, they reported that skatole levels contribute a greater amount to the off odor than androstenone compounds. In a study comparing the effects of boar taint in cooked versus dry-cured products, Banon

Table 31.8. Mean consumer sensory scores[a] and standard errors (SE) for meat samples evaluated in different weeks[b].

Sensory Attribute	Week 1		Week 2		Week 3		Week 4		P-Value
	Mean	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**	
Cooking Aroma (n=240)[c]	2.61	0.16	2.98	0.2	2.93	0.21	2.67	0.16	0.301
Flavor (n=664)	2.16[b]	0.07	2.48[a]	0.1	2.53[a]	0.11	2.41[ab]	0.09	0.014
Texture (n=664)	2.46[b]	0.08	2.70[ab]	0.1	2.89[a]	0.12	2.82[a]	0.13	0.001
Overall (n=663)	2.25[b]	0.07	2.58[a]	0.1	2.73[a]	0.12	2.68[a]	0.11	0.001

[a]Sensory scale: 1 = Like Strongly, 2 = Like Moderately, 3 = Like Slightly, 4 = Dislike Slightly, 5 = Dislike Moderately, 6 = Dislike Strongly

[b]Means with Different Letters Within a Row Differ ($P < 0.05$)

[c]Total Number of Evaluations

Source: Babol and others 2002.

and others (2003) found that although there were complex and intricate interactions among the various sensory parameters measured during boar taint analysis, that method of product preparation did not exclude boar taint occurrence (Table 31.6). Therefore, although processing methods such as fry curing alters perception of various traits, the detection of the phenomenon still occurs. Mathews and others (1997) however found no differences in eating quality of British-style sausages prepared from intact males versus females although differences readily existed in chops prepared from the same animals (Table 31.7).

Babol and others (2002) reported that in a study of Canadian consumers, the meat from intact males with low levels of androstenone and skatole was as acceptable as that from gilts initially and over time (Table 31.8). They suggested that products with a controlled level of boar taint could be readily utilized in the marketplace and that skatole analysis would be beneficial in avoiding products with excess levels. However, they cautioned that consumers might become more sensitive to boar taint over time.

CONCLUSIONS

In summary, although pork taint can be the result of improper dry curing or usage of meat from intact males, the cause in most countries outside the United States is due to the swine production methods that exclude the use of castration for various reasons. The meat from intact males is detectable by a large portion of the population regardless of cookery or processing method and even country of origin. Although both androstenone and skatole both contribute to the off odor and off flavors, it appears that more people tend to object to lower levels of skatole present in the product.

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32
Shelf Life of Fresh and Frozen Pork

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INTRODUCTION

An integrated model for consumer loyalty can be based on three components according to James Salter of Total Research Corporation (Salter 1998). One component is price, another is image, and the third is quality. Although quality encompasses many factors, this chapter will address three aspects of fresh and frozen pork shelf life quality: color, flavor, and aroma. Within each area of quality, additional characteristics, including Pale, Soft, and Exudative (PSE) or Dark, Firm, and Dry (DFD) pork, pH, L*, a*, and b* values, retail display, and characteristic and undesirable flavors and aromas, will be addressed as will technologies to improve shelf life.

COLOR

pH Decline

When an animal is harvested for meat production, metabolism soon switches from the aerobic pathway (glycolysis) used when oxygen was available, to the anaerobic pathway after death. The lack of oxygen transport from the lungs to the muscle by hemoglobin in blood reduces the amount of oxygen then transferred to the myoglobin pigment in muscle. Although a greater quantity of adenosine-5′-triphosphate (ATP) is produced through the aerobic pathway, some ATP is generated after death through the anaerobic pathway. However, the anaerobic pathway is also associated with the buildup of lactic acid. Without a functioning circulatory system, the lactic acid cannot be transported to the liver for conversion to glucose and glycogen for an energy source nor can it be taken to the heart to be converted to carbon dioxide and water. Once glycogen stores are used up and metabolic enzymes become inactive, lactic acid concentration increases and muscle pH drops (Aberle and others 2001).

At the point of death, the pH of muscle is near 7.0. In the minutes and hours postmortem, the pH decline in pork often follows one of three curves. Normal pH decline in pork resembles a curve with a gradual decrease in pH to 5.6 to 5.7 within 6 to 8 hours until the ultimate pH is reached near 24 hours postmortem. The meat produced will be reddish-pink in color. If little glycogen is available before death, an inadequate amount of lactic acid will be produced through metabolism thus pH decline will

be minimal (ultimate pH of 6.5 to 6.8). The resulting meat color will be darker than that produced by the normal pH decline. In some cases, more glycogen will be converted prior to death, resulting in more lactic acid production. This can lower the postmortem pH outside of the normal range within the first hour postmortem to near 5.4 or 5.5, and will produce an ultimate pH near 5.3. This condition will result in meat that is paler in color compared to meat produced from a normal pH decline.

Pale, Soft, and Exudative and Dark, Firm, and Dry Pork

Pale, Soft, and Exudative (PSE) pork is a muscle condition that has been extremely costly to the pork industry (Meeker and Sonka 1994). PSE can occur as a result of an inherited condition known as Porcine Stress Syndrome (PSS) or promoted by external stress factors. Although neither abnormal postmortem pH decline nor high carcass temperatures alone will guarantee the condition, both can adversely affect the quality of pork muscle. Extensive use of the aerobic pathway prior to death will provide a buildup of lactic acid soon after death and before the body heat can be removed from the carcass by chilling. This promotes muscle protein denaturation. As the pH of muscle drops, it approaches the critical pH known as the isoelectric point (pI). The pI of muscle (near 5.1 to 5.2) is a pH where the numbers of positive and negative reactive groups on the protein chains are equal. As muscle pH approaches the pI, fewer reactive groups are available, and the ability of the muscle to bind water diminishes. The resulting muscle is pale from the loss of color pigmentation, exudative from the loss of water and protein-binding capabilities, and soft in texture from the loss of protein stability (Aberle and others 2001).

Dark, Firm, and Dry (DFD) pork is also costly to the pork industry but results when an inadequate amount of glycogen/glucose is converted to lactic acid. In this case, the pH lowers only slightly and the ultimate pH is reached quickly. As the ultimate pH (6.5 to 6.8) is far from the pI (5.1 to 5.2), this muscle will bind water very well and will hold its pigmentation resulting in a dark color. The higher pH also supports or enhances microbial growth.

According to the Pork Quality Standards (NPPC 1999), pork muscle color, texture, and exudation range from pale pinkish gray, very soft, and exudative (PSE), to reddish pink, firm, and nonexudative (RFN), to dark purplish red, very firm, and dry (DFD). Fox and others (1980) found the general appearance and raw color scores decreased at a more rapid rate with storage time in PSE loin chops versus chops of normal color, thus resulting in a shorter shelf life for PSE loin chops. Topel and others (1976) also reported the few PSE chops selected over those of normal color were chosen in the first hours of display. Once cooked, most pork will resemble the marketing slogan that promotes pork as a white meat, however, the light surface color of PSE fresh muscle is commonly associated with a dry cooked whole muscle product, and may lead to an undesirable eating experience resulting in a failure to purchase pork in the future (Norman and others 2003). Although this information is widely accepted, an in-home survey by Norman and others (2003) found 20.8% of consumers surveyed still chose pale-colored pork, 26.4% chose normal reddish-pink colored pork, and 52.8% chose dark-colored pork. Topel and others (1976) found the general appearance of pork influenced consumer choices, and they preferred normal, pinkish-red lean rather than PSE or DFD meat. Buege (2001) also reported normal, RFN pork is the most desirable not only for its ability to hold water and provide juiciness once cooked, but also because color relates to appearance and these combine together to serve as an important factor in consumers' meat purchasing decisions. Brewer and McKeith (1999) found when consumers were asked to evaluate pork loin slices in a retail setting, the pinkest PSE samples and all DFD samples were ranked highest by consumers in the intent to purchase category, which generally related to overall acceptability. However, Greer and Murray (1991) reported frozen, then thawed loin chops produced a higher pH and darker color than nonfrozen, which reduced case life related to appearance as compared to fresh pork.

L*, a*, and b*

The actual color of pork can be measured by visual or spectrophotometric means. Visual appraisals may use the color standards set by the Pork Quality Standards (NPPC 1999), which range from 1.0 as pale pinkish gray to white, to 6.0 as dark purplish red, with a target of 3.0 to 5.0 (NPPC 1998). However, an objective

measure of color can be obtained using a colorimeter or spectrophotometer that uses a scale for lightness (L* value) ranging from 0 as black to 100 as white, a scale for redness (a* value) with green producing more negative values and red producing more positive values, and a measure of blueness (b* value) with blue producing more negative values and yellow more positive values. The color standards now have corresponding L* values where a 1.0 score (pale pinkish gray to white) is equivalent to an L* value of 61, an L* value of 31 is equivalent to 6.0 (dark purplish red), and normal RFN is equivalent to an L* value of 43 to 49 (NPPC 1999). Brewer and McKeith (1999) found similar results for pale pork (L* = 57.0), dark pork (L* = 38.0), and normal pork (L* = 51.5). Zhu and Brewer (1998) evaluated PSE, normal, and DFD pork over 7 days at 4°C and found PSE pork, although lighter in color initially (higher L*), did not change over time and visual redness inversely correlated with L* values. Normal and DFD pork produced increases in a* values (more red), but the same was not noted for PSE pork.

Retail Case Life of Pork

According to Kropf (1998), the key to retail pork display is to present products in an attractive and salable form. This includes a fresh appearance and acceptable quality without abnormal traits such as unattractive color, purge, or dehydration. Alterations in production practices have improved pork quality, including color, but retail outlets continue to have concerns about the quality of the product that reaches their stores (NPPC 1994). Topping their list of concerns has been excessive color variation and a short shelf life. Carr and Miller (1998) surveyed multiple retail stores across the United States to define the color that develops when pork becomes unacceptable, average shelf life, styles of retail cases in use, types of lighting, and other information related to the retail display and shelf life of pork. In the 30 stores surveyed, they concluded that the majority of the refrigerated retail cases were triple level (50%), followed by single level (coffin style; 36.7%), and finally quad level (12.5%). The same study reported the most popular description of unacceptable pork packages in the retail case as described by meat market managers was poor color (63.3% of responses). The poor color was most often gray (50%), grayish green (36.7%), or light green to brown (10%), but the color was seldom too dark (3.3%). The reason market managers removed pork products from display was reported as poor color (43.3% of responses), followed closely by poor color and past sell-by date (36.7%). This information corresponded to an expected shelf life for fresh or frozen and thawed pork products as 3.91 days in a single-level display case, 3.61 days in a triple-level case, and 3.71 days in a quad-level case.

Lighting is a necessary component of retail display, but care should be taken when selecting lighting to reduce the speed of product discoloration and to ensure an honest projection of the product in order to produce repeat customer purchases. Although the majority of research on discoloration of meat products has been on beef, lighting does affect the color changes in all fresh and frozen meats. Kropf (1998) states that one of the most influential components of lighting is the type used, which might include incandescent, fluorescent, or metal halide (mercury vapor or high-intensity sodium). Kropf (1998) also stated meat surface temperature can increase if intense lighting is used in the display. Fluorescent light is known to have surges of energy at specific wavelengths and if they correspond to wavelengths where light is strongly absorbed by myoglobin, undesirable colors can develop. Ultraviolet wavelengths caused discoloration in studies by Ramsbottom and others (1951) and Bertelsen and Skibsted (1987); therefore, it is suggested that these wavelengths be filtered by packaging film and/or retailers should use lamps designed to reduce ultraviolet emission. Emission of other wavelengths of light can also promote undesirable pork color in the retail setting. Lamps that emit low amounts of light in the red area of the light spectrum (or correspond to more light in the blue area such as a colder, bluer light emitting at 4,200°Kelvin [K]) compared to a warm light with a high proportion of red (such as one emitting at 2,600°K), will result in the undesirable appearance of blue, less red surface (Kropf 1998). The reverse will falsely intensify the appearance of red in the cut surface. Barbut (2001) reported a more red appearance to beef under incandescent lamps than fluorescent at 70 foot-candles and similar results for pork chops and chicken, but to a lesser degree because of the reduced myoglobin content. However, other research has indicated quicker discoloration with these lights related to

their tendency to increase the temperature of the products on display. Kropf (1998) estimated a 1°F rise in temperature for every 10 foot-candles of incandescent lighting in a display case with 70 cubic feet per minute air velocity. This increase in surface temperature would not only increase deterioration in color by oxidation of pigments, but could increase microbial growth. Deluxe fluorescent lights, as well as other types, can be substituted as they release about one-fifth the heat as incandescent lights for equivalent lighting. Calkins and others (1986) reported pork loin chops under deluxe cool white fluorescent lights received the most desirable visual ratings when compared to cool flood incandescent lights, cool white Surlyn-coated fluorescent, and warm white fluorescent lights. The incandescent light in that study also raised the surface temperature of the loin chops significantly and reduced the percentage of oxymyoglobin from 63% to 40% with 12 hours of light exposure each day for 5 days compared to a reduction of oxymyoglobin from 73% to 63% with 12 hours of light exposure with the other light sources. Kropf (1998) recommends using lights with a color temperature of 2,900 to 3,750°K and an intensity of 75 to 150 foot-candles, while weighing the advantages of brighter lights against the more rapid deterioration of color and shorter shelf life that will result with intense lighting. Carr and Miller (1998) found in a national survey that low wattage bulbs were the most popular in (for multilevel) or above (for single level) display cases.

If surface temperature increases are likely, the cooler the products are maintained (below 0°C or 32°F), the longer their shelf life will be while on display. Miller and Carr (2000) evaluated the development of undesirable color changes in pork displayed at various temperatures in different case types. Retail display cases were set so that the airflow temperature around the pork loin chops was at −1.1°, 1.1°, or 3.3°C (30°, 34°, or 38°F). The highest temperature setting was discontinued 3 days into the "warm-up" period before data collection could begin because a significant increase in product deterioration related to poor color developed. These authors also found single-level cases set so airflow temperature was at −1.1°C produced the least amount of product temperature gain in the hour before, throughout, and in the hour after their defrost cycle. However, if the display cases were multilevel, a setting of 1.1°C produced the least product temperature gain in the same time frame.

FLAVOR AND AROMA

Characteristics of Pork

The complexity of meat flavor is based on the thought that flavor includes taste, texture, temperature, pH, and most importantly, aroma or odor (Lawrie 1998). However, both aroma and flavor are difficult to measure by objective means. Gases produced from foods that relate to aroma may be quantified by gas chromatography, but some compounds isolated by this objective method may not be detected by most consumers. Therefore, a trained panel is often used to assess meat product aroma. Although the response to aroma is close to 10,000 times more sensitive than for taste, the role of taste, and therefore flavor, is also important to meat product satisfaction for consumers. Most will agree that the most desirable flavor and aroma for meat occurs upon cooking, as raw meat presents little odor and a serum- or blood-like taste (Ramarathnam and others 1991). It has been known for decades that when heat is applied to inosine and amino acids from glycoprotein, a "meaty" aroma will result as water soluble forms of each can be derived from muscle (Batzer and others 1962).

Undesirable Flavor and Aroma

Pork is reported to contain 50 percent or more unsaturated fatty acids. As a nonruminant, more dietary fatty acids are deposited in the body because rumen bacteria are not present to metabolize dietary fats into saturated fats. Thus, with a larger percentage of unsaturated fatty acids found in pork, the resulting meat products are more susceptible to oxidation, or oxygen attacks on the double bonds, than fat from beef or lamb, which contain less than 50% unsaturated fatty acids (Romans and others 2001). Feeding unsaturated fatty acids can increase the amount of unsaturated fatty acids in pork fat thus increasing the possibility of oxidation and off flavors (Larick and others 1992). Phospholipids also contribute to flavor and aroma, but also contribute significantly to the development of off flavors including "warmed-over flavor" (WOF; Tims and Watts 1958), which is attributed to the oxidation of unsaturated meat lipids and can be associated with reheated meats that have been refrigerated for 48 hours or less, or with precooked meats that have been frozen for as little as a few weeks (Brewer 1998). Once oxidized, the fats

often break into smaller subunits that are significant contributors to flavor of uncured meat (Ramarathnam and others 1991). These subunits, known as pentanal, hexanal, and 2-4-decadienal molecules, are responsible for the off flavor and odor. Cross and Ziegler (1965) reported considerable *n*-pentanal and *n*-hexanal concentrations in uncured hams, but barely detectable levels in cured hams. Ramarathnam and others (1991) concurred and reported hexanal concentrations in uncured pork 12 times higher than in cured pork. The compound was noticed to significantly increase over storage periods up to 8 days, particularly at 5°C compared to 1°C in research by Robson and others (1989). The flavor and closely related odors produced from these compounds are often characterized as "stale," "cardboard-like," "painty," or "rancid" (Love 1988, Vega and Brewer 1994). When the products are reheated, these compounds, stored in the fat, become volatile as the fat melts, thus heat (cooking) is the most influential cause of WOF. Other items can promote oxidation, including light. As previously mentioned, certain elements of light, such as wavelength, can alter the pigments in meat encouraging interaction with oxygen and producing darker surface color in return. The higher the energy of light (more ultraviolet), the more oxidation will be promoted and will result in a reduced shelf life for precooked, frozen meat items (Brewer 1998). Although other factors can promote oxidation and WOF, exposure to oxygen itself is a considerable contributor to the problem. As whole muscle cuts are further processed by grinding, chopping, mixing, tumbling, or mechanical deboning, oxygen is extensively incorporated into the product, thus is readily available to promote oxidation of fats (Kanner 1994).

TECHNOLOGIES TO IMPROVE PORK SHELF LIFE

Chilling and Other Temperature Influences

Muscle color is the main factor influencing consumer acceptance when purchasing meat, and thus must be maintained during storage, distribution, and retail display (Jeremiah 1982, Jeremiah and Gibson 1997). Although color stability in the retail display case is the most important factor to consumers and retail managers, processing techniques related to temperature during processing and storage can influence color stability later when pork is displayed. Investigative research into chilling effects on pork carcasses has brought mixed results related to muscle color when fabricated into wholesale and retail cuts and during retail display. Weakley and others (1986) reported lighter muscle color scores when conventional chilling (2°C) was used compared to rapid chilling but no benefit to shelf life by the latter. However, Milligan and others (1998) and Borchert and Briskey (1964) indicated accelerated chilling quickly reduced temperatures and allowed for improvements in aspects that reduced the incidence of PSE meat. Crenwelge and others (1984) also reported improvements in visual color scores were observed. Springer and others (2003) followed by reporting darker loins (lower L* values) with lower b* values (less yellow) and a lower incidence of PSE meat when accelerated chilling was used. When storage temperature was evaluated, Lee and others (2002) reported colder temperatures (−20°C compared to −10 or −15°C) promoted increased high quality life and practical quality life scores for precooked frozen pork patties. Similar results were reported by Jeremiah and Gibson (1997) when pork loin roasts retained their color stability (less surface discoloration) best when storage temperatures before retail display were at −1.5°C compared to 2 or 5°C.

Packaging

The modification of the atmosphere surrounding meat in retail display has been tested in multiple studies. Vacuum packaging can successfully reduce the amount of oxygen in contact with the meat's surface, considerably slowing the oxidation of the muscle pigment myoglobin that results in the development of a brown appearance. However, the deoxygenated myoglobin pigment (more purple in appearance) is also not as well accepted by consumers for retail display of pork cuts as the oxygenated counterpart, oxymyoglobin. Vacuum packaging systems can also promote the growth of anaerobic microbes over storage time that are lipolytic and proteolytic (Igbinedion and others 1983) or lactic acid producers (Asensio and others 1988). These lactic acid-producing microorganisms can negatively alter meat odor under vacuum packaging.

Modifications in packaging film have shown success in extending shelf life of red meats including pork. Vrana and others (1985) found pork chops displayed in high oxygen-barrier film (vacuum package) had less off odor when displayed 3 or 4 days, but not longer because odor development limited extended shelf life even though the chops were visually acceptable for 10 days. Lee and others (2003) evaluated packaging with microperforations, allowing water vapor to escape, to packaging without perforations for 14 days. At 7- and 14-day display times, the microperforated packages displayed less discoloration, portrayed a more desirable outer appearance, and were rated higher in overall acceptability at 4, 7, and 14 days by a 10-member panel.

Modifications of gases around pork surfaces have also been investigated as a means of extending shelf life. Scholtz and others (1992) modified the gas environment in packages of pork loin chops with a combination of carbon dioxide and oxygen (25% CO_2 and 75% O_2) and compared the results to 100% CO_2 or vacuum packaging. Although no differences were noticed through 4-day display, the combination modified environment extended the shelf life to 7 days as did vacuum packaging related to microbial quality, but more acceptable color evaluations were received for those chops in the combination environment package. Similar odor evaluations were found for both packaging treatments up to 7 days when moderate off odors became noticeable, but the authors reported acceptable evaluations of chops in the combination environment package up to 14 days and those in 100% CO_2 up to 21 days. Jeremiah and Gibson (1997) also reported a 100% CO_2 environment portrayed an advantage over 70% O_2/30% CO_2 in color stability for pork loin samples. Sorheim and others (1997) found similar results when, after 21-day storage, both color and discoloration scores were most favorable for pork loin sections stored with 0% O_2, followed by 0.5% and lastly by 1.0%, regardless of whether the pork was normal or PSE originally. Holley and others (1994a) reported pork loin roasts and slices stored under 50% CO_2 for 2 weeks could then be aerobically displayed for 3 days acceptably, or for 6 days if stored under 100% CO_2 for 14 days prior to display. Holley and others (1994b) indicated pork loin slices displayed at 4°C in 100% CO_2 or 50% nitrogen (N_2)/50% CO_2 could be held in retail display 7 days longer than if packaged in 100% N_2.

Vitamin E

As the oxidation of fats promotes off flavors in fresh, cooked and reheated, and frozen pork, compounds that prevent oxidation are valuable to pork products. These compounds, when fed to the pigs or added to pork items during processing, will oxidize first, delaying the oxidation of unsaturated fats in pork cell membranes (Brewer 1998). Although many natural and synthetic compounds are used for pork products, the role of vitamin E offers several advantages. Vitamin E (alpha-tocopherol) has consistently been shown to reduce lipid oxidation as measured by 2-thiobarbituric acid reactive substance (TBARS) when pigs are fed high levels of vitamin E. Monahan and others (1990) discovered pigs fed 200 milligrams per kilogram (mg/kg) produced pork with lower TBARS (less lipid oxidation) after 8 days of storage at 4°C. Cannon and others (1995) also indicated lower lipid oxidation and lower off flavor intensity scores (indicative of less WOF) for loin chops and semimembranosus/adductor roasts from pigs supplemented with 100 mg vitamin E/kg feed for 84 days prior to harvest. Dirinck and others (1996) reported fresher taste and slower lipid oxidation for loin chops from pigs supplemented with vitamin E. Some skepticism has been expressed concerning the success of adding vitamin E to muscle postmortem, as the compound may be unable to successfully integrate into the cell membrane unless added to the diet. However, some research has indicated additions can be made during processing. Lipid oxidation was slowed for cooked ground pork patties with 100 or 200 ppm vitamin E added during processing and stored at 4°C compared to cooked patties without vitamin E. Whang and others (1986) reported better sensory scores for patties with 200 ppm vitamin E. When uncooked, refrigerated ground pork was evaluated with the same treatments, vitamin E treatment provided more antioxidant ability than the control samples at 12 days storage at 4°C.

The effect of vitamin E on color stability has also been evaluated in many studies. Dirinck and others (1996) found higher redness values (a* values) for loin chops from supplemented pigs than from controls. Kerth and others (2001) reported lower percentages of PSE loins and hams from pigs supplemented with 600 or 900 IU/kg vitamin E. Results from other studies, however, have found no benefi-

cial effects of vitamin E on color (Phillips and others 2001, Cannon and others 1996, Harms and others 2003). However, vitamin E was noted to increase absorption of nonsupplemented iron without negatively affecting visual color evaluations by O'Sullivan and others (2002). The combination of vitamin E and iron supplementation reduced the oxidizing effects and promotion of WOF in *M. longissimus dorsi* and *M. psoas major* promoted by iron supplementation alone (O'Sullivan and others 2003).

Irradiation

Irradiation has been used to reduce microbial loads of food items including meats. Irradiation (low doses 3 or 5 kilogray [kGy]) can reduce microorganism counts of sausage casings (Byun and others 2001) to increase shelf life related to microbial quality, but can also increase measures of lipid oxidation when subsequently filled and displayed. Dogbevi and others (1999) reported minimal doses (1 kGy) sufficiently increased the shelf life of pork loins and reduced spoilage microbes. Ehioba and others (1987) indicated a 1 kGy dose of irradiation can increase the vacuum-packaged shelf life of ground pork by 2.5 to 3.5 days if stored at 5°C. Lambert and others (1992) found applications of irradiation in an environment without oxygen extended the shelf life of pork loin chops from 9 to 26 days at 5°C. Other successes are summarized by Thayer (1993).

One concern with irradiation use has been the generation of off odors and lipid oxidation that can occur and impact consumer acceptance. This odor has been characterized as metallic, sulfide, wet dog, wet grain, or burnt (Huber and others 1953). Irradiation may accelerate lipid oxidation as indicated by Ahn and others (1998a) when raw pork patties are subsequently stored in oxygen-permeable bags. However, Chen and others (1999) reported no influence on lipid oxidation of cooked pork during storage if irradiated before cooking. Ahn and others (1998b) noted cooking denatures antioxidants in meat, damages cell membranes, and thus exposes the lipids in the cell membrane to environmental factors that promote oxidation including irradiation doses. Other studies have not seen these effects on lipid oxidation, shelf life, or consumer acceptance. Luchsinger and others (1996a) reported no difference between pork chops irradiated at 2.5 kGy cobalt^{60} compared to controls in consumer sensory evaluations, which included overall acceptance and freshness. Higher doses (3.5 kGy) produced minimal effects on aroma, flavor, and texture of chilled and frozen pork chops. However, Sudarmadji and Urbain (1972) reported a threshold dose of 1.75 kGy of irradiation to preserve the taste of pork. Luchsinger and others (1996b) found irradiated vacuum-packaged boneless pork loin chops were redder, and possessed more color and lipid oxidation stability than controls, but oxidative rancidity increased and color stability decreased if samples were irradiated in aerobic packaging. Similarly, Nanke and others (1999) reported irradiated pork under oxygen-permeable film became less red, more yellow, more brown, and less uniform compared to nonirradiated controls.

CONCLUSION

The shelf life of pork and other meat products is dependent on its color, flavor, and aroma. These qualities can be influenced by many factors before the meat is processed (genetics, animal stress, vitamin supplementation, carcass pH, and temperature decline), but also by factors related to product handling and further processing. Carcass chilling technologies, retail lighting selections, display temperatures, packaging modifications, and product irradiation can offer color stability, microbial reductions, and delayed lipid oxidation that will extend the shelf life of pork and promote consumer satisfaction and repeat purchasing.

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Part VI
Poultry Quality

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33
General Attributes of Fresh and Frozen Poultry Meat

Lisa McKee

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INTRODUCTION

Poultry consumption has grown substantially in the past 25 years. The Economic Research Service and the United States Department of Agriculture (USDA) reported a total per capita poultry consumption of 58.2 pounds in 1980; in 2002, that total had risen to just over 100 pounds per person (National Chicken Council 2002a). Of this total, 80.5 pounds were chicken and 17.7 pounds were turkey (National Turkey Federation 2002).

Poultry is defined as any type of domesticated fowl primarily raised for meat (National Chicken Council 2002b). Chicken and turkey are by far the most widely consumed poultry products. Other domestic poultry available in limited amounts include duck, geese, squab, guinea fowl, quail, and pheasant.

A variety of fresh and frozen chicken and turkey products are available. Typical retail whole or cut-up chickens or chicken parts are from chickens called 3's and up, which weigh 3 to 4 pounds and are 40 to 45 days old. Chickens termed roasters are less than 10 weeks old and weigh 5 to 8 pounds, and Cornish game hens weigh approximately 2 pounds and are less than 30 days old. Stewing hens are actually spent layers about 15 months old and weighing about 5 pounds. Whole birds account for about one-quarter of turkey sales. Hens are about 14 weeks old and weigh anywhere from 10 to 15 pounds in the ready-to-cook form while toms are approximately 18 weeks old and weigh from 20 to 30 pounds when purchased by consumers. Sales of turkey parts, including whole breasts, breast cutlets and tenderloins, and legs, as well as ground turkey have increased in recent years as consumers look for fast and healthy food choices (National Turkey Federation 2002).

Chicken (fresh, frozen, or prepared) purchased from supermarket/retail grocery stores was consumed an average of 2.9 times over a 2-week period in a survey of 1,019 people (Brown 2003). Boneless, skinless chicken breasts were by far the most common fresh/frozen product purchased by respondents (65.6%) followed by whole chickens (33.6%), legs/leg quarters (32.8%), and drumsticks (31.1%). Turkey is reportedly consumed at least biweekly by about one-half of U.S. consumers, with lunchmeat, ground turkey, and sandwiches being some of the most popular turkey items (National Turkey Federation 2002).

SENSORY ATTRIBUTES OF POULTRY PRODUCTS

Color Characteristics

Poultry skin color generally ranges from cream-colored to yellow. The color is dependent on the type of feed used and is the result of dietary carotenoids

such as xanthophyll, which are laid down in the fat (Pérez-Vendrell and others 2001). Raw muscle typically has a pink to reddish color due to hemoglobin and myoglobin. When cooked, however, those muscles that are used more extensively, including the leg and thigh, appear dark due to the higher quantity of myoglobin while less-used muscles such as the breast are referred to as white meat. Pink discoloration remains in cooked poultry, however, possibly due to reactions of hemoglobin, myoglobin, and cytochrome c with histidine, cysteine, and methionine or their side chains (Ahn and Maurer 1990).

Since carotenoids cannot be synthesized by poultry, feed is supplemented with pigment sources such as marigolds. Such supplementation increased the pigmentation of broilers (Saylor 1986). Xanthophyll from mixed natural sources, however, was associated with lower subjective carcass color scores and objective yellowness compared to marigold concentrate. Pérez-Vendrell and others (2001) evaluated pigmentation of broilers fed a standard maize/wheat/soybean feed supplemented with either xanthophyll from isomerized marigolds or xanthophyll from conventional marigolds plus canthaxanthin. The isomerized marigolds were associated with less pigmentation and lower deposition of lutein and zeaxanthin compared to the conventional marigolds. Hunter b values were found to be a good indicator of dietary xanthophyll in breast skin while Hunter a values correlated well with canthaxanthin levels in the diet.

Poultry diets are often supplemented with other compounds such as antioxidants to improve meat quality. Such supplementation can also affect carcass color. Dietary vitamin E was associated with increases in mean color scores (less light) for refrigerated and frozen turkey breast meat as level and duration of vitamin E supplementation increased (Sheldon and others 1997). Conjugated linoleic acid at a 2% supplementation rate was associated with lighter and less red processed turkey rolls (Du and others 2002a). Although redness also decreased as the level of conjugated linoleic acid in feed increased in aerobically packaged broiler breast fillets, vacuum-packaged breast fillets were more red than controls when 0.5% conjugated linoleic acid was fed to broilers (Du and others 2002b).

Color of poultry meat is highly dependent on temperature during processing. Higher temperatures during antemortem holding (McKee and Sams 1997, Petracci and others 2001) and product holding prior to steps such as deboning and storage (McKee and Sams 1998, Molette and others 2002, Alvarado and Sams 2002) as well as delays in postmortem chilling (Rathgeber and others 1999) are associated with lighter meat colors. The pale poultry meat is often due to a defect known as pale, soft, and exudative or PSE. The condition is the result of a low muscle pH caused by rapid glycolysis at high carcass temperatures resulting in lower adenosine-5′-triphosphate (ATP) and higher lactate levels in the muscle (Woelfel and others 2002). Although values differ somewhat depending on the study, pale meat is often defined as having an L value of >53 (McKee and Sams 1997, Owens and others 2000, Qiao and others 2001). Incidence of PSE in chicken and turkey has been reported to be 0 to almost 50% (Barbut 1997, Owens and others 2000, Woelfel and others 2002).

Processing steps such as cooking and chilling/freezing also affect poultry color. Surface lightness of *pectoralis superficialis* broiler muscle heated to 70, 80, and 90°C increased as end point temperature increased while redness decreased in a study by Heath and Owens (1992). Turner and Larick (1996), however, reported chicken breasts sous vide processed to 77°C were lighter and less yellow than those processed to 94°C. Turkey roasted at 105°C was rated less brown compared to samples roasted at 163°C after reheating (Cremer and Richman 1987). A cooling temperature of 25°C was associated with lighter, less red chicken compared to lower cooling temperatures. Chilled storage of cooked chicken at 4.4°C caused a shift in the "a" value from red to green while frozen storage caused the chicken to darken and become more red and yellow over time (Heath and Owens 1992).

Irradiation of poultry was approved by the USDA in 1992 as a means of controlling *Salmonella* and other microorganisms that typically contaminate poultry products. Such processing can have a significant effect on poultry meat color. An increase in visual pinkness and/or an increase in objective redness (a values) have been noted by many researchers in both raw and cooked chicken and turkey meat (Bagorogoza and others 2001, Du and others 2001, Du and others 2002a, Du and others 2002c, Nam and others 2002). The increased redness is generally stable during refrigerated or frozen storage under anaerobic (Nanke and others 1998) and aerobic

(Millar and others 1995) conditions but is less intense in aerobic conditions (Du and others 2002c, Nam and Ahn 2002). Although Nanke and others (1999) found no change in muscle pigments when turkey was irradiated under aerobic conditions, Nam and Ahn (2002) attributed the red color to a decrease in oxidation-reduction potential and binding of carbon monoxide to the sixth ligand of myoglobin. Abu-Tarboush and others (1997) reported that although the pink/red color of irradiated chicken was considered unusual by sensory panelists, it was also rated appealing and acceptable.

The relationship between poultry color and meat functional characteristics is often of significant interest to poultry processors. Lighter broiler breast meat is typically associated with a low pH, reduced water-holding capacity (WHC), and lower emulsification capacity (Allen and others 1997, Allen and others 1998, Fletcher and others 2000, Qiao and others 2001). Raw meat color has also been found to have a linear relationship with cooked meat color (Fletcher and others 2000). Although color was not associated with a difference in psychrotrophic plate counts (Allen and others 1998), darker breast meat has been reported to have a lower shelf life based on more rapid odor development (Allen and others 1997).

Flavor and Aroma Characteristics

Poultry flavor and aroma is a complex mixture of which more than 450 compounds have been characterized. Although raw meat can have distinctive properties, the flavor and aroma components people associate with poultry develop during cooking/heating. The application of heat catalyzes chemical reactions such as Maillard browning and lipid oxidation that are responsible for the volatiles that give poultry its flavor and aroma.

A variety of hydrocarbons, aldehydes, enals, ketones, and sulfur-containing compounds contribute to poultry flavor and aroma. Gasser and Grosch (1990), using aroma extract dilution analysis, identified 16 primary compounds in chicken broth, including 2-methyl-3-furanthiol, methional, and 2-*trans*-nonenal. The compounds 2-*trans*-4-*trans*-decadienal, associated with fatty characteristics, and γ-dodecalactone, associated with tallowy characteristics, predominated in the broth. Hexanal, produced during oxidation of unsaturated fatty acids, was the most common volatile detected in both white and dark broiler meat using a vacuum distillation/concentration method (Ajuyah and others 1993). The quantity of hexanal increased in both types of meat throughout a 15-day storage period at 4°C. Taylor and Larick (1995) identified 318 volatiles in cooked chicken fat using supercritical carbon dioxide (CO_2) extraction. A trained flavor profile sensory panel detected no meaty aroma and little cooked aroma in samples. Panel detection of chicken fat aroma was correlated to compounds such as pentanal, hexanal, heptanal, and 2-octene, and chicken fat flavor was correlated to compounds such as 1,1,1-trichloroethane, 4-methylactane, and 2-heptanone.

Since heat is often an integral part of chemical reactions such as Maillard browning, application of heat is necessary for complete development of poultry flavor and aroma. Heating conditions may therefore have a significant effect on such characteristics in chicken and turkey. No difference in flavor was detected by sensory panelists for turkey cooked to 77°C in conventional, convection or microwave ovens (McNeil and Penfield 1983). An end-point temperature of 77°C, however, resulted in insufficient flavor development in turkey rolls according to sensory panelist evaluations (Cremer 1986). Chicken breasts sous vide processed at 94°C had fewer sulfur-containing volatiles and higher quantities of alcohols and hydrocarbons compared to those processed at 77°C (Turner and Larick 1996). Significantly greater aroma and flavor were noted for turkey rolls when held in an institutional convection oven for 120 minutes compared to those held for 0 minutes. Stronger roasted aroma was also noted by DiGiorgio and Setser (1987) in turkey slices held for 120 minutes at 66°C compared to those held for 0 or 60 minutes. Meaty cooked turkey flavor scores were highest for turkey cooked at 135°C compared to samples cooked at either 105°C or 163°C according to Cremer (1986), but Brown and Chyuan (1987) reported higher off flavor characteristics in turkey cooked at 135°C. Chicken flavor intensity of breasts was greater for chicken sous vide processed at 80°C than for samples prepared at the same temperature using a cook-chill system (Church and Parsons 2000). Chicken flavor was maintained in sous vide processed product during storage at <5°C while increased off flavor development was noted in stored cook-chill processed breasts.

Poultry meat is susceptible to rancidity due to a high polyunsaturated fat content. Feed supplementation

with a variety of products has been investigated as a means of improving the lipid stability and sensory properties of poultry. Thigh meat from broilers fed olive oil had higher levels of monounsaturated fatty acids and greater oxidative stability than meat from broilers fed tallow but the improvement was not reflected in better sensory scores (O'Neill and others 1998). Meat from broilers fed sunflower or olive oil was higher in unsaturated fat content, but the increase was not correlated to greater rancidity detection by sensory panelists (Ruiz and others 2001). The addition of α-tocopherol to poultry diets has been consistently reported to improve the oxidative stability of the resulting meat (Bartov and others 1983, King and others 1995, Sheldon and others 1997, Bou and others 2001, Ruiz and others 2001). Sensory evaluations correlate with the objective measurements of oxidative stability as panelists typically detect less rancidity and greater overall acceptability in poultry meat containing α-tocopherol. Other antioxidants, on the other hand, have not been effective in improving oxidative stability of poultry meat. Ascorbic acid has had little effect on rancidity development when used as a feed supplement (King and others 1995, Bou and others 2001) and β-carotene was actually reported to be a prooxidant in broiler meat (King and others 1995). Addition of extracts of oregano (Botsoglou and others 2002, 2003), rosemary, sage (Lopez-Bote and others 1998), and other herbs/spices to poultry feed has been associated with decreases in lipid oxidation as determined chemically, but few sensory evaluations of the resulting meat have been reported.

The susceptibility of poultry meat to rancidity leads to the development of a characteristic termed warmed-over flavor (WOF). Thought to be due to the oxidation of polyunsaturated fatty acids with iron as a catalyst, the reaction has become increasingly important as further processed and ready-to-eat poultry products have become more popular. Both cooking temperature and storage time affect development of WOF. A reaction temperature threshold of 74°C for WOF has been reported (Smith and others 1987). WOF-related characteristics such as sour and bitter tastes and astringent aftertaste increased while freshly cooked meat-like flavor decreased in chicken as oven cooking temperature increased from 160 to 190°C (Byrne and others 2002). Maillard degradation products also increased as cooking temperature increased and were associated with roasted and toasted notes detected by the sensory panelists. Although such degradation products have been reported to be antioxidative, they did not affect WOF development according to Byrne and others (2002). Cardboard, warmed-over, rancid/painty, burned, metallic, and other off flavor characteristics associated with WOF have been reported to increase while fresh roasted and meaty notes decrease as storage time at 2 to 4°C increases (Ang and Lyon 1990, Lyon 1993, Turner and Larick 1996).

Several compounds have been investigated for their inhibitory effect on off flavor development in poultry. Sodium tripolyphosphate and sodium ascorbate monophosphate provided only minimal reductions in lipid oxidation and stale/rancid off flavor development in vacuum packaged turkey and were associated with soapy off flavors at the 0.5% level of addition (Craig and others 1991). Sodium lactate retarded the growth of spoilage bacteria but was associated with sodium and metallic off flavors and acidic aftertastes that increased as pH decreased (Williams and Phillips 1998). Turner and Larick (1996), however, reported enhanced fresh roasted/meaty and saltiness characteristics in sous vide processed chicken breast containing sodium lactate. Chelation of iron by phytic acid and suppression of iron release from hemoglobin compounds by nitrite were associated with lower WOF scores as determined by a four-member sensory panel (Graf and Panter 1991). Calcium chloride, calcium acetate, and calcium gluconate also decreased WOF formation.

Irradiation is an effective means of reducing microbial loads in poultry. Such treatment, however, has frequently been associated with off odor development and increased production of volatiles (Heath and others 1990, Bagorogoza and others 2001, Kim and others 2002, Nam and others 2002). Dimethyl disulfide and dimethyl trisulfide, described by sensory panelists as sulfurous and foul, have been identified as two of the compounds contributing to the off odors (Patterson and Stevenson 1995, Du and others 2001). Other volatile compounds identified in irradiated poultry include 3-methyl butanal, 2-methyl butanal, *cis*-3-nonenal, and *trans*-6-nonenal. Although irradiation has often been associated with off odors, higher levels of fresh chickeny, bloody, and sweet aromas in raw chicken and chicken flavor in cooked chicken were detected by 14 trained panelists in irradiated samples compared to nonirradiated chicken (Hashim and others 1995).

Texture Properties

Texture is a complex set of characteristics that includes everything from thickness and gumminess to chewiness and fracturability. The movement of the jaws and tongue, the cutting, grinding, and tearing action of the teeth during chewing and the surfaces of the mouth all play a role in textural evaluation of foods by humans. One of the most important textural properties in meats is tenderness, which is defined as the ease with which a piece of meat can be cut and chewed. Juiciness, a property related to the fat and moisture contents of meat, and WHC are also important textural components in meats.

Age can have a significant effect on texture of poultry meat. Shear values for breasts from 8-week-old broilers (5.6 kilograms per gram [kg/g]) was about one-third of that for breasts from 16-week-old broilers 16.8 kg/g (Awonorin and Ayoade 1992). A similar trend was noted for turkey breasts from 12 (11.2 kg/g) to 25-week-old (18.9 kg/g) birds. At 16 weeks of age, chicken breast required greater force to shear (16.8 kg/g) than turkey breast (13.4 kg/g). Scores for tenderness increased slightly while those for juiciness decreased as age increased for both chicken and turkey when samples were evaluated by a 12-member sensory panel using a scale from 1 and 2 = very poor to 9 and 10 = very good. Shear values for fillets from 49- and 51-day-old broilers were equivalent to "very tough" while those from 42- and 44-day-old broilers were in the "slightly tough" to "slightly tender" portion of the texture scale (Northcutt and others 2001).

Struggling prior to exsanguination of chickens and turkeys causes increased glycolysis, greater production of lactic acid, and lower muscle pH. Since more acidic pH values are often associated with lower meat quality characteristics, stunning methods have been investigated as a means of improving poultry meat properties. Although electrical stunning has been reported to delay glycolysis (Murphy and others 1988) and anesthesia was associated with higher muscle pH and WHC and lower shear values in turkey (Ngoka and others 1982), Northcutt and others (1998) reported electrical, CO_2, and no stun treatments resulted in cooked turkey breast meat with comparable texture as well as raw and cooked muscle pH values. Mohan Raj and Gregory (1991) initially reported no differences in breast meat texture from broilers stunned by argon or electrical current and filleted under different conditions. Textural differences between stunning methods were detected in subsequent studies, however. In general, those studies indicated that although glycolysis was more rapid, argon stunning could be used to produce broiler breast meat that could be filleted earlier and required less force to shear than that produced by electrical stunning (Mohan Raj and others 1991, Raj and others 1992, Raj 1994). Ten trained sensory panelists rated broiler breast fillets from argon-stunned birds as "moderately tender" while those from electrically stimulated carcasses were rated "slightly tough," but juiciness was not different between the stunning methods (Raj and others 1992).

Muscle shortening can cause toughening of poultry meat and is influenced by rigor, temperature, and pH. Rigor mortis is the result of a series of biochemical changes that occur in carcasses postmortem. Accumulation of lactic acid causes a decrease in muscle pH, and formation of actomyosin results in stiffening of the muscle proteins. Rigor shortening was found to occur more extensively at 40°C in isolated strips of chicken *pectoralis major* muscle when the muscle pH was 6.2 and was associated with more tender cooked meat compared to that from muscle incubated at 10 to 30°C (Dunn and others 1993a, 1993b). Higher shear values, indicating greater toughness, and a wider range of muscle sarcomere lengths were reported to indicate rigor shortening in broiler carcasses chilled at 10°C while those chilled at 0°C showed no evidence of shortening nor toughness (Dunn and others 1995). Chicken muscle pH and shear values decreased as chilling time prior to deboning increased from 0 to 8 hours (Lyon and others 1985). A slight increase in both pH and shear values was noted, however, for muscle chilled 24 hours prior to deboning. Immersion chilling caused shorter sarcomeres and an increase in toughness in turkey breast muscle compared to air-chilled breasts (Wakefield and others 1989).

Postmortem treatments can also affect the texture of poultry meat. Excision of prerigor muscle, known as hot boning, is often associated with tougher meat. Stewart and Fletcher (1984) found that as postmortem pH decreased, muscle toughness decreased as holding time prior to excision increased. A holding time of at least 4 hours prior to excision was recommended. Postmortem stimulation of carcasses with low voltage decreased toughness in hot-boned broiler breast fillets, but high voltage stimulation

was needed to tenderize chill-boned fillets (Thompson and others 1987). Postmortem time prior to chilling was reported to influence WHC of both uncooked and cooked turkey breast muscle (Lesiak and others 1997). Early-boned broiler breast fillets were tougher than fillets from either aged controls or those treated with hydrodynamic pressure (Meek and others 2000). Fillets from aged controls were reported to be more tender and juicier than those from the other treatments, but hydrodynamic pressure was recommended as a potential means of tenderizing early-boned muscle.

Rigor development when carcass temperature is high may contribute to PSE poultry. The condition seems to be dependent on an interrelationship between temperature, pH, and color. When muscle is held at 30 to 40°C, lower pH, greater drip loss, and shorter sarcomere lengths typically result (McKee and Sams 1998, Alvarado and Sams 2002, Molette and others 2002). Although called soft, shear values of PSE muscle frequently indicate tougher meat. Although both lower pH and higher L values were correlated with lower WHC in turkey meat (Owens and others 2000), L values are generally considered to be the best indicator of PSE in poultry.

SUMMARY

Poultry products such as chicken and turkey are often viewed by consumers as a healthy, low-fat alternative to red meat products. This perception is, in part, responsible for the significant increase in poultry consumption seen over the last 25 years. More efficient and technologically advanced production and processing methods have resulted in both a greater availability and a wider variety of poultry products for consumers. Such processing, however, can have both desirable and undesirable effects on sensory, functional, chemical, and microbiological properties of the products. Although food safety has become a primary issue for many consumers, acceptable sensory properties remain a significant contributor to the continued consumption of poultry products.

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34
Poultry Meat Flavor

Paul L. Dawson and Nick Spineli

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INTRODUCTION

Poultry meat flavors result from interactions among proteins, nucleic acids, lipids, and other components found in the meat. Cooking promotes the development of fresh cooked flavors that are derived via amino and fatty acid reactions to form volatile and nonvolatile compounds. The Maillard reaction or Maillard-like reactions occur during heating especially when high heat and dehydrating types of cooking are used. Once poultry meat is cooked, oxidative reactions quickly initiate, first within the phospholipids fraction and within hours result in the development of detectable oxidized off flavors. These oxidized off flavors have been described as warmed-over flavors (WOF) being formed from the scission of fatty acids into shorter chain carbon compounds such as aldehydes, ketones, alcohols, and acids. Since unsaturated fatty acids oxidize at a faster rate than saturated fatty acids, poultry meat's higher degree of unsaturation compared to other meats facilitates faster oxidized flavor development. Certain strategies have been employed to slow the development of off flavors including feeding the animal high levels of antioxidants prior to slaughter or adding antioxidants prior to cooking. Exclusion of oxygen from direct contact with the meat and lowering the storage temperature also slow the rate of oxidation and off flavor development. Poultry meat flavor also varies due to muscle types within animals (i.e., breast versus leg). Dark chicken meat has a slightly higher fat content than lighter meat, however, the difference in color is due to pigment content. This chapter will discuss specific flavors of poultry meat resulting from cooking, oxidation after cooking, and flavor differences due to animal diet or species.

COOKED FLAVORS

There are several excellent reviews of the literature on poultry flavor (Wilson and Katz 1972, Steverink 1981, Ramaswamy and Richards 1982, Chen and Ho 1994). These studies discussed previous research and their own studies on both the water-soluble and lipid portions of the flavor of poultry. Most researchers used chicken broth to focus on the nonlipid portion of poultry flavor. The separation of chicken flavor into individual compounds evolved with the advent and development of gas chromatography (GC) technology (James and Martin 1952). The complexity of meat and poultry flavor was mentioned in 1937 (Howe and Barbella) and in 1948 (Crocker). Crocker (1948) published the first report attempting to link chemical composition and flavor. Bouthilet (1949, 1950, 1951a, 1951b) published the earliest in-depth work on poultry meat flavor and concluded that chicken flavor consists of at least two main fractions, a sulfur and a fatty acid fraction. This researcher further concluded that meat flavor

was a result of the muscle tissue and not primarily linked to the lipid fraction since the main meat flavor was extracted with a polar solvent. This conclusion was verified by Pippen and others (1954, 1969) when the meat, bone, skin, and composite fractions were compared for flavor and the meat fraction yielded the most meaty flavor. Using carbonyl compounds found by Pippen and others (1958) and Pippen and Nonaka (1960), Lineweaver and others (1962) reported the first use of GC to characterize chicken flavor volatiles. Pippen and Nonaka (1963) found that cooking chicken in air yielded more complex and larger volatile compound fractions compared to chicken cooked in a nitrogen gas environment. This led to the assumption that oxygen greatly affects the development of cooked chicken flavor. These researchers also identified hexanal and 2,4-decadienal as prominent volatiles in cooked chicken flavor. Minor and others (1965b, 1965c) used GC to elucidate alcohols, amines, esters, mercaptans, and sulfides in addition to ammonia, hydrogen sulfide, and 15 or so other carbonyl compounds as chicken flavor components. Minor and others (1965b, 1965c) listed 29 and 25 volatiles from cooked meat and water slurries from leg and breast muscles, respectively. These researchers also confirmed the observation by Bouthilet (1949, 1950) that chicken flavor is comprised of two major components—a "meaty" sulfur component and a "chickeny" carbonyl component—and that sulfur amino acids and lipid components are the precursors of these components, respectively. For instance, Grey and Shrimpton (1967a, 1967b) identified 20 then 27 volatile components of chicken breast muscle. The use of Kovat's indices facilitated the identification of volatile compounds by different researchers. Before the development of high-resolution GC and mass spectroscopy (MS), less than 40 compounds were identified. Nonaka and others (1967) separated 227 compounds using these technologies, but were only able to identify 62 due to interference and lack of separation. Hobson-Frohock (1970) identified about 30 new chicken flavor compounds by sequentially removing classes of compounds. Wilson and Katz (1972) added 11 new chicken flavor compounds (mostly unsaturated alcohols) listing a total of 178, and Janney and others (1974) identified 27 compounds from fried chicken including butylated hydroxy toluene, a common food antioxidant. Wilson and Katz (1972) divided chicken flavor compounds into categories of hydrocarbons, aromatic hydrocarbons, furans, alcohols, esters, ketones, ethers, fatty acids, aldehydes, sulfur compounds, inorganic compounds, amino acids/peptides, sugars, amines, lactones, and miscellaneous compounds. Harkes and Begemann (1974) found 11 new unsaturated aldehydes, and Horvat (1976) identified 30 previously unknown compounds from chicken bringing the total of identified compounds to 236 from raw, cooked, and fried chicken. Tang and others (1983) determined the flavor components of fried chicken and found that three peaks containing various cyclic compounds were related to the unique fried flavor. These researchers concluded that of the 130 identified volatiles found in fried chicken flavor, they were likely to be formed from either (1) chicken constituents during frying, (2) flour constituents during frying, (3) oxidation of the frying oil itself, and/or (4) interaction between the decomposition products of chicken or flour and the frying oil. By 1984, over 300 compounds had been identified as components of cooked chicken flavor (Maarse 1984). Further work by Noleau and Toulemonde (1986) using a Likens-Nickerson apparatus listed 197 components from roasted chicken flavor. The list included hydrocarbons (27), alcohols (17), aldehydes (47), ketones (33), acids (14), esters (6), bases (22), sulfur compounds (10), halogens (3), acetals (2), nitriles (1), phenols (5), and furans (10).

Smoking of foods was historically used to preserve meats by absorption of aromatics from the smoke into the meat. Smoked poultry meats are desired more for their flavor than for their preservation effect in modern times. Of the more than 300 compounds found in smoke, only a few have been found in smoked meats, reflecting the interaction between the smoke and the meat (Hamm 1977, Daun 1979, Clifford et al. 1980). In addition to the hydrocarbons, acids, ketones, and aldehydes found in cooked meat flavor, phenols are found in smoked meats and are responsible for smoked meat flavor (Hassan 1988, Clifford et al. 1980, Sink 1979, Shiau and Chai 1985). A specialty smoked meat item is duck, and the subcutaneous fat covered fillet known in French cuisine as "magret." The unique flavor developed in this product is a result of smoking, lipid oxidation, and the compounds forming from compound interactions. Lesimple and others (1995)

identified 62 volatiles, 34 of which were related to smoke and 7 that had not been previously reported in smoked duck meat. The smoked meat contained 15 phenolic compounds none of which were found in nonsmoked duck meat. A comparison of water-boiled and Cantonese-style roasted duck flavors revealed that roasting produced heterocyclic compounds such as pyrazines, pyridines, and thiazoles (Wu and Liou 1992), all of which are Maillard reaction products. Wu and Liou (1992) also reported that indole was a component of both water-boiled and roasted duck flavor and that indole is not found in most other meat flavors. Indole is responsible for floral notes at low concentrations (1,000–0.2 parts per million [ppm]) and acts as a positive flavor promoter dependent on other flavor compounds at concentrations below 0.21 ppm (Arctander 1969). Indole is also a primary component of certain essential oils in flowers from certain plants (lemon, bitter orange, coffee, etc.) and in some teas (Furia and Bellanca 1975).

SULFUR COMPOUNDS IN FLAVOR

Sulfur compounds formed during cooking are important flavor compounds yielding a chicken flavor. Since meat lipids do not contain sulfur, the sulfur is derived from the protein fraction, namely the sulfur amino acids (cysteine, cystine, methionine) or other sulfur-containing compounds found in meat such as glutathione. Bouthelit (1951a, 1951b) found the presence of ammonia and hydrogen sulfide in cooked chicken and claimed that sulfur compounds were important to chicken flavor. The presence of sulfides in cooked chicken volatiles was verified by Pippen and Eyring (1957) when they concluded sulfur compounds were part of chickeny flavor, and that while ammonia was present, it did not contribute to the chicken flavor. In addition to hydrogen sulfide, Mecchi and others (1964) found trithiane in cooked chicken volatiles. Minor and others (1965c) detected 13 sulfur compounds in chicken volatiles. Several researchers reported the release of substantial quantities of hydrogen sulfide during cooking of chicken (Mecchi et al. 1964, Klose 1965, Minor et al. 1965b). Pippen and Mecchi (1969) reported hydrogen sulfide concentrations of 180–730 parts per billion (ppb) in simmered, roasted, and fried chicken while only 35 ppb in chicken broth. The human threshold level of detection of hydrogen sulfide in broth is 10 ppb, thus it likely contributes strongly to chicken flavor. While storage, freezing, and reheating can reduce hydrogen sulfide to below threshold levels (Pippen and Mecchi, 1969), possible interaction with carbonyl compounds may give a more stable chicken flavor. The possible interaction of hydrogen sulfide with carbonyls was demonstrated by Pippen and Mecchi (1969) who blew hydrogen sulfide gas over chicken fat and found the resulting fat high in fixed sulfur and quite odorous. Several researchers have found that sulfur compounds decompose during cooking (Balance 1961, Mecchi et al. 1964) and that compounds released during cooking may dissolve in the fat yielding a characteristic flavor. For example, trithiane can be formed in cooked chicken from a reaction between hydrogen sulfide and formaldehyde (Mecchi et al. 1964). Swoboda (1970) detected dimethyltrisulfide at 10 ppb in roasted chicken leg meat and concluded it was a strong flavor component. A variety of sulfur compounds in chicken volatiles have been subsequently reported by various researchers (Hobson-Frohock 1970, Janney et al. 1974, Horvat 1976). Farbood and MacNeil (1979) traced the volatile sulfur compounds produced from methionine using radioactive labeling and determined that 90.7% were trapped in the protein fraction of the chicken meat slurry and 8.9% held with the lipid fraction. Bouthilet (1951b) concluded glutathione was the precursor for hydrogen sulfide. Pippen (1967) stated that poultry meat contains several sulfur-containing compounds including cystine, cysteine, methionine, taurine, and glutathione and any of these could be the source for volatile sulfur compounds. Biotin, thiamine, and coenzyme A are other sulfur-containing compounds found naturally in poultry meat, but there is little evidence that these contribute significantly to sulfur flavor volatiles. Hydrogen sulfide was produced at 200 to 1,000 ppb during oven cooking of chicken at 70 to 125°C (Parr and Levett 1969). These researchers further stated that cysteine and cystine in protein provided 90% of the sulfur for hydrogen sulfide production and that cysteine in glutathione was a minor contributor. Mecchi and others (1964) also concluded that cysteine was the major sulfur contributor for hydrogen sulfide and that leg muscle protein produced hydrogen sulfide 80% faster than whole leg muscle. Leg meat nonprotein nitrogen did

not produce hydrogen sulfide until after 2.5 hours of boiling and the glutathione was the precursor for less than 9% of the hydrogen sulfide formed during boiling. The formation of sulfur compounds was shown to occur from the primary and secondary reactions of cysteine degradation products (Boelens et al. 1975). One example is the formation of thiozoles, oxazoles, imadazoles, oxothio ketones, mercapto ketones, disulfide ketones, and 2,5-dialklthiophenes from the reaction of diketones with ethyl alcohol, hydrogen sulfide, and ammonia (Boelens et al. 1975).

Gasser and Grosch (1990) identified 2-methyl-3-furanthiol as an important compound contributing to the meaty flavor of chicken broth as well as for beef (Gasser and Grosch 1988) and canned tuna (Withycombe and Mussinan 1988). The furan ring structure can be formed by the cyclization of a 5-carbon reductone. Reductones are common intermediates of Maillard-type reactions, and their formation is enhanced by heating. The first and fifth carbons of the reductone link via the oxygen to the furan ring structure (Figure 34.1). A related Maillard pathway, the Strecker degradation of methionine, can lead to the formation of methional and dimethyl disulfide (Pippen and Mecchi 1969). In fact, the Strecker degradation has been identified as the likely pathway for the production of carbonyl flavor compounds involving amino acids (Schonberg and Maubacher 1952). Ambler (1929) and Schonberg and Maubacher (1952) demonstrated that acetaldehyde could be formed via the Strecker degradation of alanine, aspargine, or aspartic acid. The presence of thiols and sulfides linked to alkyl groups suggest that sulfur-containing amino acids undergo breakdown by more complex mechanisms than the Strecker degradation (Pippen 1967).

2-Methyl-3-furanthiol (Figure 34.2) and a similarly structured dimer have been created by heating thiamine and cysteine hydrochloride with hydrolyzed vegetable protein (Evers et al. 1976). These meat flavor compounds have also been detected in volatiles from heated yeast extract (Ames and MacLeod 1985) and heated thiamine (van der Linde 1979, Hartman et al. 1984, Reineccius and Leardon 1985) verifying that thiamine is one likely source of fresh cooked meat flavor. In fact Giacino (1968) patented a method to produce chicken flavor by heating thiamine with sulfur-containing polypeptides and alkanones followed by addition of hexanal and diacetyl. Simple sugars (i.e., ribose) and nucleic acids (i.e., inosine monophosphate [IMP]) when reacted with cysteine or glutathione yielded 2-methyl-3-furanthiol. The pathway to forming 2-methyl-3-furanthiol and its dimer bis(2-methyl-3-furanthiol) disulfide can occur when heating liberates hydrogen sulfide from either cysteine or glutathione, which in turn reacts with a 5-carbon ketone from IMP or ribose (Figure 34.3). Glutathione is a major source of hydrogen sulfide in the initial stages of heating (Ohloff et al. 1985), while cysteine is a major contributor of hydrogen sulfide during long-term cooking (Mecchi et al. 1964).

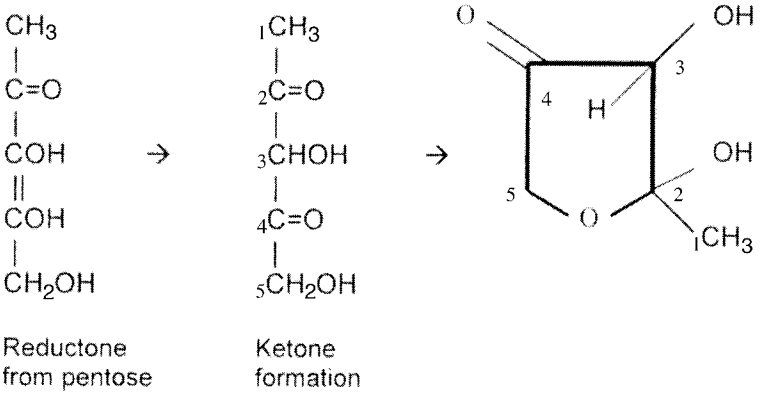

Figure 34.1. Pathway for formation of methyl-furan.

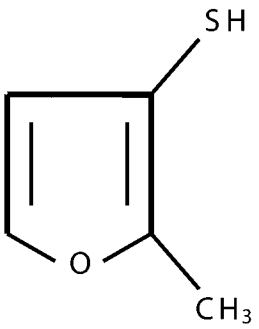

Figure 34.2. Structure of 2-methyl-3-furanthiol.

CARBONYL COMPOUNDS IN FLAVOR

Carbonyls are a diverse group of compounds that include ketones, aldehydes, and acids. Compounds containing a carbonyl functional group have been considered as a major source for poultry meat flavors (Lineweaver and Pippen 1961, Minor et al. 1965c). The carbonyl flavor compounds are derived from oxidative reactions of lipids, which are promoted by cooking and storage. Thus, the type and amount of carbonyls produced from poultry meat are affected by type of heating and length of storage. The development of oxidized carbonyls can be somewhat controlled by the addition of antioxidants and by exclusion of oxygen via packaging. The single digit ppb threshold values of some carbonyls (Lea and Swoboda 1958, Meijboom 1964) make them potent flavor compounds. Meijboom (1964) associated the flavor perception of carbonyls to their chemical structures, degree of saturation, and chain length. For example, 2-trans-alkenals had higher threshold values than their saturated counterparts, and aldehydes with an even number of carbons had a higher threshold value than ones with an odd number of carbons. Specific odor volatiles in turkey skin aroma were linked to several flavor/aroma descriptors by expert sensory panelists (Table 34.1) (MacNeil and Dimick 1970a). The conversion of acetoin to diacetyl was reported to create a freshly cooked chicken aroma (Pippen et al. 1960) since acetoin (Figure 34.4a) presence did not contribute to chicken broth odor while diacetyl (Figure 34.4b) made a significant contribution to fresh cooked chicken flavor. Narasimhan and others (1993) reported isolating 1,2-dibutylcyclopentane, 2,6-bis (1,1,-dimethylethyl)-4-methylphenol, and 1,12 -dodecadiol as unique chicken flavor compounds compared to beef and pork.

Carbonyl compounds are also a source of oxidized off flavors or rancidity of chicken. Nutter and others (1943) found that raw, frozen turkey fat became rancid in 3 weeks while chicken fat was stable to 4 months based on measurements of fatty acid content, acetyl value, iodine value, and thiocyanogen numbers. Fat stability also varies according to which part of the animal the fat was derived from (Pool et al. 1950) with back fat, neck fat, and visceral fat sequentially decreasing in stability (most to least) (Klose et al. 1951). The formation and content of specific aldehydes (malondialdehyde and hexanal) have been employed to measure the degree of meat oxidation. The thiobarbituric acid (TBA) value (also known as thiobarbituric acid reactive substances [TBARS]) and malondialdehyde value (MDA) are chemical tests measuring the presence of malondialdehyde in meat and other substances including human blood. Harris and Lindsay (1972) reported that off flavor development in cooked chicken was linked to malondialdehyde formation using the TBA test. Furthermore, a trained sensory panel rejection of stored poultry skin was directly related to high levels of 2-enal and 2,4-dienal carbonyls (MacNeil and Dimick 1970b). By capturing volatiles in air bubbled through a heated mixture of chicken meat and water, Pippen and others (1958) identified diacetyl, acetone, (C_2-C_6, C_8, and C_9) saturated aldehydes, (C_5-C_7, C_{10}, C_{11}) 2-alkenals, and 2,4-heptadienal. Subsequently, Pippen and others (1960) identified many more carbonyls in a steam distillate from fresh chicken including 2-alkanones and 1,2-dicarbonyls with the predominant compounds being diacetyl, acetaldehyde, n-hexanal, and 2,4-decadienal. In a later study, Pippen and Nanoka (1963) reported that when chicken and turkey meat were cooked in a nitrogen atmosphere, fewer carbonyls and smaller amounts of those produced were detected compared to turkey meat cooked in an air atmosphere. Furthermore, rancid chicken meat contained the same volatiles but in larger quantities than fresh chicken meat. Rao and others (1976) found the addition of polyphosphates to poultry meat prior to cooking could reduce the amount of carbonyls (mostly dicarbonyls, methyl ketones, 2,4-dienals) produced.

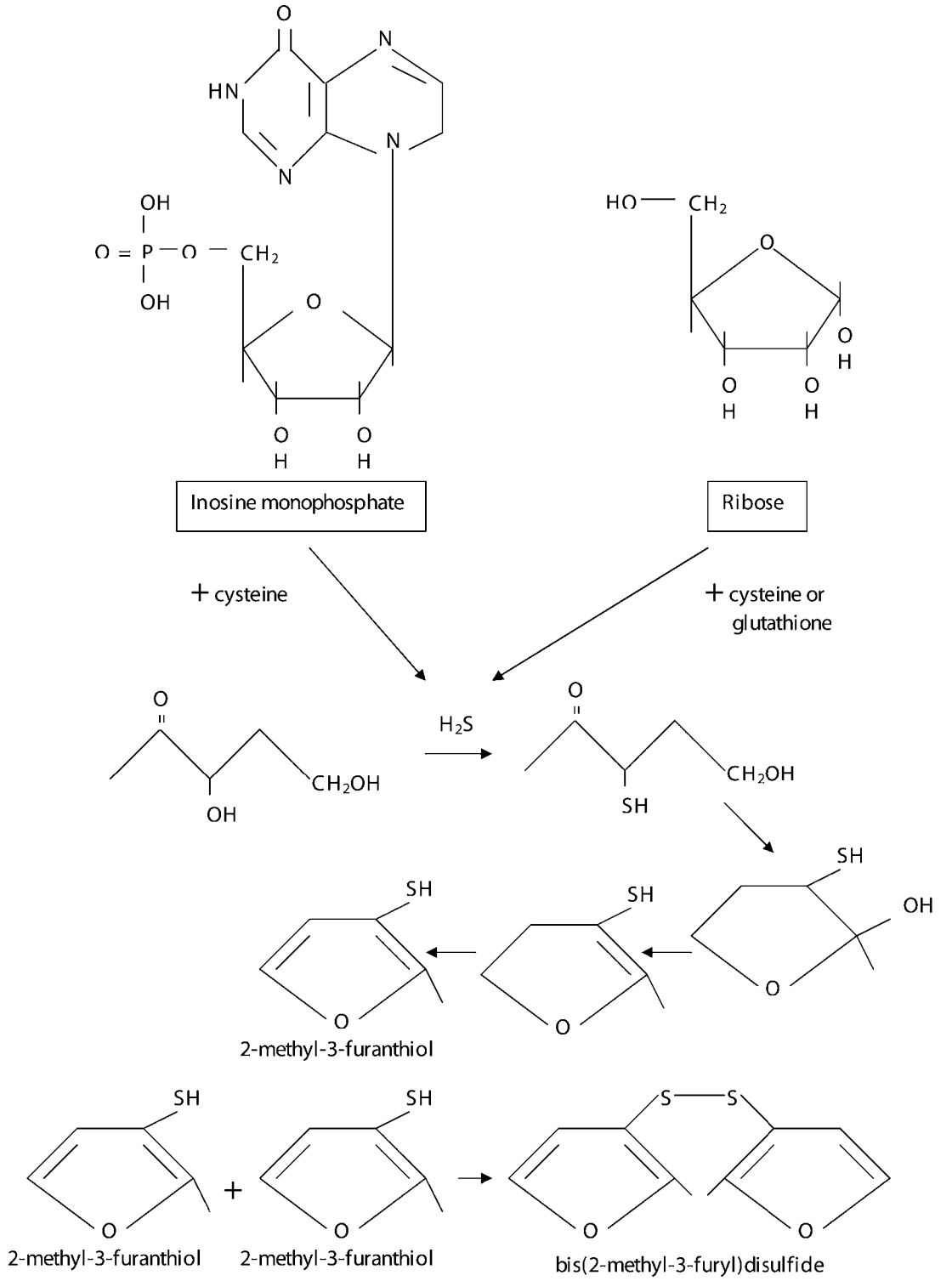
Inosine monophosphate
Ribose
+ cysteine
+ cysteine or glutathione
H_2S
2-methyl-3-furanthiol
2-methyl-3-furanthiol
2-methyl-3-furanthiol
bis(2-methyl-3-furyl)disulfide

Figure 34.3.

Table 34.1. Response of expert sensory panelists to specific volatile carbonyls generated from turkey skin (Adapted from MacNeil and Dimick 1970a).

Turkey-skin volatile	Expert sensory panel descriptor
Methyl ketone	oily, minty
Alkanal	meaty, turkey-like
Alk-2-enal	strong, oxidized, broth-like
Alk-2,4-dienal	strong, painty, nutmeg-like, spicy

PRODUCTION/PROCESSING FACTORS

Various production and processing factors can affect the flavor of poultry in addition to the complexity of flavor in culinary applications. Production factors include animal diet, age, gender, and genetics while muscle type, chilling method, thermal processing, irradiation, storage, antioxidants, and microbiology are some processing factors affecting poultry flavor. Although animal genetics has some effect on flavor of meat, it is minimal when compared to other production and processing factors. Hanson et al. (1960) demonstrated this by making over 600 comparisons between "slow-growing" 1930s genetic strains and "faster-growing" 1950s broilers and reporting no difference on flavor.

Microbiological Effects on Flavor

The microbiological content and makeup of meat can have a significant effect on flavor during storage (Adamcic and Clark 1970, Carlin et al. 1957, Freeman et al. 1976). In fact, Harris and others (1968, 1970) reported significant flavor differences between germ-free and conventional chicken. Grey and Shrimpton (1967a) matched flavor compounds found in chicken breast muscle with ones found in the animal caecum (part of the digestive tract). Klinger and Basker (1977) found that chickens fed 1,3, b-p-chlorobenzylidine aminoguanidine hydrochloride (an anticocidial agent) from birth to slaughter had a longer storage life and better flavor than controls. Sheldon (1979) further demonstrated that chicken meat flavor could be altered by changing the intestinal flora and resulting microbial fatty acid production via antibiotic administration during growth.

Several studies have confirmed that poultry meat spoilage is largely due to gram-negative, psychrotrophic bacteria (McMeekin 1975, 1977; Daud et al. 1979). Hawke (1966) established that bacteria could produce methyl ketones from lipids. Aerobic lipophilic bacteria with the capacity to hydrolyze lipids including *Psuedomonas* spp., *Achromobacter* spp., and *Micrococci* spp. have all been associated with poultry meat (Frazier 1967, Jay 2000). Microbial enzymes may also degrade lipids (Smith and

Figure 34.4. Structure of acetoin (a) and diacetyl (b).

Alford 1968) resulting in carbonyl off flavor compounds. Off flavor due to bacteria have been linked to chicken breast and leg meat (McKeekin 1975) and to chicken skin (Daud et al. 1979). McKeekin and Patterson (1975) linked hydrogen sulfide-producing bacteria to chicken processing plants and Freeman and others (1976) associated spoilage odor volatiles to refrigerated chicken. *Pseudomonas* spp. has been identified as a major spoilage strain linked to poultry meat, and several researchers have isolated volatile compounds from pure *Pseudomonas* spp. cultures. For example, Freeman and others (1976) detected dimethyl sulfide, dimethyl disulfide, and methyl thiolacetate from a *P. fluorescens* culture grown on previously irradiated chicken meat. In this study, dimethyl disulfide was the only sulfur compound detected from sterile chicken meat when inoculated with *P. putida.* Lee and others (1979) reported that acetone, 2-butanone, methyl thiolacetate, dimethyl disulfide, toluene, dimethyl trisulfide, and 2-nonanone were found when *P. putida* was grown on either standard microbiological growth media or chicken breast meat and that acetone, toluene, methyl thiolacetate, dimethyl disulfide, dimethyl trisulfide, 2-nonanone, and 1-undecance were detected for *P. fluorescens* under the same conditions. Pittard and others (1982) found 12 of the same volatiles out of 25 total compounds were produced by four different fluorescent *Pseudomonas* strains and that only 4 compounds out of 25 were unique to one strain.

Animal Dietary Effects on Flavor

Animal diet has a strong impact on poultry flavor. Objectively, animal dietary effect on meat flavor is dramatic when the flavor of wild game birds is compared to the flavor of birds grown under controlled dietary conditions. The effect of diet on flavor was documented as early as 1935 by Maw who reported that poultry fed a corn diet were more flavorful than those fed a diet consisting of barley, oats, and wheat. The addition of dried chicken feces (Lewis 1955) or dairy products (Weisberg 1956) to broiler feed was reported to produce a more flavorful meat.

Considerable work has been reported on off flavor especially related to "fishy" off flavors. The transfer of fishy off flavor from dietary fish meal or fish oil to poultry meat was observed many years ago (Cruickshank 1939, Murphy 1939). Asmundson and others (1938) found that 25% fish meal in turkey diets did not result in a meat with fishy taste but that 2–5% fish oil added to turkey diets did result in meat with a fishy flavor. The fishy flavor in poultry meat has been attributed to the inclusion of highly unsaturated fatty acids (three or more double bonds). These highly unsaturated fatty acids are metabolized and deposited in the fatty tissue of the animal that when cooked produce fishy flavor volatiles (Klose et al. 1953). Klose and others (1951) found that feeding linseed oil (highly unsaturated fat) to turkeys resulted in cooked meat with a fishy off flavor. Crawford and others (1975) further reported that fish off flavor in turkey meat could be induced by feeding turkeys highly unsaturated fatty acids, and later it was determined that these off flavors developed during cooking (Crawford and Kretsch 1979). Crawford and Kretsch (1976) identified 71 unique volatile compounds in turkeys fed with tuna oil and that 21 of those were likely to be the cause of fishy off flavor. Since the fishy flavors were a result of the oxidative breakdown products of unsaturated fatty acids, fishy flavor in poultry fed highly unsaturated fatty acids could be reduced by the addition of antioxidants (α-tocopherol) (Crawford et al. 1975). Sheldon and others (1997) studied the effects of various dietary vitamin E levels on the oxidative stability, flavor, color, and volatile compounds of refrigerated and frozen turkey meat. Turkeys were fed 5, 10, or 25 times the National Research Council recommended amounts, which are 12 and 10 IU/kg body weight from 0–8 and 9–18 weeks of age, respectively. Oxidation rate was inversely proportional to dietary vitamin E as indicated by TBA values and sensory panels.

Animal diet alterations other than degree of unsaturated fatty acids or antioxidants in the diet of poultry have little effect on meat flavor. Variations in poultry rations using 12.5% raw peanuts (Offiong 1976), 45% field beans (Grey et al. 1972), or 38.2% dried poultry waste (Cunningham 1976) had no significant effect on cooked meat flavor. Furthermore, Carlson and others (1962) found no difference in the flavor of meat from turkeys fed varying levels of fat. Thus the percentage of highly unsaturated fat in poultry feed is the only dietary factor playing a significant role in poultry meat flavor. Long chain unsaturated fatty acids such as those found in fish have been linked to improved cardiovascular health and the addition of these to meat has been accomplished

through animal diets. Van Elswyk and others (1995) compared the incorporation of docosahexanoic acid (DHA) into chicken meat by adding the fatty acid to the diet as menhaden oil or as natural marine algae. Meat from broilers fed the algae source of DHA were more acceptable and stable than meat from birds receiving feed supplemented with menhaden oil.

Effect of Animal Gender and Age on Flavor

There are conflicting reports as to the effect of gender on poultry meat flavor, and the age of the animal sometimes confounds these results. For instance, Sweetman and MacKellar (1954) found that meat from male birds had stronger flavor compared to meat from female birds while Carlson and others (1962) reported no flavor difference in meat from male and female turkeys. Fry and others (1958) found chicken broth flavor was affected by the gender of the meat source for chickens 10–14 weeks old, however, they found no measurable difference in this meat when baked. Fry and others (1958) also reported flavor differences in broth and baked chicken due to hormone levels in the meat from 6- to -14-week-old birds. MacNeil and Dimick (1970a) reported higher carbonyl levels in meat from male turkeys compared to female turkeys.

Meat from older chickens was found to be more flavorful (Sweetman and MacKellar 1954, Vail et al. 1967). This was supported by Minor and others (1965a) who reported that meat from older chickens had higher concentrations of flavor compounds compared to meat from younger birds. Between 9-week-, 19-week-, and 28-month-old chickens, Wells and others (1962) reported that cooked freeze-dried meat from the 28-month-old chickens was most flavorful. The generation conclusion is that meat from older chickens and turkeys tends to taste more flavorful.

OXIDATION-DERIVED FLAVORS

Off flavors via lipid oxidation is a common concern in poultry products due to the relatively high degree of unsaturation of the fatty acids compared to other meats. Turkey meat is even less stable than chicken (Wilson et al. 1976, Einerson and Reineccius 1977) partly due to the lower levels of natural antioxidants in turkey lipids (Nutter et al. 1943) even when animals were fed the same amount of tocopherol (Mecchi et al. 1956). Criddle and Morgan (1951) reported that tocopherol content in turkey meat could be greatly increased by feeding tocopherol during growth. Warmed-over flavor (WOF) was a phrase introduced by Tims and Watts (1958) to describe the rapid onset of rancidity in cooked meats detected when reheated after refrigerated storage. The main components in meat that promote the onset of oxidation leading to rancidity are heme, nonheme iron, unsaturated fatty acids, and phospholipids. St. Angelo and others (1988) identified volatile compounds responsible for WOF as hexanal (grassy), propanal (alcoholic), pentanal (pungent), 2,3-octadione, nonanal (soapy), and 2-pentylfuran. Johnson and Civille (1986) and later Byrne and others (2002) found a decrease in the meaty and sweet notes on cooked refrigerated chicken meat and an increase in WOF notes such as cardboard, linseed oil, rancid, and sulfur/rubber. Byrne and others (2002) reported compounds as products of different reactions including 3-methylbutanal (malty), 2-methylbutanal (roasted corn), and 3-methylthiopropanal from the Strecker degradation; dimethly trisulfide (garlic) and dimethyl tetrasulfide (cabbage) from thermal sulfur amino acid degradation; and 1-octen-3-ol (mushroom), hexanal (grassy), 2-heptenal (bitter, almond), 2,4-heptadienal, and 2,3-octanedione. Ruenger and others (1978) reported that WOF in cooked turkey was detected equally by sensory panelists in dark and light meat contradictory to Wilson and others (1976) who reported that dark meat developed WOFs to a greater degree than light meat. Ruenger and others (1978) further found that three compounds increased in reheated samples. Two of the compounds were identified as heptaldehyde (flavor characterized as harsh, pungent, and unpleasant fatty) and *n*-nona-3,6-dienal (dienal flavors characterized as fishy).

Mechanically deboned chicken meat (MDCM) is particularly susceptible to rancidity (Dimick et al. 1972) due to mixing of the meat with bone marrow and cellular constituents during deboning, in particular the heme pigments (Froning and Johnson 1973). Mixing of meat tissue with air at this point will also contribute to accelerated oxidation (Dawson et al. 1990). Oxidation of MDCM results in off flavors that will carry through to the final product making oxidized meat less valuable.

Poultry by-product meal is used in pet foods and is derived from the rendering of parts of the animal

not typically used for human consumption (head, viscera, legs, feathers). These parts are prepared into a dry meal via the rendering process and are an inexpensive source of protein. Greenberg (1981) characterized the flavor volatiles from poultry by-product meal and concluded that the majority of the flavor volatiles in the meal were derived from lipid oxidation. These compounds are formed from the 14% fat contained in the final dried meal, and the formation of these compounds is promoted by the heat and oxidative environment used to render the meal safe for use as feed.

Table 34.2. Categories of different types of cooking.

Dry Heat	Moist Heat	Combination
Grilling or broiling	Steaming	Stewing
Barbecuing	Poaching	Braising
Roasting or baking	Simmering	
Poeleing	Boiling	
Sautéing		
Pan-frying		
Deep-fat frying		

CULINARY ASPECTS OF COOKED POULTRY MEAT

The application of heat to food in various ways is a crude definition of cooking. Meat is cooked to render it safe, easier to digest, and more flavorful (McGee 1984). Cooking can be divided into the following three categories: dry heat; moist heat; and a combination of dry and moist heat with several different types of cooking possible within each category (Table 34.2). The dry heat methods produce a variety of results (Culinary Institute of America 1996). For example, grilling and broiling results in highly flavored exteriors with moist interiors while roasted meats develop a rich roasted aroma, well-developed color, and tender texture. Sautéed foods develop a specific degree of browning while pan-fried and deep-fried items have a crispy exterior. In general, dry heat does not tenderize meat to the degree moist heat does thus is not normally used for tough meat cuts. Moist heat cooking can be used to tenderize meat and are combined with a flavorful liquid in the preparation. The combination method is sometimes called "slow cooking" with a preliminary searing step (dry heat) or conversely a blanching step (moist heat). The combination of the two methods develops complex flavor profiles not possible with single methods. Imafidon and others (1994) linked the five basic meat tastes of sweet, salty, acid, bitter, and umami to acids, peptides, and salts as follows: sweet (certain amino acids); bitter and umami (peptides and hypoxanthine); acid (lactic, inosinic, orthophosphoric, and succinic acids); sweet and salty (glucose and sodium salts of glutamic and aspartic acids).

The flavor of chicken allows for other flavors to develop and blend together. The chicken is used in numerous cultures\cuisines. It is extremely versatile due to the subtle profile it has and the manner in which flavors, from simple to complex, can be developed with it. Chicken can be prepared in many ways, including baking, broiling, boiling, roasting, frying, sautéing, braising, barbecuing, and stewing. Each of these means of preparation provides a different flavor profile, yet it is easily enhanced by any number of flavor combinations in any of the cooking techniques. Therefore, the cooked flavor of chicken is greatly influenced by numerous factors.

The cooked flavor of chicken, when not influenced by a cooking method or seasoning, is very pleasant, unlike poultry with more dominant flavors, such as duck, turkey, goose, and Cornish game hens. This cooked flavor can best be experienced when boiling fresh chicken with its bones. Meat that is refrigerated for too many days or thawed from the freezer has a discernable different flavor. Professional kitchens will often intensify the cooked poultry flavor notes. One method is to reduce poultry liquids, otherwise referred to as stocks and broths. These liquids are further enhanced by creating a thickening agent that is made from heated poultry fat.

The use of particular herbs and vegetables aid the common poultry flavor in the European and North American cultures. The herbs used are rosemary, thyme, marjoram, parsley, and sage, plus the addition of the vegetables consisting of carrots, onions, leeks, and celery.

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35
Color of Fresh and Frozen Poultry

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INTRODUCTION

In several regions in some countries, consumers prefer the flavor of raw and processed poultry as compared to other meat species. The reasons for this preference vary, including lower fat content, flavor, ethnic tradition, and religion. Nonetheless, poultry meat and meat product quality are frequently altered mainly due to color variation originated from conditions that differ from those found in red meats. The low concentration of heme pigments results in considering poultry as "white" meat. Color in poultry is a result of several parameters such as heme pigment concentration as well as its chemical state, altering the light reflectance; preslaughter factors (diet, heat stress, handling, and stunning, among others); slaughtering conditions; and postmortem treatment during refrigerated and frozen storage. Final pH seems to be a factor highly correlated to poultry meat color. Processing, such as additive addition—intentional or unintentional—and processing temperature also determine color.

MEAT COLOR

From an optical point of view, meat can be considered as an anisotropic translucid tissue. Perpendicular light incidence with respect to muscle fibers increases reflectance because meat is a light-scattering matrix of cellular material, myofibrillar proteins, connective tissue, and light-absorbing pigments. Myoglobin and its derivates are the main responsible compounds for the selective absorption of meat pigment's selective absorption (Mateo-Oyagüe 2001). Meat color varies due to (1) myoglobin quantity and its inherent differences depending on animal species, age, and muscle; (2) myoglobin chemical state; (3) pH; and (4) other conditions such as muscle situation before or after bleeding and surface dehydration. Acceptance of poultry meat and meat products by the consumer is mainly associated with color, since this appearance is related to freshness, eating quality, texture, and flavor. Poultry is merchandized either before or after skinning, complete, or in pieces (breast, thigh, and leg, etc.) or separation of white and dark meat. In all cases, lacking the appropriate color results in a failure in merchandizing (Northcutt 1997).

Poultry Meat Color

Poultry meat color is affected by factors such as bird age, sex, strain, diet, intramuscular fat, meat moisture

content, preslaughter conditions, and processing variables. Poultry discoloration is related to the amount of muscle pigments—myoglobin and hemoglobin—and can occur in an entire muscle or be limited to a specific area such as areas around bruises or broken blood vessels. Higher myoglobin concentration is necessary in those muscles subjected to fast movements, therefore producing a more intense red color. Because muscles differ greatly in activity, oxygen demand also differs accounting for variations in myoglobin concentration (Northcutt 1997, Maurer 1998). Some of the main color defects in poultry meat are listed in Table 35.1.

Color as Related to Muscle Type

Highly and specifically organized tissues, both morphologically and biochemically, are made up of striated muscles, with the conversion of chemical energy to mechanical energy (work) as their main function. Muscles can be classified by color and enervation type. Two main muscle types are distinguished: red muscle (high mitochondria and myoglobin content, aerobic oxidative metabolism, and abundant blood irrigation); and white muscle (low mitochondria and myoglobin content, anaerobic metabolism, and low blood irrigation). White muscles (W) undergo a fast contraction (or α), whereas red muscles may present fast (α) or slow (β) contraction. Slow contraction muscles use, in addition to glucose, fatty acids in the presence of oxygen. The slow contraction red muscles (Rβ) have more abundant blood irrigation and smaller diameter as compared to red fast contraction (Rα) muscles. In general, red muscles are those involving energetic movements. Conversely, white fast contraction (Wα) muscles have scarce blood irrigation. In these, muscle energy is generated from anaerobic sugar metabolism. The main differences between red and white muscles are listed in Table 35.2 (Carballo and López de la Torre 1991). Poultry muscle composition is shown in Table 35.3.

COLOR MEASUREMENT

Pictorial color standards, supporting specific color scales, have been developed. However, according to the objectives for a specific study, appropriate scales and standards must be chosen. These study methods can be based on trained (descriptive) panels, consumer

Table 35.1. Main color defects detected in poultry meat (adapted from Maurer 1998).

Color	Possible causes
Bone darkening	Hemoglobin from bone marrow leaches through the spongy bone wall and deposits on bone surface in young birds where bones are not fully calcified. Cooking oxidizes and denatures hemoglobin changing color into brown and black hues, also increased by freezing and thawing.
Gray-black skin	Caused by incomplete cooking caused by faulty equipment. Chlorinated water dissolves copper from water pipes; the metal is absorbed during scalding and combined with sulfur compounds released during cooking, black cupric sulfide is produced. Similar situation occurs with high iron-content water.
Black spots	Due to molds and bacteria growth.
Green muscle	Deep pectoral myopathy. Heavy-breasted broilers or turkey flap their wings excessively during catching and handling, increasing pressure on breast muscles, reducing blood supply and eventually causing necrosis.
Blue, pink or red and greenish bruise	Due to harmed muscles and rupture of blood vessels originated by improper catching or shank.
Green iridescence	Caused by the micro structural diffraction.
Green patches	Excessive use of nitrates or nitrites.
Pinkness	Ammonia leakage.
Pink–red color	Undercooked poultry.
Pink	Cooking uncovered birds in poorly ventilated ovens; effect of salt and seasonings added to curing solution.

Table 35.2. White and red muscles main characteristics (adapted from Carballo and López de la Torre 1991).

Characteristics	Red muscle	White muscle
Myoglobin	Abundant (red color)	Poor (white color)
Energy metabolism	Aerobic, oxidative	Anaerobic, glycolytic
Contraction speed	Slow (β), fast (α)	Fast (α)
Transverse section	Small	Large
Blood irrigation	Abundant	Scarce
Mitochondria	Abundant	Scarce
Energy source	Glucose, fatty acid, keto compounds	Glucose
Post mortem acidification	Slow (β), very fast (α)	Fast (α)
Adipose and conjunctive tissue associated	Very abundant	Scarce

(acceptance) panels, or both. The most suitable scales are usually constructed by preliminary studies in which the product is treated under defined conditions, as scales reflecting color changes due to specific experiments are necessary. Scales developed in this way will encompass the spectrum of sample colors that most likely appear, and can be used for panel training as reference standards during visual appraisals (AMSA 1991).

Objective color measurements may be used for various reasons: (1) to support descriptive visual appraisals; (2) as a basis for product acceptance or rejection; (3) to document color deterioration over time; and (4) to estimate the proportion of myoglobin in different chemical states. However, the most important aims for objective color measurements are to support visual observations and to provide unbiased evidence of treatment effects that can be statistically analyzed. To fulfill these aims data expression as color coordinates (e.g., L, a, b -values) is probably enough, but such data must only be used to represent relative color differences, not "absolute" descriptions of color (AMSA 1991).

In the last decade, a number of research workers suggested the possibility of using color measurement to predict functional properties of poultry meat. Specifically, pale, soft, and exudative (PSE)-like conditions and water-holding capacity (WHC) are the most common functional properties mentioned. Many of these studies are based on the use of commercial colorimeters that express meat color in terms of color differences (from a designated color standard as opposed to an absolute color determination) such as the Commission International de L'Eclairage, or International Commission of Illumination system (CIE-Lab) based on three coordinates: lightness (L*); redness (a*); and yellowness (b*). Because of the nature of color difference measurements, as well as differences in sample presentation and measurement conditions, comparison of absolute color values between laboratories is difficult. In order to compare meat color studies by

Table 35.3. Red and white fibers percentage in several thigh and breast poultry muscles (adapted from Xiong 1994).

Muscle	βR	αW	αR
Thigh			
Anterior *Longissius dorsi*	100	0	0
Femoralis pars medalis	51–100		0–49
Sartorius	31–32	15–20	49–53
Breast			
Pectoralis	0–12	67	21
Pectoralis superficialis	0	100	0

different authors and the potential of using computer-based vision systems applying color discrimination criteria to evaluate meat quality in poultry processing plants, a better understanding of the influence of measurement condition on the instrumental color response is needed. These results indicate that under practical conditions, broiler and turkey meat color measurements are influenced more by sample thickness than by background color. Such background color is only important in measuring thin sample and evaluating and reporting breast meat color. These results also indicate that the development and application of online machine vision color systems to measure and sort broiler and turkey breast meat prior to further processing must take into account variations in thickness, location of color reading on the fillet, and background (transfer belt) surface color (Bianchi and Fletcher 2002).

Similarly, the development of instrumentally standardized tests to objectively differentiate between dark-colored and normal-colored broiler chicken carcasses (cut-off points) was studied by Boulianne and King (1998). Poultry line inspectors separate carcasses into normal or dark, although this is a dichotomous classification. The choice of cut-off point is important as it is applied in deciding whether the carcass is considered normal-colored and thus kept for human consumption, or dark-colored and condemned. The threshold or cut-off point value determine the sensitivity and specificity of the test in which a* value was more likely to correctly classify dark-colored versus normal-colored broiler chicken breast fillets than L* and b* values.

Liu and others (2004) reported that some of the commonly applied analytical methods (shear force measurement, CIE-Lab color measurement, or sensory evaluation) are destructive, time consuming, and unsuitable for on-line application. The development of fast, nondestructive, accurate, and on-line/at-line techniques is critical to increase processing efficiency. Visible/near infrared spectroscopy (NIRS) could be the basis for such a technique due to its speed, simplicity to use, and low interference of moisture or color in meat samples. Partial least squares regression models were developed using different spectral regions. This study suggests that visible/NIR spectroscopy might have the feasibility to predict color and pH in broiler muscles. As expected, predictive models for CIE-Lab coordinates and pH are more accurate than those based on individual sensory attributes.

Kranen and others (1999) developed a method based on analysis of hemoglobin and myoglobin content in broiler muscle, since red poultry muscle color depends on myoglobin content. Extractability of hemoglobin and myoglobin from muscle tissue are different. Myoglobin and hemoglobin concentrations are very low in the glycolytic muscles such as *Pectineus* and *P. superficialis*, and higher in oxidative *Adductor* muscle. The mixed type (glycolytic-oxidative) muscles such as *Sartorius* and *Adductor* differ considerably with respect to myoglobin level, but hemoglobin levels are essentially the same. The results reported by these authors indicate that heme protein levels, especially myoglobin, are correlated with muscle fiber composition. Hemoglobin quantification and myoglobin content of chicken muscles, using a combination of spectrophotometric analysis and size-exclusion chromatography, is accurate and reproducible to determine the concentration of these compounds in poultry meat.

FACTORS AFFECTING POULTRY COLOR

Similar to other meat animals designed for direct consumption or further processing, poultry undergoes a number of changes promoted by environmental factors as well as by feeding and inherent genetic characteristics. These intrinsic and extrinsic parameters are reflected in biochemical changes in the living tissues, and can be minimized or increased by slaughtering, processing, packaging, and storage conditions.

Several variables must be considered in the production of poultry and poultry products free of color defects, including those related to *in vivo* handling and utilization of high quality feeds and water supply. Slaughtering practices, such as age, diet (mainly nitrite or moldy feed intake), premortem transportation and handling (stressing conditions such as exhaust gases from motor engines, high temperature, and prolonged transportation periods), stunning, evisceration, and cleaning are also determinants for optimum meat color. Carcass washing using metal and nitrate-free water also prevents meat discoloration (Maurer 1998). Meat aging, cooking in metal containers, and nitrite/nitrate-containing water usually results in poultry color alteration (Smith and Northcutt 2004a).

Preslaughter Conditions

Effect of Stunning

Extreme light or extremely dark color are found in breast meat, resulting from short-term antemortem stress-altering protein, ash, and free fatty acid content related to genetic predisposition in commercial poultry varieties. Long-term antemortem factors such as diet, management, etc., also contribute to extreme color differences (Qiao and others 2002b).

Defective bleeding can occur in broiler processing conditions. Electrical stunning has been found to result in a higher incidence of hemorrhagic leg syndrome indicated by excessive blood around the femur. The viable alternative is whole body stunning, since blood has a higher contribution than marrow in broiler breast discoloration (Smith and Northcutt 2004a). Although different stunning or killing (irrecoverable stunning) methods induce different response in live birds during slaughter and result in different meat quality characteristics, there has been no direct comparison of using carbon dioxide (CO_2) for recoverable and unrecoverable stunning with respect to electric stimulation. No significant difference in pH or expressible moisture was observed between CO_2 stunning, electrical stunning, and CO_2 killing. In spite of this, L, a, and b values were observed in 1-hour postmortem poultry fillet, CO_2 killing gave darker meat (lower L* values, 51.97±2.2) than those form birds stunned with CO_2 (51.92±2.5). Fillets from poultry stunned with CO_2 became lighter during storage, as compared to those obtained from poultry killed with CO_2. Fillets from electrically stunned poultry were redder (higher a* values, 5.10±0.80 versus 4.56±0.92 and 4.71±0.73) at 1 hour postmortem as compared to CO_2 treatments; whereas no difference was observed among the three treatments at 24 hours of storage. CO_2 killing gave darker meat at 24-hour postmortem (Kang and Sams 1999). Color defects can be prevented by using a close surveillance and control of all preslaughter and process parameters (Fronning 1995).

Postmortem Conditions

The main factor determining poultry color change is the ultimate pH. Chicken breast meat can be divided into three color types: dark (L*<47); normal (L* = 47 to 51); and light (L*>50) (Qiao and others 2001, Woelfel and others 2002, Holownia and others 2003b). The ultimate pH values of the breast meat from muscles undergoing fast glycolysis (pH ≤5.80 after 15 minutes postmortem) were not different from those of the normal glycolysis (pH >6.0 at 15 minutes postmortem). Breast meat color was not affected by postmortem glycolysis rate, but delays in carcass chilling produced lighter, redder, and yellowier meat than carcass immediately refrigerated (Rathgeber and others 1999). Dark chicken breast fillets have pH, myoglobin, and iron contents higher than normally colored fillets. The establishment of a cut-off value for color parameter a* provides a reliable instrumental method to differentiate between dark- and normal-colored carcasses. Normal meat color parameters at pH = 5.99±0.14 are a* = 1.4±0.4; L* = 49.2±1.4; and b* = 10.3±1.3. Dark muscle color parameters at a pH = 6.04±0.12 are a* = 5.6±0.4; b* = 9.08±0.9; and L* = 39.9±0.8. Threshold a* is 2.72 at 97% sensitivity and 90% specificity (Boulianne and King 1998).

Refrigeration

L*-values for refrigerated poultry fillets decrease as storage proceeds for 24 to 48 hours postmortem, suggesting considerable drip losses; further L* value decreases are related to meat shrinkage (Galobart and Moran Jr 2004).

Irradiation

Irradiation has been applied to extend poultry and poultry products' shelf life, although with detrimental effects on quality. Oxidative changes such as rancidity development, off flavor production, and discoloration are major concerns in cooked poultry meat storage. Irradiation of cooked poultry meat can accelerate development of lipid oxidation and off flavor production, therefore extreme care is needed in dealing with irradiated precooked poultry meat. Irradiation, packaging, and storage deeply influence surface color of precooked turkey breast. However, redness of vacuum-packaged precooked turkey breast increases with irradiation and seems to be dose-dependent. Color changes occur throughout the breast resulting in a very stable color during a 3-month frozen storage. Irradiation has little effect on aerobically packaged precooked turkey breast; pink color is very liable to undergo oxidation.

Irradiation, as well as cooking, promotes displacement of carbon-containing gases from the meat tissue, such as carbon monoxide (CO) and methane; the volume of displaced gas is irradiation-dose dependent, higher in vacuum than in aerobic packaging but decreases in both packaging types after 3 months of storage although a considerable amount still remained in the meat tissue. Increased redness in irradiated precooked turkey could be caused by the CO-myoglobin formation as CO has a strong affinity to heme pigments. Reducing conditions promoted by irradiation also contribute to high a* values (Nam and others 2002). Millar and others (1993) reported redness was observed to be stable during the refrigerated storage in irradiated poultry meat.

Meat color stability is closely related to the inherent oxygen consumption rates, oxidation-reduction potential, metmyoglobin-reducing capacity, and metmyoglobin reductase activity of muscles. Packaging also prevents color changes. Kim and others (2002) reported no alteration in L*-values of nonirradiated turkey samples under aerobic or vacuum packaging stored from 0 to 7 days, whereas a*-values increased in aerobic packaged samples and b*-values decreased in vacuum-packaged turkey. A 3-kilogray (kGy) dose resulted in no changes in L*-values and b*-values but higher a*-values in both packaging systems. Aerobic packaging gave more total color difference (δE) values than vacuum packaging; no significant difference was observed with respect to storage time.

Cooking

Cooking to high temperature endpoint reduces red/bloody discoloration. Reduced discoloration with low endpoint temperatures is also possible if the product is frozen before cooking (Smith and Northcutt 2004b). Current studies provide evidence that discoloration (both in dark and redder than normal muscles) is induced in broiler breast meat. Addition of bone marrow or blood produces darker and redder cooked meat. Discoloration is observed as burgundy (reddish-brown) or dark color; no red color or blood drip was produced (Smith and Northcutt 2004a).

Skin Color

Color is highly important to consumers' acceptance as poultry is sold skinned or with the skin. Dietary xantophyll pigments deposited in the epidermis are primarily responsible for skin color as well as for poultry color changes during storage. Broiler skin pigmentation has long been recognized as a critical quality attribute. Soap stock added to the diets caused different levels of pigmentation of broiler skin. Soybean soap stock promotes intense pigmentation due to its high yellow pigment concentration in such a way that it is generally recommended in broiler diets as vegetable oil substitute, and as polyunsaturated fatty acids (PUFA) and xantophyll pigment sources (Pardio and others 2001). Color differences (δE) have several implications regarding poultry color changes, both in earlier changes associated with processing as well as longer-term changes associated with storage. Regardless of scalding conditions or anatomical location of measurement, skin color changes dramatically during the first 2 hours postmortem, especially for the subscalded carcasses. These changes are mainly an increase in lightness, with more lightness apparent in skin areas with higher fat deposits where xantophyll deposits, and areas associated with thicker skin areas of feathers. Skin and meat color changes are more pronounced during the first 4 hours postmortem when the carcasses are still in the processing plant. After this time, the color continues changing but at a slower rate up to 12 to 24 hours postmortem. Color changes from day 1 to day 8 of storage vary depending on processing or storage. Effects of color changes during storage are less critical but still important for possible effects on product uniformity and consumer acceptance (Petracci and Fletcher 2002).

PINK COLOR DEFECTS

White poultry meat displays areas retaining pink color, even after heating to internal temperatures exceeding 70°C. Although safety is not related to pink color defects, as no reports of illness have been associated with pink color development in poultry, this condition may be responsible for substantial losses to the poultry industry due to consumers' rejection, rework, and discards. Factors related to pinking include the presence of several pigments; preslaughter factors such as genetics; feed; hauling and handling; heat and cold stress and exhaust gases in the environment; stunning methods; incidental nitrate/nitrite contamination through diet, water supply, freezing,

and processing equipment; and processing ingredients. Industry procedures include the use of nonmeat ingredients and cooking methods and endpoint cooking temperature and, recently, irradiation of precooked products. The well-documented pink discoloration may also be attributed to specific *in situ* conditions such as pH, reducing agents, chemical state and reactivity of pigments, pigment-protein denaturation degree, and reactivity of endogenous meat compounds (Holownia and others 2003a). Pink color defect (pinking, pinkness, or pink tinge) is perceived by consumers as an undercooked and unsafe-to-eat product in cooked poultry white meat. The factors associated with pinking have been related to the presence of four pigment classes: (1) undenatured myoglobin or oxymyoglobin; (2) reduced globin hemochromes of well cooked meats; (3) the pigment in cured meats nitrosyl hemochrome; and (4) carboxy myoglobin, a pigment results from the reaction between carbon monoxide and myoglobin.

Good manufacturing practices concentrate on efforts to reduce or eliminate external contamination by nitrate and nitrite such as nitrosyl pigments, but reduction of this compound does not significantly contribute to pink discoloration prevention.

Because no significant processing effect has been found in plants where recurrent pinking was observed, alternative explanations have been studied. The natural variation in raw color of breast muscle affects pinking occurrence (Holownia and others 2004a, 2004b). Raw muscle's initial L*-values are the most critical condition for pinking occurrence; raw muscle lightness indicates meat initial endogenous conditions. It is recommended that poultry processors ensure the shortest time to processing (Holownia and others 2003b). Regardless of the muscle color (i.e., light, normal, or dark), pinking occurs when 1 parts per million (ppm) or more of sodium nitrite is introduced into the meat during processing. Although pigments in their native chemical state may be responsible for pinking in cooked meat under particular conditions, this is not the only factor involved. The presence of sodium nitrate had the most significant effect on pinking, even at very low levels such as 1 ppm. When sodium nitrite concentration was lower than this concentration, meat intrinsic conditions such as high pH and low oxidation-reduction potential were the more important factors affecting pigment reactivity. No meat additive alone, except sodium nitrate, significantly induces pinking. Even residual nitrite amounts may be present in the meat depending on preslaughtering factors and processing conditions. In the absence of nitrites, other chemicals that promote similar reactions are sodium chloride, sodium tripolyphosphate, and sodium erythorbate. Schwarz and others (1999) explained pinking formation as the result of ligand binding to position 6 of the heme moiety of the pigment. If this ligand is nicotinamide or nitrite, pinking is produced. Holownia and others (2004b) imitated pinking onset by adding sodium tripolyphosphate, sodium erythorbate, and sodium nitrite to raw chicken breast imitates pink color defect in the cooked meat. Pinking simulation was most effective in the dark muscles, followed by the normal and light muscles.

PALE, SOFT, EXUDATIVE POULTRY MEAT

The term pale, soft, exudative (PSE) is a descriptor for a meat product, typically pork or turkey, which has abnormal light color, flaccid consistency, poor WHC, and substantially reduced cooking yield. PSE meat is a common defect in poultry, mainly in turkey. Although the undesirable appearance of fresh meat cuts exhibiting the PSE condition may lead to product rejection by consumer, it is the poor protein functionality in processed meat products that is considered to be the primary cause of financial loss associated with turkey PSE meat in the processing industry. It is generally accepted that PSE meat is closely associated with rapid early-postmortem glycolysis in muscle tissue, which, in turn, result in accelerated rigor mortis development and low pH (<5.8). This combination promotes muscle protein and consequent loss of protein functionality, denaturation leading to paler meat color, decreased WHC, and softer texture. McKee and Sams (1998) reported that higher postmortem carcass temperatures ($>20°C$) in turkey resulted in lighter meat with higher drip loss and cook loss.

Stressing conditions accelerate postmortem glycolysis in turkeys, producing PSE meat (Strasburg and Chiang 2003). These conditions include extreme ambient temperatures, inadequate transportation and handling, and faulty stunning methods. They accelerate rigor development in some carcasses (Woelfel and others 2002), whereas PSE incidence was higher in the warm months (15.6% in

summer, 11.3% in autumn, and 2.7% in winter) (Petracci and others 2004).

Genetic factors are likely to play a role in poultry relative stress susceptibility and its inability to adapt to stressors, thereby influencing the probability of PSE development. Evidence for a genetic component of PSE turkey is limited; it is suggestive rather than direct. Turkeys could be classified with fast or slow postmortem glycolysis. The striking similarity in development of PSE condition in turkey and pork, together with the identification of a genetic component or stress susceptibility in pigs, suggested that there is a genetic basis for stress susceptibility in turkeys. Based on the apparent similarities of the basis for PSE pork and PSE turkey, one or more mutations exist in the turkey ryanodine receptor (RYR).

Since the PSE-susceptible turkey is thought to be associated with the rapidly growing commercial turkey industry, genetic selection can result in increased frequency of altered RYRs in modern commercial turkeys. Strasburg and Chiang (2003) compared PSE incidence between a random-bred, genetically unimproved line and a commercial line of turkeys intensively selected for a rapid growth and increased muscling. It was observed that over 50% of improved turkeys are PSE, while only 25% of the random-bred birds had this condition. The genetic basis for PSE turkey was not clear, but it seems that ryanodine receptors may predispose birds to PSE development. Mutations could exist in either the αRYR or βRYR isoforms or in both isoforms.

Succinylcholine, a neuromuscular depolarizating muscle relaxant, and certain anesthetics such as halothane have been successfully used to screen pigs for the presence of the genetic mutation that results in PSE meat. These drugs are able to induce porcine stress syndrome (PSS) and subsequently the PSE conditions by triggering the calcium release from the RYR in the muscle. Although birds exhibited reactions to the halothane gas, this screening test in conjunction with succinylcholine injection was not predictive of detecting 4-week-old broilers prone to developing PSE meat when processed at 7 weeks (Cavitt and others 2004).

PSE broiler breast meat appears different from PSE in turkey and pork, as protein denaturation does not seem to be the main cause of paleness and low WHC. Ultimate pH of PSE broiler meat is lower than pH of normal breast meat and appears to be the cause of paleness and low WHC. Thus, procedures that limit pH decline or increase pH of PSE to that of normal meat during processing may be effective in reducing the incidence and improving functionality of PSE broiler breast meat (Van Laack and others 2000). Several research workers have demonstrated high correlation between breast meat color and raw meat pH. A thorough understanding of the difference in quality properties related to color is important for further processing to reduce meat color variation on processed products (Qiao and others 2001).

PSE can also cause problems during cooking in certain types of further processed turkey products, such as formed breast loaves and rolls by increasing purge in cook-in bags, decreased cook yield, and dry texture that is unacceptable to the consumers. L*-values seems to be of more predictive value than pH. It is easier and faster than pH and could therefore be used to sort chicken meat in commercial processing plants (Woelfel and others 2002).

Carcass Chilling and PSE Development

Although temperature is an important determinant to PSE meat development, the relationship between pH and postmortem temperature is even more critical. Low pH combined with high postmortem temperatures can cause more severe protein damage than low pH at low temperatures. Therefore, poor chilling conditions can lead to PSE conditions in normal glycolysis carcasses (Alvarado and Sams 2002).

Poultry cuts including bones must be chilled within the shortest time, because they are very susceptible to quality changes due to temperature fluctuations due to the low thermal transference rate of bones. Leg quarters are particularly affected due to dark meat concentration and percentage of bone. Visual appearance of processed poultry, especially color, chilled at temperatures ranging from +4 to −18°C is of interest to consumers and processors. Discoloration of raw cooked tissue occurs due to cell disruptions and blood migration caused by slow chilling rates.

Lyon and Lyon (2002) studied L*, a*, and b* values in uncooked and cooked leg quarters refrigerated at 4 to −18°C for 7 days. These authors reported that surface color under the skin was not influenced by refrigerated storage. Conversely, color of cooked meat adjacent to the femur was affected by a combination of temperature history and internal temperatures; in-

tense red meat was obtained by cooking at 75°C internal temperature and stored at 4°C, whereas the least red samples were those stored at −18°C. No significant difference was observed in samples cooked at 85°C regardless of storage temperature.

Alvarado and Sams (2002) studied the effect of storage temperature on *Pectoralis* color and pH decline. The pH decline during the first 60 minutes postmortem was faster at temperatures around 25°C where L* values were higher, than at 0°C with lower L* values; however, at 24 hours of storage there were no significant differences in L* values.

Paleness of PSE meat is the result of increased sarcoplasmic protein denaturation, increasing light scattering. Alvarado and Sams (2004) studied protein denaturation and fillet paleness as a function of carcass temperature, particularly in turkey *Pectoralis* muscle. These authors reported that at 26°C or above PSE meat is more likely to develop. Their results show that slow or inadequate chilling can contribute to PSE development, and recommended to turkey processors to cool *Pectoralis* muscle at 25°C or lower in less than 60 minutes postmortem to reduce PSE meat. The same authors reported a relationship between time and temperature, holding turkey meat at 30°C for 90 minutes postmortem resulting in PSE meat.

COLOR CHANGES PREVENTION

Ingredient Addition

Schwarz and others (1999) studied the effect on preventing pinking of the following compounds injected into turkey breast:

1. trans-1, 2-diamino-cyclohexane-N,N,N',N' (CDTA)
2. diethylen triamine pentacetic acid (DTPA)
3. ethylene dinitrilo tetracetic acid disodium salt (EDTA)
4. nonfat dry milk (NFDM)
5. 2% NaCl
6. 0.5% sodium tripolyphosphate
7. sodium nitrate and nicotinamide

The results showed that low levels (50 ppm) reduced pinking associated with prolonged storage, whereas higher levels (100, 200 ppm) were not significantly better to decrease pinking as compared to low concentrated solutions. The most efficient pinking reducer was DTPA. NFDM is an approved nonmeat ingredient. Other dairy proteins or their components reduce or eliminate the pink defect (Slesinski and others 2000a, 2000b).

Slesinski and others (2000a) studied the effectiveness of seven commercial dairy proteins (NFDM; sodium caseinates; whey protein concentrates [WPC]) in ground turkey breast samples treated with 10 ppm nitrite or 1.0% nicotinamide pink coloration inductors. When no pink color generated ligand was added, NFDM had no effect on a* values as compared to the control with no ligand added. Similar results were observed in samples added with WPC. Increase in turkey breast products lightness may improve the products overall appearance. NFDM and WPC increased lightness regardless of ligand treatment. NFDM and WPC can be added to processed turkey to potentially increase product yielding, increase lightness, and reduce pink color generation caused by variations in nicotinamide content or nitrite contamination. Slesinski and others (2000b) reported that addition of 200 ppm EDTA eliminated pinking regardless of ligand treatment, although this chemical is not approved for use in meat products.

Marination

Qiao and others (2002a) studied the effect of marination on broiler breast fillet color. Marinade was made of water (92.5%), salt (5%), and phosphates (2.5%) and applied for 24 hours. Results showed considerable variation in meat color related to differences in pH that, in turn, affect functional properties relative to marinade intake and cooking yield. Lightness of raw fillets was positively correlated with cooking loss and negatively correlated with WHC. pH and marination were neither additive nor synergistic. Dark meat with high pH had poor marinade uptake and high cooking yield. Alvarado and Sams (2003) reported that marination of pale and normally colored breast fillets in prerigor condition can reduce the negative effect of PSE meat.

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36
Shelf Life of Fresh and Frozen Poultry

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INTRODUCTION

The shelf life of a food can be defined as the time the food is safe to consume and/or has an acceptable quality to consumers (Fu and Labuza 1993). Poultry meat is one of the most popular muscle foods in the U.S., where the per capita meat consumption has increased from 56.1 pounds in 1985 to 95.8 pounds in 1999 (USDA 2000). Overall, per capita consumption of red meat and poultry has not changed significantly, but when beef, pork, and chicken are examined separately, beef appears to be losing market share to chicken. The report results indicate that consumer concerns regarding beef were related to cholesterol, calorie content, artificial ingredients, convenience, characteristics of how beef is displayed in the store, and price (Resurrección 2003). Other authors reported differentiation by taste, healthiness, and convenience, and by process characteristics like organic production and animal welfare (Grunert and others 2004). Whereas straightforward meat lovers focus mainly on taste as the decisive criterion, indifferent consumers are strongly price oriented (Verbeke and Vackier 2004). Besides, cholesterol has been implicated in diseases associated with modern life, especially in developed countries. These include various cancers and especially coronary heart diseases (Wood and others 2004).

Extending the shelf life of poultry products is a major concern for the poultry industry. The shelf life of poultry depends on several factors, particularly initial bacterial loads, storage temperature, and the gaseous environment around the product (Mead 1990). Teratanavat and Hooker (2004) examined the characteristics of U.S. meat and poultry recalls between 1994 and 2002, and highlighted the public health implications of these trends. Most recalls involve serious health consequences including biological hazards. This may be explained by improving inspection methods for detecting microbial pathogens, greater consumer awareness, and/or better surveillance of food-borne illness.

Food shelf life is not totally dependent on time but also on environmental conditions such as temperature, humidity, light, and oxygen. The prime factors, temperature and humidity, may increase deterioration as they rise, or slow the process as they decrease. However, their impact depends on how widely they vary and on the product itself. Although more difficult to measure in certain cases, sensory quality is the most important characteristic for consumers and processors alike, and in some cases with foods with long shelf life, this attribute may also be an indicator of nutrient quality. More than 75% of further processed chicken products are frozen for distribution. Many of these products are thawed prior to preparation. Ice crystals alter muscle cell

integrity and enable fluid losses that vary with the nature of crystallization.

DETERIORATION MODES OF FRESH AND FROZEN POULTRY

Microbes usually are not a problem since they cannot grow at freezing temperatures unless subjected to extensive temperature abuse above the freezing point. For any specific frozen product, which mode determines its shelf life depends on the product characteristics, prefreezing treatment, freezing process, packaging film and process, and a course of storage conditions. The quality deterioration and potential hazards are usually exaggerated or complicated by a fluctuating time-temperature environment during storage. On the other hand, the shelf life of a frozen food can be extended through ingredient selection, process modification, and change of package or storage conditions (Fu and Labuza 1993). The main modes of fresh and frozen poultry meat deterioration are shown in Table 36.1.

Microorganisms

Microbiological food safety standards have been around for a long time, but reasons for their development and the differences between countries and jurisdictions lead to ambivalence to them by industry and trading countries. There is an underlying assumption that there is a need to protect the public by reducing the contamination level of pathogens in raw meats and poultry even if they are not completely eliminated. But there is little apparent connection between public health goals and standards or guidelines except in a general way on reducing or limiting contamination (Todd 2003). Alvarez-Astorga and others (2002) studied the microbiological quality of retail chicken parts in Spain. The quality of chicken parts was generally regarded as unacceptable since psychrotrophs, *E. coli,* and *S. aureus* counts were higher than the maximum limits established in the guidelines for poultry meat. Other studies (Northcutt and others 2003) revealed that feed withdrawal may have increased prechill carcass counts for *E. coli* and *Campylobacter*, particularly in older broilers, but it had no effect on postchill counts when chlorine was used.

Salmonella and other food-borne pathogens acquire antibiotic resistance by random chromosomal mutations, mutation of existing genes, and through specific mechanisms such as transduction, transformation, and conjugation. In a study, approximately 25% of salmonella serotypes isolated were antibiotic resistant, and throughout the study *S. heidelberg* was identified most often for antibiotic resistance (Nayak and Kenney 2002). Wang and others (2004) studied the sous vide treatment to prolong the shelf life of chicken wings and concluded that the sous vide treatment retarded the microorganism growth within 7 weeks. Jones and Richardson (2004) suggested that salmonella contamination rates are related to poultry feed as a result of mill management practices. Goksoy and others (2000) inoculated fresh chicken breast with *Escherichia coli* and *Campylobacter yeyuni* and exposed it to microwaves. The results indicated that short-time exposure of microorganisms in chicken meat to microwaves had no significant effect on their numbers or growth. *Campylobacter*, a major food-borne pathogen found in poultry products, remains a serious problem for poultry processors, and has been recognized as an important cause of human illness. Case control studies have estimated that 50 to 70% of *Campylobacter* contamination in human food is due to poultry and poultry products. Bashor and others (2004) evaluated washing systems including combinations of inside/outside carcass washers and homemade cabi-

Table 36.1 Mode of deterioration of fresh and freezing poultry.

Food Product	Mode of Deterioration	Critical Environmental Factors	Average Shelf Life
Fresh poultry	Pathogen growth, Microbial decay Rancidity	Oxygen, Temperature, Light	2–7 days at refrigerated temperature
Frozen poultry	Protein denaturation, Color change	Temperature	6–12 months

net washers. These results suggest that carcass washer systems provide minimal reductions in *Campylobacter* populations found on poultry in processing plants. The population of yeast in fresh meat poultry during storage at refrigeration temperature was studied and results suggested that yeast, particularly *Y. lipolytica*, may play a more prominent role than previously recognized in the spoilage of fresh and processed products of poultry stored at 5°C (Ismail and others 2000). Microbial contamination is a serious problem and many decontaminant treatments have been proposed (Table 36.2).

Rancidity

Lipid oxidation is considered to be one of the major problems in the meat industry, due to the resultant flavor deterioration and loss of nutritional value (Ahn and others 1992). Lipid oxidation is a complex process whereby unsaturated fatty acids react with the molecular oxygen via free radicals, and form peroxides or other primary products of oxidation. Secondary oxidation products, such as aldehydes, ketones, and esters, are responsible for the increased deterioration and rancid flavor during frozen storage. Activity of antioxidant depends on the concentration of active compounds present in the samples available to scavenge the free radicals formed during the storage period. Supplementation with antioxidants could be an alternative method to prevent oxidative degradation of the meat during frozen storage when vacuum packaging is not practical (Mielnik and others 2003). In a sensory evaluation study, the descriptive profiling indicated that warmed-over flavor was described by an increase of rancid and sulfur/rubber sensory notes and a concurrent decrease of chicken meaty characteristics (Byrne and others 2002).

Russell and others (2003) studied the effect on the quality of frozen duck meat with the supplementation of tocopheryl acetate. This was done because poultry meat, particularly that of duck, has relatively high levels of unsaturated fatty acids and low levels of antioxidants. Ducks consume twice as much feed as broilers during growth. Other authors (Batifoulier and others 2002) reported that the supplementation of turkeys with α-tocopheryl acetate increased the vitamin E content of microsomal membranes and had a protective effect on lipid oxidation. The supplementation with vitamin E significantly protected free thiols from oxidation but had only a small effect on carbonyl group formation. Beltrán and others (2003) reported that salt and mechanical processing had a greater prooxidant effect on pressurized samples. Oregano supplements to chicken meat protected against stress-induced increase in thiobarbituric acid reactive substances (TBARS) in different muscles with no effect on water-holding capacity (Young and others 2003). Rosemary extract had an antioxidant effect, EDTA strongly inhibited oxidation,

Table 36.2 Mean poultry decontamination treatments (adapted from Capita and others 2002).

Chemical	Physical	Microbiological
Organic acids (lactic acid, acetic acid)	Water (rinse, spray)	Lactic acid bacteria
Inorganic acids	High pressure	Bacteriocins
Chlorine and related compounds	Irradiation	Microbial parasites
Carbohydrates (sucrose, mannose)	Electrical methods	
Organic preservatives (benzoates, propionates)	Air ions	
Bacteriocins or bio peptides (nisin, magainin)	Ultrasonic energy	
Oxidizers (hydrogen peroxide, ozone)	UV light	
Inorganic phosphates	High temperature	
Salts (Nitrite, NaCl)	Freeze-thaw cycling	
Antibiotics		

and hexamethaphosphate also showed antioxidant ability (Beltrán and others 2004).

Protein Denaturation

Protein denaturation is a consequence of the meat microstructure disruption, caused by changes in temperature and/or humidity (Molina-García and others 2004). In a study by Yoon (2002), no significant texture toughening was observed in frozen chicken breast after 10 months of storage at −20°C, suggesting that toughening is not a determinant factor in quality loss of frozen chicken breast.

Color Change

Color, appearance, and texture are important factors that consumers will consider before making a decision to buy poultry. The effect of stunning and decapitation is an important factor for meat quality (Mcneal and others 2003). Rodbotten and others (2004) investigated if meat from different species could be described and related to each other by sensory analysis. Sixty-eight percent of the sensory variation was contained in the first component, which was dominated by color attributes. Qiao and others (2002) reported that extreme variations in breast meat color were related to differences in muscle pH. Instrumental shear force measurement and CIE-Lab color parameters or sensory evaluation techniques can provide reliable information about poultry meat quality. However, these techniques except CIE-Lab color parameters are destructive, time consuming, and unsuitable for on-line application. Visible/near infrared spectroscopy could form the basis for such techniques due to speed, ease of use, and lesser interference from moisture or color of meat samples (Liu and others 2004). When poultry meat is frozen and thawed, an accentuated L value can be achieved, usually indicating a poor quality because of their soft character and extensive water loss, which is similar to the pale, soft, and exudative (PSE) condition in swine white muscle (Galobart and Moran Jr 2004). Variation in the color of raw poultry meat, very light or very dark, has been reported. Barbut (1997) reported the occurrence of PSE pattern in broiler breast muscle to be between 0 and 28%. PSE is associated with meat such as pork.

METHODS TO PROLONG SHELF LIFE

The methods to prolong shelf life of fresh and frozen poultry meat and products are mainly packaging and irradiation.

Packaging

Packaging materials used for meat products are usually plastics, in which polymers with good oxygen-barrier properties are incorporated with polymers with good humidity barrier and sealing properties such as polyethylene and polypropylene. Nam and Ahn (2003a) studied the effects of the combination of aerobic and anaerobic packaging on color, lipid oxidation, and volatile production to establish a modified packaging method to control quality changes in irradiated raw turkey meat. Lipid oxidation is the major problem with aerobically packaged irradiated turkey breast. The same authors (Nam and Ahn 2003b) also studied the effects of double packaging and antioxidant combinations on color, lipid oxidation, and volatiles of irradiated raw turkey breast during refrigerated storage and after cooking. They concluded that the combination of double packaging and antioxidants was more effective in reducing sulfur volatiles and lipid oxidation, when compared with aerobic packaging. In a study to prove the effectiveness of microperforated film on minimizing microbial contamination and evaporative weight loss of fresh meat during chilled storage, Lee and others (2003) concluded that the use of microperforated film was an effective packaging technology for preserving the raw meat during chilled storage. Pettersen and others (2004) investigated the effects of natural and synthetic antioxidants in packaging material under different packaging atmospheres on the oxidative stability of mechanically deboned turkey meat. They observed that a natural antioxidant (α-tocoferol) was used to manufacture the polyethylene layers in the package material, and when meat was stored in such packages in a vacuum or modified atmosphere, the lowest TBARS values and hexanal content were obtained in almost all samples.

The applicability of time-temperature indicators for the quality control of modified atmosphere packaged broiler chicken cuts was evaluated at various

constant and variable temperature conditions. It was found that microbiological shelf life could be considerably improved when the cold-chain was carefully maintained. Temperature had a critical effect on the amount of enterobacteriaceae, proteolytic bacteria, hydrogen sulfide-producing bacteria, and clostridia, the microbial groups most likely to have an effect on the sensory quality. The results also indicated that time-temperature indicators seemed to be useful tools for evaluation of the quality of broiler chicken cuts (Smolander and others 2004). The quality changes in modified atmosphere (MA) packaged broiler chicken cuts during cold storage were monitored using four different method groups, the comparison of the sensitivities of the quality indicating method group was performed using principal component analysis. According to this study, the microbiological analysis and the time-temperature indicators gave the same result and were more critical than either the quality indicating metabolites or sensory evaluations (Vainionpaa and others 2004). Nannerup and others (2004) studied the influence of different packaging and storage parameters on the color stability of modified atmosphere packed, cured, and cooked ham. A multiplicative analysis of variance model was developed. The critical parameters investigated were the percentage of residual oxygen, temperature, light intensity, and oxygen transmission rate. The model illustrated that all the investigated parameters interacted, but especially the percent of residual oxygen influenced the degree of discoloration. Pexara and others (2002) evaluated shelf life by comparing products stored in vacuum and modified atmospheres and concluded that the use of MA packaging in these tests did not extend nor reduce the product shelf life in comparison to vacuum packaging.

Irradiation

The chronology of food irradiation events in poultry started in 1976, when the U.S. Army contracted with commercial companies to study the wholesomeness of irradiated ham, pork, and chicken. In 1990, the FDA approved the irradiation of poultry to control salmonella and other food-borne bacteria. Maximum permitted dosage was set at 3.0 kilogray (kGy), and in 1992 the USDA approved irradiation for poultry to control salmonella and other food-borne bacteria. Although irradiation is the best method to ensure the microbiological safety of raw meat, it caused a few radiolytic meat quality defects. Irradiated pork and poultry meat accelerate lipid oxidation (Katusin-Razem and others 1992). Color changes in irradiated fresh meat occur because of the susceptibility of the myoglobin molecule, especially the iron, to alterations in the chemical environment and to energy input. The potential for iron electrons to exist in various states makes the environment adjacent to the iron atom particularly vulnerable to the presence of electron-donating compounds and high-energy inputs (irradiation). Generation of stable red pigments or brown pigments that become red over time appears to be due to binding of irradiation-generated reactive oxygen species or gasses that become ligands bound by iron under altered reducing conditions. Generation of green pigments appears to be due to breakdown of the porphyrin integrity and/or formation of sulfmyoglobin. Maintenance of ideal meat color during irradiation can be enhanced by many combinations of preslaughter feeding of antioxidants to livestock, optimizing the condition of the meat prior to irradiation, addition of antioxidants, gas atmosphere, packaging, and temperature control (Brewer 2004).

Gomes and others (2003) reported that the irradiated mechanically deboned chicken meat showed higher values for a* (redness) than the nonirradiated samples as from the fourth day under refrigeration. Considering the sensory analysis, color, physochrotrophic bacterial counts and TBARS analyses as a whole, the samples irradiated with doses of 0.0, 3.0, and 4.0 kGy were acceptable under refrigerated storage for 4, 10, and 6 days, respectively. Nonetheless, Nam and others (2003) reported that the redness of meat decreased during the 7-day storage, but irradiated meat maintained redder color than nonirradiated. Irradiated meat produced more sulfur volatiles and aldehydes than nonirradiated meats. Liu and others (2003) reported that a relative amount of oxymyoglobin increases as a result of irradiation. In the same way, Zhu and others (2003) studied the effect of irradiation on the quality of turkey ham during storage. Their results showed that up to 2 kGy of irradiation had had limited effects on the color and oxidation of vacuum-packaged commercial turkey ham. However, irradiation had a significant influence on the odor/flavor of vacuum-packaged turkey ham. Both

sensory panelists and volatiles analysis showed that there were significant changes in sulfur-related odor/flavor in ready-to-eat (RTE) turkey products by irradiation. Irradiation of fresh chicken pieces with a dose of 3 kGy appeared to be able to extend the microbial shelf life by a factor of 2. When the chicken was marinated and irradiated at 3 kGy or when irradiated at 5 kGy without marinating, the microbial shelf life was extended by a factor of 7 to 8. A presence of Salmonella was found in samples irradiated at 5 kGy under vacuum (Mahrour and others 1998).

CONCLUSIONS

It is necessary to prolong the shelf life of fresh poultry products. All available technological tools can be used to comply with strict government regulations to avoid microbiological contamination, mainly pathogens, from slaughter to the consumer. For example, Hazard Analysis and Critical Control Points (HACCP) implementation is a good alternative to achieve this goal.

With regards to frozen poultry, the main problem seems to be rancidity, and there are many studies about antioxidants incorporation. Color and protein denaturation are troubles that can be avoided if the frozen procedure is correctly performed.

Radiation seems to be the newest and safer procedure to prolong shelf life, but more research about the effect of this procedure on flavor and other sensory characteristics is needed.

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Packaging of Fresh and Frozen Poultry

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References

INTRODUCTION

Food packaging constitutes the main tool by which produce could reach consumers from the production centers through distribution and commercialization. For perishable foodstuffs, such as poultry meats and related products, packaging also contributes to keeping the freshness by controlling microbial or chemical alteration during their transport and display on retail, home storage, and up to preparation and serving.

Regarding freezing, the objective of packaging poultry meats also includes maintaining acceptable sensory and functional characteristics of the tissue for as long as possible. Many factors could alter the quality of this product. Among these factors are the intrinsic quality of the meat, including slaughter conditions, onset, developing and resolution of the rigor mortis, and the aging conditions before freezing. In fact, the freezing procedure is an important factor in the subsequent quality of the product. The selection of packaging material is important, particularly for its gas permeability, resulting in important quality attributes such as myoglobin oxidation and lipid rancidity in the meat. Finally, handling conditions are important to preserve the quality until the final destination.

Generally, food packaging aims to maintain quality and protect hygiene during storage and transport. The choice of suitable packaging materials and in particular the suitability of processing conditions should ensure that the impact of undesirable influences in the contents is minimized as much as possible. Packaging used to be regarded as a protection against being touched or a means of protecting the hygiene of the product, but now packaging increasingly serves additional functions. For example, see-through packs enable the buyer to visually judge the content, portion packs can help a consumer to avoid concerns over any leftovers, and modified atmospheres reduce microbial growth.

PACKAGING FUNCTIONS

The main function of packaging fresh and frozen poultry meat is to protect the product against bruising, physical and chemical changes, and microbial contamination; to convey information to retailers and consumers; and to attractively present the product to the consumers. Packaging systems are designed to keep the natural quality of poultry throughout the food chain in a series of events that ends with the preparation and consumption by the consumers. The required shelf life depends on the way in which poultry is commercialized. The shelf life is also influenced by how and where the product was processed, from the farms, including feeding and transportation, until slaughtering, identifying the time and temperature storage parameters (Lundquist 1994).

Most importantly, different markets will have different requirements and a different set of standards and specifications for package performance and shelf life. Packaging is one of the most widely used strategies to extend product life, and is normally used in combination with other technologies (Woods and Church 1999). Meat and poultry packaging can be divided into four levels according to the type of product and the distribution channel. First, the retail packaging is designed to function as protection for cuts or pieces and also as an attractive way to induce consumers to buy it in the marketplace. Secondly, the institutional packaging is employed to supply poultry and meat to the catering sector, including hotels and restaurants. There is also the distribution packaging, used to distribute products or carcasses to consumption centers. Finally, for processed products, the packaging has the main function to prolong the shelf life of these products (Hotchkiss 1995).

Development of Packaging

Packaging is mostly needed during the transfer from production to the retail outlet and on retailing to the consumer. Meat needs to be protected in the meat case, and in the early stages of packaging technology, cellophane, which was later improved by the application of nitrocellulose lacquer to reduce moisture loss and for heat sealing. This was still not a good oxygen barrier. Stretchable plasticized polyvinyl chloride (PVC) films provided a cheaper alternative with better oxygen (O_2) permeability. Case ready packaged poultry with PVC overwrapping on an expanded polystyrene (EPS) tray became the choice system during the 1970s. The success of convenient poultry case ready packaging over other meats was due to less demanding color requirements. While red meats require an oxygen supply on the surface to maintain color, only minced chicken and poultry required it for short periods. Additionally, aging of poultry was limited to 8–24 hours before packaging (Jenkins and Harrington 1991).

A future development consisted of the polyolefin film stretched over the tray, and the heat shrunk film that allowed a tight and hermetically sealed product with additional visual appeal.

The Packing Process

A typical poultry packing process involves weighing and grading whole carcasses before trussing by folding the wings behind the back and tucking the shanks into the open cavity of the body. There are two main systems of chilling:

- For immersion chilling, the giblets, prepacked in polythene bags, are inserted into the body cavity. The carcass is then placed into a polythene bag and taped or clip-sealed. The process is similar for turkeys, but the bag is oxygen-impermeable and shrink-wrapped with the air evacuated and shrinking is done in a hot water shrinking-tunnel, before passing into the freezing step. This practice of giblets handling, which commonly involves pooling and contributes to contamination, is likely to be abandoned (ACMSF 1996).
- For air chilling, poultry is packed without the giblets, and commonly in polystyrene trays and wrapped in film, or sometimes bulk packed in polyethene-lined cardboard boxes.

Application of Robotic Packaging

Mechanization and the development of robotics application to the meat industry have enabled food manufacturers to use advanced robots integrating vision and sensing systems and even intelligence systems to a range of applications. Harsh working conditions, health-related issues, high labor turnover, and stringent requirements of hygiene make the meat-processing sector the logical place for application of automated systems. Deboning and cutting systems have been operating successfully, but some of the

packing applications are being developed for the poultry industry. Primary assembly and packaging, including inspection identification and handling, involve the assembly of items from primary processing into individual containers. Previous steps include weighing, sorting, and grading. Packing applications are in use for sausages and processed meats. A system that could bring considerable savings to the producer is a system to prepare fixed-weight-fixed-price packaging in which trays of product are assembled from individual portions such as drumsticks or thighs by a robotic system that minimizes excess weight per packet and maximizes productivity (Khodabandehloo 1993).

More widely developed is the application of robotics for secondary assembly and packaging, which involves collation of cartons or trays into larger boxes, and palletization and wrapping of pallets with cling film and further deployment (Dore and Sharp 1993).

PACKAGING MATERIALS

A great variety of packaging materials are employed for packaging of fresh and frozen poultry. In most cases, the substance is elaborated by the combination of different materials to obtain a composite material with unique properties. Factors to take into consideration regarding these composed materials are the control of the oxygen permeability, humidity, hardness, and stability, besides the capacity of impression and sealing properties, plus heat resistance properties, market requirements, and costs (Lundquist 1994).

For retail of fresh or frozen poultry meat, the packaging can be rigid, semirigid, or flexible, depending on the materials and packaging needs. One of the more important catalysts for packaging advancement has been the development of multifunctional plastics. These advancements are partially due to the developments in high-barrier plastics (Balasubramaniam and others 1997). Oxygen permeation is the most important parameter to consider when selecting the packaging material.

Synthetic Materials

Synthetic packaging has many advantages in fresh and frozen poultry meat packaging, mainly due to their water and gas permeability properties, in addition to their mechanical qualities. The latter properties include tension resistance, thermal properties such as sealing and shrinking, and opacity to ultraviolet (UV) radiations among others. In this way, plastic films are those materials with a thickness up to 25.4 μmeter (m), and below this thickness the materials are named sheets. When two or more films are stuck together, they form a laminated sheet or film, but if they are extruded jointly the result is a composed film (Hanlon 1992).

Many interesting modifications to synthetic films have been reported recently. Murphy and others (2002) studied the effect of the packaging thickness on the heat transfer and the microorganisms' lethality in vacuum-packaged cooked poultry breast. Employing two different thicknesses, 0.0762 and 0.2032 millimeter (mm), they found that during pasteurization (68°C internal temperature) the packaging thickness affected the heat penetration and consequently the thermal inactivation of microorganisms. The thicker film had a lower heat transfer and consequently a lower thermal lethality than the products packed within a thinner film. The functions, properties, and applications of the packaging materials were reviewed by Dawson (2001), but as this is an area that is in constant development, commercial suppliers maintain up-to-date product information and specifications (i.e., Anon 1995, Anon 2004a, Anon 2004b).

For fresh case ready packaging, the following materials are used: stretchable and shrinkable films, absorbent pads, trays, bags for whole birds, pouches for leg quarters and breasts, thermoform rollstock, and chub films for ground poultry. The absorbent pads are an innovation that improves appearance and acceptability, but also by controlling the free water within the pack, the spoilage rate is reduced. Some trays have an absorbent layer built in within the tray. A recent development is the use of hermetic tray packages, which in the UK is driven by guidelines that aim to reduce potential cross contamination due to juices leaking from case ready packages. The materials for postpackaging cooking could be barrier shrink or barrier nonshrink, in a selection of adhesion characteristics, or barrier or nonbarrier.

Another alternative that allows hermetical sealing are the barrier foam trays that have a barrier film laminated onto the inside surface, resulting in a tray with a transparent sealed film on top that envelops the product attractively. Some barrier films could be formulated with antifog properties, some are microwave

heating compatible, and some applications incorporate a UV light-activated oxygen scavenging system on the film.

Toxicity of Packaging Materials

The contact of the packaging material with foods could in some cases allow for transfer of material from the packaging into the product, causing tainting or food safety issues. When this is a known possibility, these materials will normally not be used, and legislation and regulations have been developed to protect consumers. Some cases of particular concern have been irradiation and microwavable packaging. Microwave cookware and packaging materials are a concern because contaminant migration rates could be increased in relation to high temperatures or thermal degradation or products from benzene formed in or by contact with susceptors. The mechanisms of transfer could include self migration, leaching, or chemical attack, and has been reviewed by Davies (1991). In the U.S., the United States Department of Agriculture's (USDA) Food Safety and Inspection Service monitors the meat packaging materials to be compliant with the Food and Drug Administration (FDA) requirements.

Edible Films

In the last several years, interest in edible films has increased because of environmental concerns and issues about plastic degradation. In addition, the search for new storage procedures and packing techniques creates opportunities and new markets for underutilized agricultural products (Ouattara and others 2000). The edible films require modifications to improve their physical and mechanical properties and avoid chemical changes. This can be achieved with the incorporation of plasticizers that reduce the polymer intermolecular forces increasing the flexibility and extensibility of the film. On the other hand, the addition of plasticizers increases the gas, water vapor, and solutes permeability, but decreases film elasticity and cohesion. Edible plasticizers such as mono-, di-, or oligosaccharides, lipids and their derivates, and polyols have been evaluated (Choi and Han 2001).

One example of a preparation of edible films is one with a concentration of 5–15% of isolate protein in distilled water with an added plasticizer (commonly glycerol) and a functional additive such as an antioxidant or antimicrobial agent (i.e., organic acids or bacteriocins) could be added, adjusting the pH (acid near to 2.5 or alkaline, up to 10). The mixtures are degasified and the film formation is generally at room temperature (23°C) with controlled conditions of humidity.

TYPES OF PACKAGING

According to their function, the packaging can be divided in nonpreservative, preservative, and double-phase packaging. Nonpreservative packaging protects the product against contamination and loss of water, without creating different conditions to the external environment. In this way, unless the product will be frozen or refrigerated, it continues to be highly perishable. Preservative packaging is characterized by its ability to extend the poultry meat shelf life by modifying or restricting the microbial growth by creating or maintaining different environmental conditions (Bell 2001). The preservative packaging employed in meat freezing has three kinds of modified packaging conditions that limit the microbial growth. Those conditions can be considered as one because all three employ a modification of the surrounding environment, mostly the gas atmosphere: vacuum, modified atmosphere, and gas. The primary function of a meat package is to contain the meat and prevent its contamination and is easily accomplished with the range of plastic materials available today. The composition of the atmosphere around the product determines the meat color and the nature of spoilage that develops (Balasubramaniam and Chinnan 1997). The one packaging system that combines the extended shelf life and the potential for retail sale is based on the change of the gaseous environment that surrounds the meat between the storage and the commercialization stage. This can be achieved by removing part of the oxygen and replacing it with carbon dioxide as a preservative atmosphere and is denominated double-phase packaging (Bell 2001).

Modification of the Packaging Atmosphere

Modified Atmosphere Packaging or Gas Packaging

Modified atmosphere packaging (MAP) is defined as the enclosure of a food product in gas-barrier ma-

terials while providing an environment that has been changed or modified to inhibit the action of spoilage agents. This results in either maintenance of the quality of a perishable food during its natural life, or the actual extension of the shelf life. In gas packaging, the product is generally stored in an atmosphere containing an appropriate composition of nitrogen (N_2), carbon dioxide (CO_2), and oxygen (O_2). Nitrogen is an inert gas used primarily to prevent package collapse and with foods that absorb the CO_2, or simply to displace the oxygen to retard oxidation (Balasubramaniam and Chinnan 1997, Hood and Mead 1993).

The shelf life of poultry packaged in MAP is dependent on several factors such as the gas composition, storage temperature, the degree of initial carcass contamination, the film permeability to oxygen and carbon dioxide, and the headspace volume in the package. Several short-chain organic acids, which are generally recognized as safe (GRAS), have been applied for poultry carcass and meat cut decontamination, by spraying or immersing samples in test solutions. Jiménez and others (1999) employed 1% acetic acid in combination with MAP (70% CO_2/30% N_2), during 21 days at 4°C. The use of MAP, or its combination with acetic acid decontamination, was sufficient to suppress growth of pseu-domonas and resulted in an extended lag phase of lactobacilli up to 7 days. Similar growth delay was revealed for total viable counts and in enterobacteria only when both hurdles were used simultaneously. None of the samples showed production of slime at the end of the storage periods, but untreated samples did have off odors. Acetic acid decontamination suppressed the off odor production in samples stored for 21 days. Therefore, MAP with acetic acid decontamination could be used successfully to improve the shelf life of fresh chicken breast during chilled storage. Vainionpää and others (2004) used time temperature indicators to monitor the quality of broiler chicken cuts at various constant and variable temperature conditions and under MAP (80% CO_2/20% N_2). They found that microbiological shelf life could be considerably improved when the cold chain was carefully maintained. Temperature had a critical effect on the microbiological quality and a consequent negative effect on the sensory quality. Higher temperature (7.7°C) samples were rejected after 5 days of storage. Lower temperatures (6.6°C) extended the rejection time to 9 days, and the lowest temperature employed (5.5°C) extended the rejection until 12 storage days. Church (1993) reviewed the applications of MAP to poultry products. In pale cooked products, the color is not as relevant as in cured products or those from darker meats, where color stability is an issue. A possible spoilage potential from lactic acid bacteria has also been widely discussed. Packaging systems offer a good solution to maintain oxidative stability in cooked or fried products.

Vacuum Packaging

Vacuum packaging involves placing a product in a film of low-oxygen permeability, removing air from the package, and applying a hermetic seal. The O_2 level is reduced to less than 1% by vacuum packaging. The method has been shown to be effective in inhibiting bacterial growth at low temperatures, resulting in reduced spoilage and extended product shelf life (Balasubramaniam and others 1997).

Skin Packaging

Skin packaging is essentially a modified form of vacuum packaging. A preheated film is dropped onto the product that is supported on a lower web of the same film. The air between the two films is withdrawn and an upper web forms around the food to produce a skintight package that is then heat sealed in a vacuum chamber. It is used primarily for packaging frozen meat products (Balasubramaniam and others 1997). A study by Kartika and others (2003) concluded that in vacuum-skin packaged chicken, a brine rinsing pretreatment contributed to the yield and overall quality of the product, with lower bacterial counts and a significant interaction between film and rinse for coliform levels. The barrier properties of the film (oxygen transmission rate) significantly influenced the redness of the product. Bureau (1986) reviewed the effects of permeability on microbial growth and compared the carbon dioxide and vacuum contribution to bacterial spoilage. When products are going to be pasteurized after film packaging, it should be considered that film thickness influences the rate of heat transfer during the heating and cooling process (Murphy and others 2002).

Active Packaging

While some of the principles of active packaging date back many decades, most of the current

technologies were developed during the 1990s, but some claims still need to be sustained and the innovations need to be proved for commercial viability (Rooney 1995). This innovative concept in packaging technology can be defined like a system in which the poultry meat, the packaging material, and the environment interact to extend the shelf life, ensuring the innocuousness and the sensory properties (Suppakul and others 2003). There are basically two kinds of active packaging. The first is called "active packaging" and coats the package with compounds that remove or scavenge the oxygen, absorb and control the humidity, and/or generate ethanol or carbon dioxide. Protein-active packaging films could be employed to avoid the superficial application of preservatives in foodstuffs, maintaining a relatively high and constant inhibitory action of the liberated compounds (Teerakan and others 2002). A shelf extension system (Ageless FX100 and PVDC-coated nylon) was used to achieve a 3-week shelf life of precooked chicken nuggets stored at 0–4°C, preventing discoloration and rancidity (Smith and others 1995). There are some concerns regarding the use of oxygen-absorbent technology in relation to the potential growth of a range of microbial pathogens that would be able to grow under anaerobic conditions, and some of them (i.e., *Listeria monocytogenes* and *Clostridium botulinum*) at refrigeration temperatures. The UK's Advisory Committee on the Microbiological Safety of Food reviewed the risks of vacuum packing of products including poultry, and prepared a report that identifies *C. botulinum* as the major risk, and recommended a number of control measures in relation to the application of the technology, but also for products intended to have a shelf life of more than 10 days, the application of combined preservation technologies, and temperature control to minimize risks (ACMSF 1992).

A second type of active packaging is called "antimicrobial packaging" because this system applies controlled liberation systems of an antimicrobial agent from the transporter (the film), to the semiliquid foodstuff phase, in order to keep a predetermined constant concentration of the active component for the storage time. In this way it is possible to control or prevent the growth of nondesirable bacteria responsible for packed food degradation and pathogens (Bouncore and others 2003). Ming and others (1997) demonstrated the possibility of reducing the risk but not controlling growth of *L. monocytogenes* by application of pediocin to the film's surface. Polymeric films control the diffusion of low molecular weight compounds from outside to inside or in the opposite direction (Del Nobile and others 2003). Edible films can be used as well as packaging materials acting like "delivery ingredient systems" in the application or liberation of preweighed ingredients during processing to avoid errors in the measurement and handling of these compounds (Kim and Ustonol 2001). Alteri and others (2004) had proposed to produce oxygen scavenger films using aerobic microorganisms as the "active compounds."

Irradiation Plus Packaging

Ionization radiation had been proposed as a food preservation method since 1930. Nonetheless, a great number of reactions result from the irradiation due to the complex meat and poultry composition. Chemical and nutritional effects on the amino acids profile are not detectable at 10 kilogray (kGy) doses. The absence of free radicals in meat at low radiation doses prevents a more significant loss of amino acids (Elias 1985).

Double Packaging

Packaging technologies could be used to overcome some of the issues with flavor development in irradiated poultry meats. Modifying packaging conditions may minimize the quality deterioration of irradiated meat. A new alternative is the process called "double packaging," which aims to control the accumulation of volatile off flavors. There are two proposed models:

- In the first model, poultry is first packed in oxygen-permeable bags and after a period of time, the product is repackaged in oxygen-impermeable plastic bags and irradiated. The exterior bags can then be removed to expose the product, still packed, for a few days during retail.
- In the second model, the product is aerobically packaged and irradiated, and vacuum packaged after a few days' storage.

The advantages of this double-packaging concept are (a) complete off odor elimination, (b) pink color in the poultry meat surface could be reduced, and (c) the use of additives can be eliminated (Nam and Ahn 2002).

The color and odor changes in meat due to irradiation are highly dependent upon packaging conditions. An appropriate combination of aerobic and vacuum packaging can be effective in minimizing lipid oxidation and off odor volatiles in irradiated turkey breast during storage. It may also be effective in reducing pink color irradiated meat. The volatiles profile of irradiated turkey breast meat was highly dependent upon packaging conditions during irradiation and storage. Under aerobic conditions, almost all S-components disappeared during a 10 day-storage period. Therefore, aerobic packaging was more effective than vacuum packaging in reducing volatiles responsible for irradiation off odor. However, aerobic packaging promoted lipid oxidation in meats as detected by the increased amount of propanal and hexanal as well as thiobarbituric acid reactive substance (TBARS). When lipid oxidation and irradiation off odor were considered, double packaging of turkey breast meat was more desirable than aerobic or vacuum packaging alone. Double packaging volatile characteristics were somewhere between the aerobic and vacuum packaging meats depending upon the number of days. Exposing the double-packaging irradiated meat to aerobic conditions by removing the outer vacuum bag 3 days before the "sell by" or "use by" date would be desirable to reduce S-volatiles and minimize lipid oxidation in turkey breast meat. Therefore, double packaging alone was not enough to solve both lipid oxidation and off odor problems simultaneously in irradiated thigh meat (Nam and Ahn 2003).

From irradiated poultry tests, Bagorogoza and others (2001) reported that packaging had no significant effects on any sensory attributes evaluated by a sensory panel. However, an interaction was found between the packaging and irradiation effect on stale flavor of cooked turkey breast. Samples irradiated in air packaging had significantly higher scores than those nonirradiated in air packaging, whereas both irradiated and nonirradiated nitrogen-packaged turkey breasts had similar scores. During frozen storage, raw ground meat undergoes several changes that can reduce its quality, such as lipid peroxidation. The addition of butylated hydroxytoluene (BHT) or tocopherol to minced chicken meat retarded the onset of oxidative rancidity during storage, as measured by thiobarbituric (TBA) value, carbonyl content, and general acceptability. Addition of antioxidants to chicken meat prior to low-dose (2.5 kGy) gamma irradiation lowered TBA values, carbonyl, and free fatty acid content as compared to irradiation treatment alone (Kanatt and others 1999). Du and others (2002) found that changes in color and aroma of irradiated vacuum packaged broiler fillets were higher than in the aerobic packaging. Ahn and others (2001) found a similar situation in turkey, when aerobic storage resulted in lower cholesterol oxidation products produced during cooking and storage. Eilamo and others (1998) reported an experiment that studied the effects of headspace volume, oxygen transfer of the package, then residual carbon dioxide and residual oxygen on raw chicken legs, packed in film-wrapped trays. Most of the odor development was related to the time and temperature of storage, but carbon dioxide concentration and headspace are significant packaging parameters. Large headspace inhibited sulphite-reducing clostridia.

FROZEN POULTRY PACKAGING IMPLICATIONS

Besides the poultry packaging, some recent advances have been made in this step of the process. The use of evaporated air chilling, where the carcasses are sprayed with a thin water film and cooled by blowing air onto them at low temperature (2 to 4°C), had been proposed as a quick and effective chilling method since the evaporation of water removes heat from carcasses (Mielnik and others 1999). Another important problem during poultry carcasses chilling is the remaining water produced. Two processes have been suggested. One is the advanced oxidation process of UV-enhanced ozonation as a treatment method for improving the microbiological safety, turbidity, and water use of overflow poultry chiller water. This process allows the water to be reconditioned for reuse (Diaz and others 2002). The other process is the chlorination of water in spray washers or chillers. This method has been used to reduce microbial contamination and cross-contamination of chilled carcasses in poultry processing plants. Electrochemically activated solutions containing a mixture of chlorine, peroxide, and chlorine oxides (50 parts per million [ppm] of free chlorine) used in in/out and chilling treatments reduced contamination (Yang and others 1999).

Changes in Packaging Poultry Due to Frozen Storage

Enzyme Activity

Low temperature in meat conservation causes structural changes in myofibrils during freezing, due to remaining activity of enzymes and the interaction among lipids, formaldehyde, and other proteins (Matsumoto 1980). Storage at low freezing temperatures can slow but not inactivate these enzymes in the tissue. In poultry meat, the enzymes that remain active are lipolytic (lipases and phospholipases) and proteolytic. Hydrolytic enzymes in frozen foods can result in quality denaturation, and the only way to avoid the consequences of lipolysis in frozen foods is to use processes involving fast freezing and minimization of ice recrystallization in the product (Sista and others 1997).

Lipid Oxidation

The major strategies for reducing lipid oxidation in mechanically deboned poultry meat (MDPM) are using free radical terminators, such as phenolic antioxidants, and restricting the access of oxygen during storage. Packaging material used for meat products are usually plastics, in which polymers with good O_2-barrier properties are incorporated with polymers with good humidity barrier and selling properties. The samples were MAP (60% O_2/ 40% N_2), plus α-tocopherol. Based on the results of the current work, it may be concluded that oxidation on MDPM was affected by the presence of oxygen (i.e., packaging atmosphere) and storage time. However, the most important factor was packaging atmosphere; the rancidity of the MDPM stored in air was significantly higher than that stored in a vacuum or modified atmosphere. MDPM stored in packages where a natural antioxidant (α-tocopherol) was used in production of one of the polyelectrolyte (PE) layers, had, in almost every instance, the lowest TBARS values and hexanal content when stored in a vacuum or modified atmosphere. However, the difference was not proven to be statistically significant. Neither TBARS values nor hexanal content showed dependency on the temperature profile of freezing, or freezing/thawing refreezing investigated in this study (Pettersen and others 2004). The use of onion cultivars rich in quercetin as a natural food antioxidant might be promising for extending the shelf life of cook-chill chicken meat, as an alternative to using synthetic antioxidants in lacquered cans stored at −5°C (Karastogiannidou 1999).

Kilic and Richards (2003) reported inhibition of lipid oxidation in poultry doner kebab by sodium ascorbate and vacuum packaging, and they observed a prooxidant effect of the ascorbate in the presence of mechanically recovered turkey meat.

Moisture Migration

Moisture migration is the principal physical change in frozen foods and has major effects on the chemical and biochemical properties of frozen poultry. Moisture migration or moisture loss can be present in several ways: moisture loss by sublimation, moisture absorption and redistribution in foods or food components, recrystallization of ice, and drip loss during thawing. Apart from appearance, loss of moisture will also affect the juiciness and texture of meat, as well as have a significant effect on weight loss. On the other hand, during the chilling of meat, some surface drying is necessary to prevent or retard microbial growth by reducing the surface water activity, as well as to avoid a glassy appearance when the meat is frozen (Phan and Mawson 1997).

Freezing and Thawing

The main problem reported in relation to the process of freezing and thawing is the formation of large protein aggregates within the meat structure, resulting in water displacement, proteins moving closer together, and cross linking and incomplete rehydration, when the protein-water affinity is the same or less than the protein-protein affinity (Matsumoto 1980). Yoon (2002) reported no significant texture toughening in frozen chicken breast after 10 months of storage at −20°C, suggesting that toughening is not a determining factor in the quality loss of frozen chicken breast when the samples were treated with 10% trisodium phosphate or sodium tripolyphosphate solution before frozen storage. The improvement of the water-binding ability of chicken meat without the ice crystal formation during frozen storage is most important for preserving the eating quality of frozen chicken breast.

Freeze-crack

There are two types of freeze-crack: (a) surface only cracks and (b) cracks originating from inside the product, which then progresses to the surface. The mechanical damage is induced by cryogenic freezing due to volumetric changes associated with water-ice phase transition (Hung 1997).

Freezer Burn

Freezer burn can be reduced if poultry carcasses or cuts are suitably packaged in a tight-fitting film that is impermeable to water and vapor or if the cold storage facility is maintained at a high relative humidity (i.e., storage over ice). Freezer burn is most commonly associated with damaged packaging, because the loss of package integrity allows areas of the product surface to be exposed to the external environment (Taylor 1985).

The selection of films for packaging primarily considers the functionality in relation to the food to be protected and the suitability for the application, in relation to the use and consumer needs. However, Stiles (1991) suggests that the environmental acceptability should be a major concern for the meat industry and that the methods of disposal should be considered, avoiding chloride-containing polymers if the refuse is likely to be incinerated.

The technology and materials available for poultry packaging have been constantly evolving, and this discipline is becoming more specialized requiring expertise and know-how from a range of technologists and engineers, equipment and materials, and manufacturers and suppliers. Some innovative systems are being tested and developed to provide even more convenience to consumers, more flexibility and safety, and a wider range of attractive products.

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38
Microbiological and Sensory Properties of Fresh and Frozen Poultry

Lisa McKee

Introduction
General Microbial Types and Loads on Poultry
    Common Pathogens on Poultry
    Common Spoilage Organisms on Poultry
Microbial Loads and Sensory Properties of Fresh and Frozen Poultry
Methods for Reducing Microbial Loads and Sensory Properties of Poultry
    Atmosphere Modifications
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Conclusion
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INTRODUCTION

Poultry consumption has risen dramatically in the United States over the past 25 years. (See Table 38.1) Although the term poultry can include everything from chicken and turkey to duck and geese, approximately 98% of poultry consumption is in the form of chicken and turkey (National Chicken Council 2002).

As consumption of poultry has increased, so has concern over consumer handling of such products. Numerous factors are involved in outbreaks of poultry-borne illness, including cross contamination (Brown and others 1988, Bryan and Doyle 1995), inadequate cooking (Bean and Griffin 1990, Bryan 1980), preparation of food well in advance of consumption (Worsfold and Griffith 1997), improper handling of food (Altekruse and others 1996, Shiferaw and others 2000), and temperature abuse during transport, preparation, or storage (Worsfold and Griffith 1997). A particular problem that is consistently reported is consumers not washing their hands or cutting boards after handling raw poultry. Although a variety of educational strategies have been designed to improve consumer food safety knowledge and safe food handling skills, poultry-related food-borne illness problems persist. (See Table 38.2)

GENERAL MICROBIAL TYPES AND LOADS ON POULTRY

Raw poultry is often highly contaminated. The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) recognizes the challenge in reducing the microbial contamination of such products. The Pathogen Reduction, Hazard Analysis and Critical Control Point (PR-HACCP) Systems rule, published in 1996, requires poultry plants to develop and implement HACCP plans and sanitation standard operating procedures. All plants must test for generic *Escherichia coli* as an indicator of fecal contamination and must ensure *Salmonella* rates are below standards set in the rule (USDA-FSIS 2000b, Schlosser and others 2000).

A number of microorganisms can be found in raw poultry meat. Lahellec and others (1975) isolated 5,920 strains of psychrotrophic bacteria from chicken carcasses in three processing plants, including

Table 38.1. U.S. per capita consumption of poultry and other meats.

	Pounds per capita per year			
Year	Chicken	Turkey	Beef	Pork
1980	32.7	8.1	72.1	52.1
1990	42.4	13.8	63.9	46.4
2001	52.4	13.8	63.1	46.9

Compiled from Economic Research Service/USDA data

Pseudomonas, *Achromobacter*, *Flavobacterium*, enterobacteriaceae, and *Corynebacterium*. Pathogens such as *Salmonella* and *Campylobacter* are also frequently reported contaminants on poultry. Loads vary widely, ranging from less than $\log_{10}$ 1.0 colony-forming units (CFU)/square centimeters (cm^2) to greater than $\log_{10}$ 7.0 CFU/cm^2, but are reported to most commonly be on the order of $\log_{10}$ 4.0 CFU/cm^2 (National Advisory Committee on Microbiological Criteria for Foods 1997). Sources of poultry microorganisms also range widely and include everything from farm management practices and transportation to processing operations such as defeathering, scalding, and chilling.

Common Pathogens on Poultry

Poultry products are often thought to be a significant source of *Salmonella*. There are thousands of recognized serovars of this rod-shaped, gram-negative, facultative anaerobe. The bacteria have been reported to grow in a wide range of temperatures and pH levels, are typically inhibited by 3% to 4% sodium chloride (NaCl) and generally require an

Table 38.2. Selected food-borne illness outbreaks associated with poultry.

Year	Potential Source	Organism	Place(s)
1982	poultry giblet gravy	*Salmonella enteritidis*	Maine
1983	chicken liver paté	*Salmonella heidelberg*	Maine
1986	improperly thawed and cooked chicken	*Salmonella*	Oklahoma
1988	turkey franks	*Listeria monocytogenes*	Oklahoma
1990	improperly thawed and cooked turkey	*Salmonella reading*	Connecticut
1995	chicken salad	*Cryptosporidium parvum*	Minnesota
1996	lettuce cross contaminated with raw chicken	*Campylobacter jejuni*	Oklahoma
1999	chicken	*Campylobacter jejuni*	England
2000	chicken salad sandwiches	Group A rotovirus	District of Columbia
2000	deli turkey meat	*Listeria monocytogenes*	New York, Georgia, Connecticut, Ohio, Michigan, California, Pennsylvania, Utah, Tennessee, Wisconsin
2002	deli turkey meat	*Listeria monocytogenes*	Pennsylvania, New York, Delaware, Maryland, New Jersey, Connecticut, Michigan, Massachusetts

Compiled from Morbidity and Mortality Weekly reports with the exception of 1999 outbreak from Pearson and others 2000.

a_w of at least 0.93. *Salmonella* infections resulting in enterocolitis/gastroenteritis produce symptoms ranging from abdominal pain and diarrhea to nausea and vomiting. Other syndromes associated with salmonellosis include enteric (typhoid) fever and chronic conditions such as arthritis and Reiter's syndrome.

Although many consumers commonly associate *Salmonella* with poultry products, detection rates for the organism in such products is often low. In a baseline study conducted prior to implementation of the PR-HACCP rule, Schlosser and others (2000) reported an average rate of 15.9% in chicken carcasses and 11.9% in turkey carcasses for the four most frequently detected *Salmonella* species. *Salmonella heidelberg* and *Salmonella hadar* were two of the most common species detected in both chicken and turkey carcasses. Only 10.9% of the 5,697 rinse samples taken from broiler carcasses collected from 124 large processing plants during the first year of HACCP implementation were *Salmonella* positive (USDA-FSIS 2000a). Slightly higher rates of *Salmonella* were detected in raw chickens evaluated in England 25%; (Jorgensen and others 2002) and Spain 35.8%; (Dominguez and others 2002), but only 6.8% of chicken carcasses were *Salmonella* positive in a study conducted in the Accra, Ghana, metropolis (Sackey and others 2001).

Campylobacter spp. were not identified as a significant human pathogen until 1977. The organisms are gram-negative, asporogenous rods that may be aerobic, anaerobic, or microaerophilic depending on the strain. Low pH levels, temperatures less than 30°C, high oxygen levels, and drying inhibit growth of *Campylobacter*. *Campylobacter* infections cause fever, abdominal pain, and diarrhea. Incidence is generally highest in infants, but a potential high risk of campylobacteriosis has also been reported for 18 to 30 year olds (Bryan and Doyle 1995, Rosenquist and others 2003).

Detection rates for *Campylobacter* spp. in poultry are typically much higher than those for *Salmonella* and are frequently reported to be greater than 50% of samples evaluated (Park and Stankiewicz 1981, Rayes and others 1983, Moore and others 2002, Pezzotti and others 2003). Populations of up to 10^7 organisms per uneviscerated chicken carcass (Hood and others 1988) and 10^6 organisms per eviscerated chicken carcass (Stern and others 1985, Waldroup and others 1992, Tokumaru and others 1991, Jorgensen and others 2002) have been reported. Case-controlled studies have implicated handling raw poultry, consumption of chicken and turkey, consumption of undercooked poultry, and cross-contamination of poultry with salads and ready-to-eat foods as sources of *Campylobacter* illnesses (Hopkins and Scott 1983, Hopkins and others 1984, Harris and others 1986, Deming and others 1987).

Testing for generic *Escherichia coli* is required by the PR-HACCP systems rule for all poultry slaughter plants as an indication of proper processing and sanitation procedures (USDA-FSIS 2000b). Detection rates for the organism in poultry have varied widely, with reported levels ranging from 11.9% in turkey and 38.7% in chicken (Zhao and others 2001) to nearly 100% in chicken (Northcutt and others 2003). *Listeria* spp. have also been detected in poultry. Studies by Genigeorgis and others (1989, 1990) indicated a rate of 40.6% in chicken and 45.0% in turkey for overall *Listeria*, with *Listeria monocytogenes* being the most prevalent type. The wings of both chickens and turkeys are frequently the most contaminated.

Common Spoilage Organisms on Poultry

As with most meat processing, poultry slaughter involves a shift from a mesophilic environment at the start of processing to a psychrotrophic environment at the end of the production cycle. Spoilage of poultry is therefore most often associated with psychrotrophic microorganisms. Storage and packaging conditions also influence the microorganisms associated with spoilage in poultry products.

Pseudomonas spp. are the most common organisms associated with spoilage of refrigerated meats such as poultry stored under aerobic conditions. In addition to being psychrotrophic, the pseudomonads are able to grow at pH levels of 5.5 to 7.0 and are highly oxidative, which gives them the ability to use nitrogen compounds as an energy source. These characteristics give pseudomonads a competitive advantage in spoilage of poultry and other meats.

Lactobacillus spp. are often associated with spoilage of refrigerated poultry stored under microaerophilic or anaerobic conditions. These gram-positive rods are common on many foods but are generally present in very low levels on freshly slaughtered poultry. Cold temperatures and vacuum packaging, however, encourage the growth of *Lactobacillus*.

Yeast loads have been reported to increase in both chicken and turkey during cold storage (Ismail and others 2000) and yeasts were the fifth most common organism detected in a study of 5,920 psychrotrophic organisms isolated from chickens (Lahellec and others 1975). Proteolytic and/or lipolytic yeasts such as *Candida zeylanoides* and *Yarrowia lipolytica* are some of the most common species isolated from fresh and spoiled poultry (Viljoen and others 1998, Diriye and others 1993).

MICROBIAL LOADS AND SENSORY PROPERTIES OF FRESH AND FROZEN POULTRY

Spoilage of fresh and frozen poultry meat is associated with changes in the organoleptic properties of the products. The psychrotrophic microorganisms responsible for spoilage of poultry meat are typically present at low levels immediately after slaughter but flourish rapidly during cold storage to produce changes in the aroma, appearance, and texture of the meat.

Off odors are typically one of the first organoleptic characteristics to be detected in spoiling poultry and become noticeable when microbial levels reach 10^6 to 10^8 CFU/cm^2. Pseudomonads are by far the most commonly reported microorganisms associated with the off odors of spoiled poultry. Most of the microbial load in chicken used in a study by Chen and others (1991) was found to be pseudomonads. When rated on a scale of 1=absent or excellent to 9=extremely poor, aroma scores of both raw and simmered chicken were positively correlated with microbial loads. Intensity of aroma increased as aroma became less desirable. Controlled spoilage at 3°C resulted in odors described subjectively as "sulfur," "dishrag," "ammonia," "wet dog," "skunk," "dirty socks," "rancid fish," and "canned corn" (Russell and others 1995). These odors were attributed primarily to *Shewanella putrefaciens* A, B, and D; *Pseudomonas fluorescens* A, B, and D; and *Pseudomonas fragi*. Subjective detection of sulfur-like odors associated with pseudomonads such as *Pseudomonas fluorescens* and *Pseudomonas putida* has also been reported by other researchers (Viehweg and others 1989, Russell 1997).

Off odors detected subjectively in spoiling poultry are associated with sulfur-containing compounds. Dimethyl sulfide, dimethyl disulfide, and propylene sulfide are some of the compounds frequently identified in spoiled poultry (Pittard and others 1982, Bowman and others 1983). Although these compounds are typically associated with organisms such as *Pseudomonas fluorescens* and *Pseudomonas putida*, the specific strain of bacteria, the storage conditions, and the substrate will influence the exact compounds produced during low temperature spoilage of poultry.

Sliminess on poultry typically occurs after off odors are apparent and has been associated with microbial levels of 10^6 to 10^9 CFU/cm^2. Vareltzis and others (1997) noted slime formation under the wings and between the leg and breast of whole chicken carcasses after 5 days of storage in oxygen-permeable packaging at 4°C. *Pseudomonas* populations on the carcasses were reported to be 10^6 CFU/cm^2 at the time slime was detected. A positive correlation between subjective raw sliminess and microbial loads was reported by Chen and others (1991). The slime smear test was positively correlated to both sensory evaluation of sliminess and to pseudomonad, total aerobic plate, total aerobic psychrotrophic, and fluorescent pseudomonad counts.

METHODS FOR REDUCING MICROBIAL LOADS AND SENSORY PROPERTIES OF POULTRY

Atmosphere Modifications

Since it is primarily a surface phenomenon, poultry spoilage is most commonly the result of growth of aerobic bacteria. Modification of the environment around the meat can therefore influence the microflora as well as subsequent spoilage characteristics. Vacuum packaging, carbon dioxide, and nitrogen are some of the most common atmosphere modifications made to increase the shelf life of poultry.

Vacuum packaging involves evacuation of air from the package. The lack of oxygen inhibits growth of gram-negative psychrotrophs such as *Pseudomonas* but results in the growth of facultative anaerobes. Lactic acid bacteria, in particular *Lactobacillus* spp., have been reported to be the predominant spoilage microorganism in poultry packaged under vacuum (Bailey and others 1979, Thomas and others 1984, Sawaya and others 1993). Although vacuum packaging undoubtedly extends the shelf

life of poultry, the effects of such atmosphere modification are dependent on several factors.

Storage temperature has a significant effect on shelf life of vacuum-packaged poultry. Although a panel of five to six judges rated odor as unacceptable in vacuum-packaged chicken carcasses after 17 days of storage at 4°C, color, texture, and overall appearance were acceptable through 20 days of storage (Sawaya and others 1993). Vacuum-packaged carcasses stored at 7°C were rated unacceptable for all characteristics after 17 days and those stored at 9°C were unacceptable at 10 days. Carcasses stored in conventional packages at the same temperatures were judged unacceptable 3 to 4 days sooner than vacuum-packaged samples. Vacuum packaged whole broilers were free of slime through 13 days of storage at about 5°C, but objectionable ammonia-like odors were noted by judges at day 9 in a study by Thomas and others (1984). A less appealing appearance was also noted in vacuum-packaged samples due to yellowing of the skin. Lactic acid odors and greening were reported by Patterson and others (1984) in vacuum-packaged chicken breast portions after 42 days and in leg and thigh portions after 35 days when stored at 1°C. However, off odors were detected after only 28 days in breasts and 15 days in legs and thighs when stored at 4 to 5°C.

Interactions between storage temperature and treatments applied to poultry prior to vacuum packaging also affect the shelf life of the products. Potassium sorbate has generally been found to reduce microbial growth and increase shelf life when vacuum-packaged poultry is stored at temperatures of 1 to 2°C (McMeekin and others 1984, Patterson and others 1984). The positive effects of the sorbate, however, are negated when storage temperatures increase. An untrained taste panel could not detect flavor changes in sorbate-dipped, vacuum-packaged breast fillets through 6 weeks of storage at 2°C nor could the panel differentiate between sorbate-dipped fillets and control, vacuum-packed or untreated fillets (McMeekin and others 1984). Lactic acid also increased the shelf life of vacuum packed chicken legs and thighs stored at 1°C, but the skin and meat of the pieces were described as "gray and unattractive" while those dipped in the potassium sorbate were described as having "an attractive white appearance" (Patterson and others 1984).

Flushing packages with gases such as CO_2 and nitrogen generally results in an increase in shelf life of poultry products by extending the lag phase of aerobic microorganisms. Facultative and true anaerobic microorganisms, however, often find such environments acceptable and are commonly found in high levels in spoiled products stored under such atmospheric modifications. The shift in microflora from aerobic bacteria such as *Pseudomonas* to facultative/anaerobic bacteria such as *Lactobacillus* results in changes in the organoleptic properties and spoilage characteristics.

As with other treatments, shelf life of CO_2-treated poultry is temperature dependent. An environment of 65% CO_2 resulted in lower microbial loads and a slightly longer shelf life (19 days) than environments of either 20% CO_2 (18 days) or air (14 days) when chicken samples were stored at 2°C (Bailey and others 1979). Spoilage was defined as "strong off odor" and was associated with microbial loads of 10^6 CFU/cm^2 with *Lactobacillus* comprising more than 90% of the microorganisms in the spoiled chicken. Thomas and others (1984) noted off odors in CO_2 treated whole chickens at 20 days and cut-up broilers at 17 days when stored at about 5°C. Microbial loads were reported to be 10^6 for mesophiles and 10^4 for lactobacilli at the time of spoilage. Breast portions had a shelf life of 42 days and leg/thigh portions a shelf life of 35 days when treated with either CO_2 or nitrogen and stored at 1°C (Patterson and others 1984). Microbial loads were 10^7 CFU/cm^2 at the end of storage for both breasts and legs/thighs. *Listeria monocytogenes* growth was arrested by storage at 1°C regardless of atmosphere and inhibited by environments containing CO_2 when chicken breasts were held at 6°C (Hart and others 1991). Pseudomonads predominated in breasts stored aerobically and were associated with strong, fruity odors. *Carnobacterium* and *Lactobacillus* spp. predominated in breasts stored under CO_2. The odor of spoiled poultry stored under CO_2 is often described as acid or sour (Bailey and others 1979, Patterson and others 1984, Hart and others 1991). Hart and others (1991) also reported cheesy odors. Such odors are typically attributed to the presence of lactobacilli. Patterson and others (1984) also noted sulfide odors in gas-flushed packages of legs/thighs after 15 days of storage at 4°C. Fecal odors detected in breast portions at 42 days when stored at 1°C and 28 days when stored at 4°C were associated with increases in *Enterobacteriaceae*. Chicken thighs treated with a combination of potassium sorbate and

CO_2 were described as slightly sour at a total microbial load of 10^7 CFU/cm^2 while thighs treated with sorbate and vacuum packaging were described as putrid at the same microbial level (Elliott and others 1985).

Modified atmospheres also affect poultry color. Turkey meat stored under 100% CO_2 plus an O_2 scavenger had the most stable red values, the lowest myoglobin oxidation rates, and the lowest microbial loads compared to turkey stored under 100% O_2, 100% N_2, or 25% CO_2/9% N_2/66% O_2 (Sante and others 1994). Yellowness of the CO_2-treated turkey also increased slightly over 21 days of storage at 3 to 4°C. A 100% CO_2 concentration has also been reported to result in a whitening of chicken skin (Elliott and others 1985).

Use of Organic Compounds

The antimicrobial activity of acids such as lactic, acetic, and citric is associated with both a decrease in pH and the presence of undissociated molecules (Ouattara and others 1997). Weak organic acids tend to be more effective than strong acids, but concentration, the acid dissociation constant, and the type of acid all affect the antimicrobial activity. The use of acids can also have significant effects on spoilage characteristics and organoleptic properties of the poultry.

Acetic acid reportedly has greater antimicrobial activity than either lactic or citric acids (Kim and Marshall 2000). Chicken wings immersed in a 1% acetic acid solution for 10 minutes and stored at 4°C had lower aerobic plate counts for 12 days compared to wings treated with 1% solutions of either lactic or citric acids. Untrained sensory panelists were unable to detect color differences among the treatments even though Hunter color values indicated wings immersed in acetic acid were lighter, less red, and less yellow than untreated wings. Odor of acid-treated wings was always rated higher by panelists compared to controls with those treated with acetic acid having the most acceptable odor over the 12-day storage period.

Broiler carcasses treated with food-grade vinegar (0.6% acetic acid) darkened/yellowed and the feather follicles puckered according to Dickens and others (1994). No differences in Warner-Bratzler shear values and sensory triangle tests were detected for treated and untreated muscle cooked by either boiling in sealed bags or oven roasting in foil boats. Although acetic acid was not effective in reducing total aerobic counts, it did significantly reduce the enterobacteriaceae counts.

Chicken legs treated with a 10% lactic acid buffered system were judged acceptable for smell and visual appearance by 10 trained judges after 12 days' storage at 6°C (Zeitoun and Debevere 1990). Untreated legs stored for 6 days at 6°C were judged unacceptable even though aerobic psychrotrophic loads were similar to those of the treated samples at 12 days' storage. Hydrogen sulfide and other sulfide-like flavors were detected after eight days' storage at 6°C in cooked legs that had been treated with 2% lactic acid. Van der Marel and others (1988) reported typical sulfide-like odors in chicken legs treated with 5% lactic acid after 9 days' storage at 6°C. No adverse sensory effects were reported due to the lactic acid.

Spoilage bacteria, including total aerobic loads, *Brochothrix thermosphacta, Pseudomonas,* and coliform counts, were always lower for chicken carcasses treated with sodium tripolyphosphate compared to controls (Vareltzis and others 1997). Sensory evaluation using a trained panel found treated carcasses had a putrid odor after 7 days of storage at 4°C while untreated carcasses were putrid after 4 days. Judges detected slime formation after 5 days in control carcasses, but slime was only beginning to form on treated carcasses after 8 days at 4°C.

Irradiation

The use of irradiation in doses of 1.5 to 3.0 kilogray (kGy) to eliminate bacteria on poultry was approved by the U.S. Food and Drug Administration (FDA) in 1992 (*Federal Register* 1992). Studies have shown that such treatment is effective in eliminating pathogens and extending the refrigerated shelf life of poultry. Factors such as irradiation dose, storage temperature, and poultry characteristics also affect the organoleptic properties of the irradiated poultry.

An irradiation dose of as little as 1.0 kGy was effective in eliminating coliforms, generic *Escherichia coli, Salmonella, Campylobacter,* and psychrotrophs on boneless, skinless chicken breasts and reduced total aerobic bacteria from $\log_{10}$ 4.6 to $\log_{10}$ 2.23 (Lewis and others 2002). A consumer taste panel found no differences in appearance, texture, flavor, color desirability, and overall acceptability

between irradiated and nonirradiated breast fillets stored at 0°C at 0 days' storage, but irradiated samples had lower texture and flavor scores at 14 days' storage. Texture, flavor, and overall acceptability of the irradiated breasts were even less desirable at 28 days' storage. Objective measurements also indicated lipid oxidation increased and breasts became redder with increasing irradiation level and storage time.

Viable *Listeria monocytogenes* was still present in chicken breasts cooked to an internal temperature of 71.1°C under vacuum (sous vide) but was undetectable in samples treated with a combination of sous vide and an irradiation dose of 3.1 kGy (Shamsuzzaman and others 1995). Evaluation of odor, flavor, and texture indicated unirradiated sous vide breasts spoiled at 16 days when stored at 8°C while those treated with an irradiation dose of 1.0 kGy spoiled at 23 days. Breasts treated with 2.0 and 3.0 kGy were acceptable through day 35. No significant differences in odor, flavor, or texture were associated with any of the irradiation doses evaluated.

Abu-Tarboush and others (1997) also reported few differences in the sensory properties of raw and cooked chicken breasts and thighs taken from whole carcasses irradiated with doses of 2.5, 5.0, 7.5, and 10.0 kGy. Panelists rejected samples treated with 2.5 kGy, however, at day 21 when stored at 4°C. As has been noted in other studies, a pink color in raw samples and elimination by cooking of some of the volatile compounds associated with odor deterioration were reported for irradiated samples. Although panelists were able to detect specific problems in raw meat, including rancidity in thighs and odor deterioration in breasts, that resulted in rejection, color, appearance, odor, juiciness, taste, and tenderness of cooked samples were acceptable for samples irradiated at doses above 2.5 kGy through 21 days of storage at 4°C.

A radiation dose of 2.5 kGy reduced the aerobic microbial load on chicken breast halves packaged on polystyrene trays overwrapped with polyvinyl chloride (PVC) film by two log cycles compared to untreated controls (Lescano and others 1991). Although the aerobic count reached 10^6 CFU/g at the 19th day of storage at 2°C, *Escherichia coli,* enterococci, and presumptive *Salmonella* were not detected in irradiated breasts. Lynch and others (1991) reported negligible microorganism growth in chill stored turkey breast fillets irradiated at 2.5 kGy.

In contrast to other studies, a strong irradiation odor was detected in the raw treated meat and was related to an unpleasant odor detected by trained sensory panelists by both Lescano and others (1991) and Lynch and others (1991). Odors were described as sour, rancid, manure, bad meat, and putrid when turkey breast fillets were stored in oxygen-impermeable films (Lynch and others 1991). A slight pink color developed in irradiated chicken breasts over a 22-day storage period (Lescano and others 1991) while an intense pink color was reportedly associated with irradiation of turkey breast fillets (Lynch and others 1991). Lescano and others (1991) also reported tenderness of cooked, irradiated chicken was lower than that of untreated samples and was related to a decrease in water-holding capacity. Flavor and overall acceptability of irradiated samples were similar to controls through 22 days' storage. Both rancidity (by peroxide value) and free fatty acids decreased in the chicken samples irradiated with 2.5 kGy.

CONCLUSION

Microbial contamination of poultry products remains a problem for the food industry. Pathogens such as *Campylobacter*, *Salmonella,* and *Listeria* and spoilage organisms such as *Pseudomonas* can play significant roles in the safety and shelf life of poultry products. Although washing and sanitizing, treatment with organic compounds, modified atmosphere packaging/storage, irradiation, and other processes have been investigated as potential methods for reducing microbial loads in poultry, the effect of such methods on color, flavor, texture, and other sensory properties is often undesirable. New technologies such as hydrodynamic pressure and ozone treatment are only now being investigated and may provide a means in the future to produce safer poultry products with acceptable organoleptic properties.

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VII
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39
Fish and Sensory Analysis in the Fish Chain

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INTRODUCTION

Fish are different from all other food commodities regarding method of harvesting, fragility of the product during transport to processing sites, and further in the chain, temperature dependency and variety of species. This sensitivity makes it necessary to monitor the product quality in the whole chain, and sensory analysis is ideal for this monitoring. Sensory assessment of fish and fish products has therefore for years played a natural part in the fishery chain.

Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water with fins consisting of a variable number of skeletal elements called fin rays (Thurman and Webber 1984).

Fish are the most numerous of the vertebrates, with at least 20,000 known species, and more than half of the species are found in the marine environment. Marine fish are most common in warm and temperate waters of the continental shelves with more than 8,000 species. In the cold polar waters, about 1,100 species are found. In the oceanic pelagic environment well away from the effect of land, there are approximately 225 species. Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1,000 meters' [m] depth), the number of species increases. There are some 1,000 species of so-called mid-water fish (Thurman and Webber 1984).

Each species is identified by a scientific name that has two parts—the genus and the specific epithet (binominal nomenclature). As an example, the scientific (species) name of the common cod is *Gadus morhua*.

THE LIVING FISH

During growth the size of each muscle cell increases, but not the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a size characteristic of the species and this is not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to produce female fish in aquaculture.

Every year mature fish use energy to build up the gonads (the roe and milk). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake. The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity, while others have ripe ovaries for nearly the whole year. The depletion of the reserves of the fish during gonadal development can be extremely severe, especially if reproduction is combined with migration to the breeding grounds. Some species, e.g., Pacific salmon (*Oncorhynchus* spp.), eel (*Anguilla anguilla*), and others, manage to migrate only once, then they degenerate and die. This is partly because these species do not eat during migration; in the case of a salmon, it can lose up to 92% lipid, 72% protein, and 63% of the ash content during migration and reproduction (Love 1970). Other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about 8 years before spawning causes its death, and other species can live even longer (Cushing 1975).

The Skeleton of Fish

Fish (vertebraes) have a backbone composed of segments (vertebrae) and a cranium covering the brain. The backbone runs from the head to the tail. The vertebraes are extended dorsally to form neural spines, and in the trunk region they have lateral processes that bear ribs. The ribs are cartilaginous or bony structures in the connective tissue between the muscle segments (see also Chapter 42 regarding texture of fish, fish product, and shellfish). Usually, there are also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones have to be removed if a bone-free filet is the goal.

Muscle Anatomy of Fish

Fish have muscle cells (Figure 39.1) running parallel and connected to sheaths of connective tissue (myocommata) anchored to the skeleton and the skin. The bundles of parallel muscle cells are called myotomes. The myocommata run in an oblique

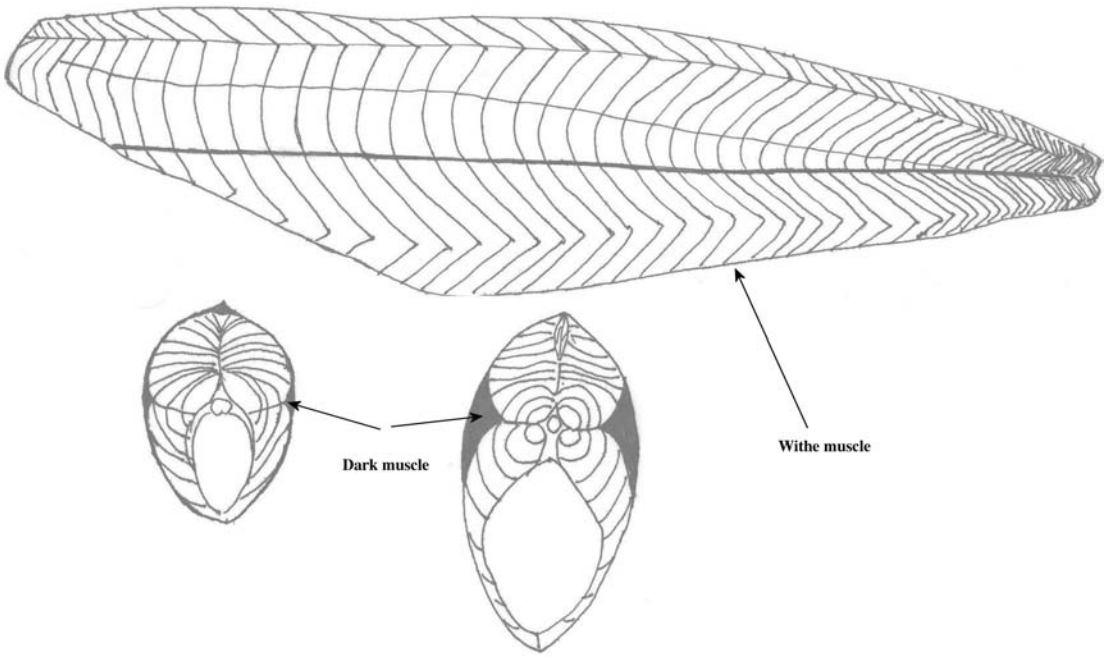

Figure 39.1. Muscle cells in fish.

pattern perpendicular to the long axis of the fish, from the skin to the spine. This anatomy is ideally suited for the flexing muscle movements necessary for swimming through the water. All muscle cells extend the full length between two myocommata, and run parallel with the longitudinal direction of the fish. The muscle mass on each side of the fish makes up the fillet, of which the upper part is termed the dorsal muscle and the lower part the ventral muscle.

Difference Between Fish Species

Difference in the fish muscle has consequence for the commercial value due to the effect on shelf life. In many species, most of the muscle is white or has a light color, but depending on the species, many fish will have a certain amount of dark tissue of a brown or reddish color. The dark muscle is located just under the skin along the body side (Figure 39.1). A typical pelagic fish as herring can contain nearly 50% of dark muscle required for prolonged aerobic muscle activity. The proportion of dark muscle varies with the activity of the fish. The more active the fish is, the larger the amount of dark muscle. There are many differences in the chemical composition of the two muscle types (e.g., higher levels of lipids and myoglobin in the dark muscle). From a technological point of view, the high lipid content of dark muscle is important and can give high intensities of desirable sensory notes, but a shorter shelf life due to lipid oxidation resulting in train-oil and rancid notes among others. Demersal species such as cod have a white flaky and tender muscle; textural changes such as toughness and chewiness are the most noticeable problem during storage. The white muscle is used for quick attacks or escapes, not for long destinations.

The reddish meat color found in salmon and sea trout does not originate from myoglobin but is a result of the red carotenoid, astaxanthin. The function of this pigment has not been clearly established, but it has been proposed that the carotenoid may play a role as an antioxidant. Further, the accumulation in the muscle may function as a depot for pigment needed at the time of spawning when the male develops a strong red color in the skin and the female transports carotenoids into the eggs. It is clearly seen that the muscle color of salmonids fades at the time of spawning. The fish cannot synthesize astaxanthin and is thus dependent on ingestion of the pigment through the feed. Some salmonids live in waters where the natural prey does not have high concentrations of carotenoid, e.g., in the Baltic Sea, thus resulting in a muscle color less red than salmonids from other waters.

Difference Between Individuals

There are not only differences between species, but also a considerable variation between individuals. These variations must be taken in to account when setting up experiments using sensory analysis to characterize fish and fish products. In farmed salmon for example, the lipid content can differ from 10 to 19% lipid in salmon from the same farm.

Also for herring there is a wide variation in lipid content within catches. A single catch can contain herring with lipid content ranging from 1 to 25%. This variation is due to heterogeneity caused by mixing between stocks (Nielsen and others 2005).

CHEMICAL COMPOSITION OF WILD CAUGHT FISH

The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment, and season.

The variation in the chemical composition of fish is closely related to feed intake, migratory swimming, and sexual changes in connection with spawning. Fish will have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Usually spawning, whether occurring after long migrations or not, calls for higher levels of energy. Fish having energy depots in the form of lipids will rely on this. Species performing long migrations before they reach specific spawning grounds or rivers may use protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish. Most species, in addition, do not usually ingest much food during migration and are therefore not able to supply energy through feeding.

During periods of heavy feeding, at first the protein content of the muscle tissue will increase to an extent depending upon how much it has been depleted. Then the lipid content will show a marked

and rapid increase. After spawning the fish resumes feeding behavior and often migrates to find suitable sources of food. Plankton-eating species such as herring will then naturally experience another seasonal variation than that caused by spawning, since plankton production depends on the season and various physical parameters in the oceans.

The lipid fraction is the component showing the greatest variation. Often, the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of spawning.

Although the protein fraction is rather constant in most species, variations have been observed such as protein reduction occurring in salmon during long spawning migrations (Ando and others 1985, Ando and Hatano 1986) and in Baltic cod during the spawning season (Borresen 1992).

A possible method for discriminating between lean and fatty fish species is to term fish that store lipids only in the liver as lean, and fish storing lipids in fat cells distributed in other body tissues as fatty fish. Typical lean species are the bottom-dwelling ground fish like codfish and flatfish species. Fatty species include the pelagics like herring, mackerel, and sprat. Some species store lipids in limited parts of their body tissues only, or in lower quantities than typical fatty species, and are consequently termed semifatty species (e.g., barracuda, mullet, and shark).

The lipid content of fillets from lean fish is low and stable whereas the lipid content in fillets from fatty species varies considerably.

Whether a fish is lean or fatty, the actual fat content is important for the technological characteristics postmortem. The changes taking place in fresh lean fish may be predicted from knowledge of biochemical reactions in the protein fraction, whereas in fatty species the changes in the lipid fractions have to be taken into account. The implication may be that the storage time is reduced due to lipid oxidation, or special precautions have to be taken to avoid this.

AQUACULTURE FISH

As demonstrated above, the chemical composition of the different fish species will show variations depending on season, migratory behavior, sexual maturation, feeding cycles, etc. These factors are observed in wild, free-living fish in the open sea and inland waters. Fish raised in aquaculture may also show variation in chemical composition, but in this case, several factors can be controlled, and the chemical composition may be predicted. To a certain extent the fish farmer is able to design the fish by selecting the farming conditions. It has been reported that factors such as feed composition, environment, fish size, and genetic traits all have an impact on the composition and quality (Reinitz and others 1979, Einen and Skrede 1998, Nortvedt and Tuene 1998).

In salmon aquaculture, astaxanthin is included in the feed, to compensate for the red color from crustaceans normally found in the natural feed of the wild fish. The red color of the flesh is one of the most important intrinsic quality criteria for salmon.

The world's production of fish from aquaculture has been steadily increasing in the last decades. One generation ago salmon was considered to be a primary luxury only consumed at special occasions or by the very wealthy. This has changed dramatically and now the price of salmon is below the price of cod. Chile and Norway have now a total production of more than 1 million tons of fish per year from aquaculture farms. The increasing growth has not been without drawbacks coming from parasites and virus. This in combination with a heavily fluctuating price on fish meal and oil due to climate phenomena such as El Nino has made the market for salmon very complex. An example of how aquaculture is meeting the new challenges is the Danish aquaculture sector.

The Danish aquaculture sector has been going through a major change in the last decade. The spotlight has been on the environment as the sector contributes to pollution. Recent consumer studies, however, have shown that aquaculture now is perceived according to the risk and benefit of eating the product. This is in contradiction to 10 years ago when the products were categorized at the same level as egg from battery hens or bacon from industrial pig farms.

This new status for the Danish aquaculture sector opens up new opportunities, both in credibility and new products. One of the future limitations of aquaculture is the access to a sustainable resource of fish or other sea living organism that can be used as part of the feed to aquaculture fish. Tests are already going on to use organisms from a lower tropic level than the traditional fish species used for fish meal

and fish oil production. Another option is to use components that have a vegetable origin such as soy oil or rapeseed oil. This in combination with the use of more vegetable components such as vegetable proteins means that it is possible to produce a fish such as rainbow trout, which has been fed a diet consisting of a high degree of vegetable origin, and make a "green" rainbow trout. At the same time new legislation has opened up the possibility to produce organic fish in the Danish aquaculture sector.

SLAUGHTER AND PROCESSING OF FISH

In some fisheries, bleeding of the fish is very important as a uniform white fillet is desirable. Bleeding is more affected by time onboard prior to bleeding/gutting than by the actual bleeding/gutting procedure. The best bleeding is obtained if live fish are handled, but it is of major importance to cut the fish before it enters rigor mortis since it is the muscle contractions that force the blood out of the tissues. However, it should be pointed out that the effect of bleeding must be weighted against the advantages of having a fast and effective handling procedure resulting in rapid chilling of the catch. A practical solution could be handling small quantities of fish at the same time.

Discoloration of the fillet may also be a result of rough handling during catch and catch handling while the fish is still alive. Physical rough handling in the net (long trawling time, very large catches) or on the deck (fishermen stepping on the fish or throwing boxes, containers, and other items on top of the fish) may cause bruises, rupture of blood vessels, and blood oozing into the muscle tissue (haematoma).

Heavy pressure on dead fish, when the blood is clotted (e.g., overloading of fish boxes) does not cause discoloration, but the fish may suffer a serious weight loss.

Changes in Raw Fresh Fish

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 hours (Azam and others 1990, Proctor and others 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor the fish body will be completely stiff; the filleting yield will drop significantly, and rough handling can cause gaping in the fillets. If the fillets are removed from the bone prerigor, the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink up to 52% and white muscle up to 15% of the original length (Buttkus 1963). If the fish is cooked prerigor the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Postrigor the flesh will become firm, succulent, and elastic.

SPOILAGE AND SHELF LIFE OF FISH

The spoilage rate and shelf life of fish are affected by many parameters and fish spoil at different rates. In general it can be stated that larger fish spoil more slowly than small fish, lean fish keep longer than fatty fish under aerobic storage, and bony fish are edible longer than cartilaginous fish (Table 39.1). Several factors probably contribute to these differences and whereas some are clear, many are still on the level of hypotheses.

Rough handling will result in a faster spoilage rate. This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. The surface/volume ratio of larger fish is lower than that of smaller fish, and, as bacteria are found on the outside, this is probably the reason for the longer shelf life of the former. This is true within a species, but may not be universally.

The skin of the fatty pelagic fish is often very thin, and this may contribute to the faster spoilage rate. This allows enzymes and bacteria to penetrate more quickly. On the contrary, the thick skin of flatfish and the antibacterial compounds found in the slime of these fish may also contribute to the ability to keep flatfish. As described earlier, the slime of flatfish contains bacteriolytic enzymes, antibodies, and various other antibacterial substances (Hjelmland and others 1983, Murray and Fletcher 1976). Although large differences exist in

Table 39.1. Intrinsic factors affecting spoilage rate of fish species stored in ice.

	Relative spoilage rate	
Factors affecting spoilage rate	Fast	Slow
Size	Small fish	Larger fish
Post mortem pH	High pH	Low pH
Fat content	Fatty species	Lean species
Skin properties	Thin skin	Thick skin

the content of trimethylamine oxide (TMAO), this does not seem to affect the shelf life of aerobically stored fish but rather the chemical spoilage profile of the species.

OFF FLAVORS RELATED TO FISHING GROUND

Occasionally fish with off flavors are caught, and in certain localities this is a fairly common phenomenon. Several of these off flavors can be attributed to their feeding on different compounds or organisms. The planktonic mollusk, *Spiratella helicina*, gives rise to an off flavor described as "mineral oil" or "petrol." It is caused by dimethyl-β-propiothetin, which is converted to dimethylsulphide in the fish (Connell 1975). The larvae of *Mytilus* spp. cause a bitter taste in herring. A very well known off flavor is the muddy-earthy taint in many freshwater fish (Howgate 2004). The flavor is mainly caused by two compounds: geosmin and 2-methyl-iso-borneol, which also are part of the chemical profile of wine with cork flavor. Geosmin, the odor of which is detectable in concentrations of 0.01-0.1 μgram(μg)/liter (L), is produced by several bacterial taxa, notably the actinomycetes *Streptomyces* and *Actinomyces*.

An iodine-like flavor is found in some fish and shrimp species in the marine environment. This is caused by volatile bromophenolic compounds, and it has been suggested that the compounds are formed by marine algae, sponges, and Bryozoa and become distributed through the food chain (Anthoni and others 1990).

Oil taint may be found in fish flesh in areas of the world where offshore exploitation of oil is intensive or in areas where large oil spills occur. The fraction of the crude oil that is soluble in water is responsible for the off flavors. This is caused by the accumulation of various hydrocarbon compounds, where particularly the aromatic compounds are strong flavorants (Martinsen and others 1992).

THE SENSORY ANALYSIS IN THE FISH CHAIN

The fish chain is the flow that a fish follows from when it is caught or taken out of a fish cage in a fish farm to consumption. The numbers of links are determined by fish species and/or product type. The links can for instance be the fishing vessels, fish processors, transports, storage, and retail stores. (See Figure 39.2.) In each link the fish is exposed to different factors that all influence the sensory quality and the sensory characteristic of the product. These factors (extrinsic) can be environmental conditions like temperature but also different handling/processing steps like packing, cutting, or smoking. Besides the factors that fish are exposed to in the chain, the raw material also has a key influence on the product quality and characteristic. To ensure product quality, it is important to follow and control the quality in each chain link. Sensory assessment of fish and fish products are an ideal way of controlling the quality and should therefore be an integrated part of the working routine. Until now, sensory analysis has mostly been used in quality control and quality assurance in some part of the fish sector, and to a certain extent, in product development and optimization (Jonsdottir 1998). There has however until now been very little integration between the descriptive/discriminative analysis and marketing test. Sensory analysis in the fish chain has mostly been used to describe the intrinsic product qualities such as smell, taste, and texture, while consumer choice is based both on intrinsic and extrinsic qualities such as convenience and price.

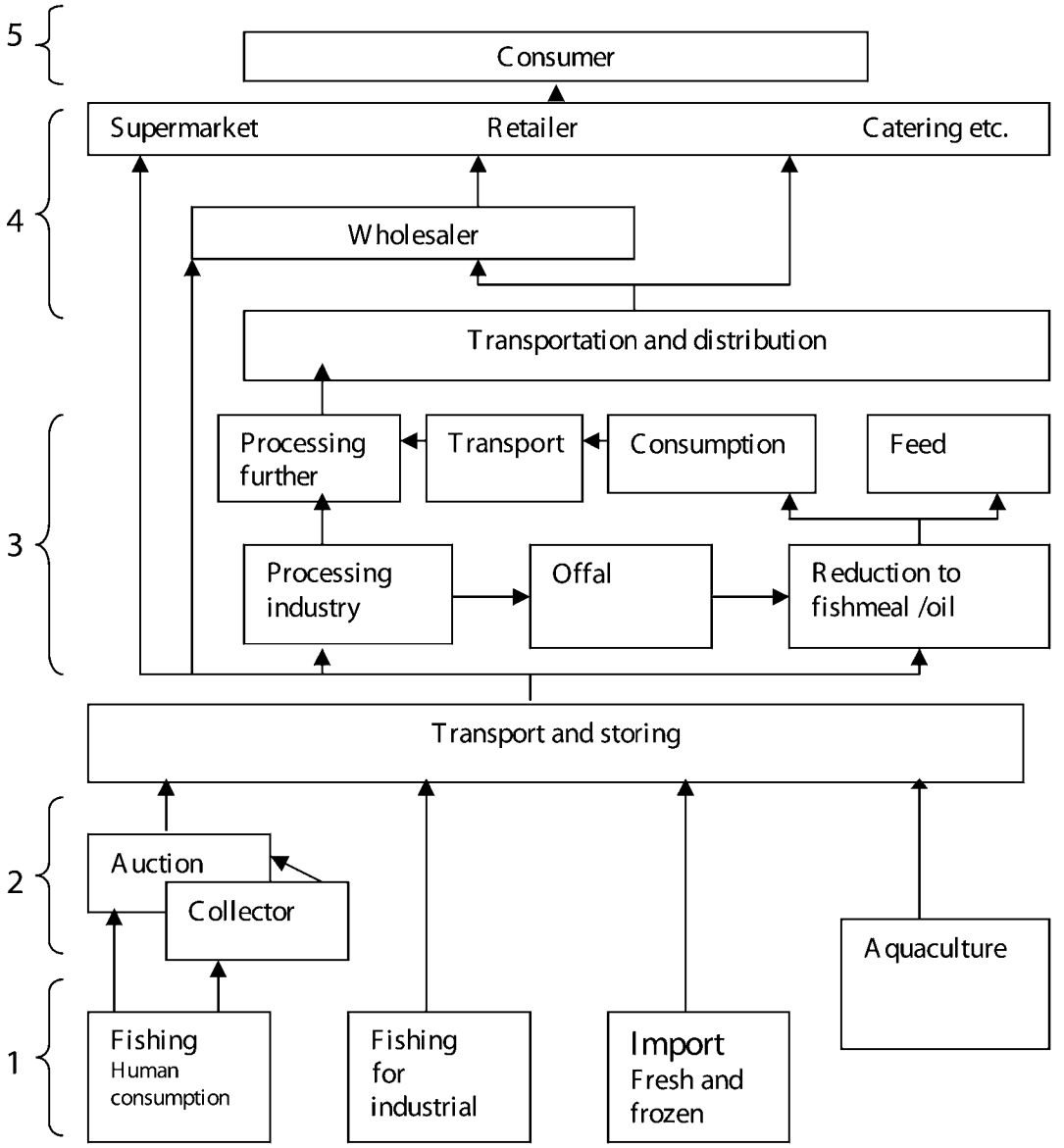

Figure 39.2. Overview of the fish chain from catch to consumer. 1) Primary producers, 2) First sale, 3) Processing, 4) Final sale, and 5) consumption.

Sensory analysis has primarily been used to control a batch of fish when it is received in a link. An example could be the entrance control in a processing plant with the purpose to evaluate quality in accordance with the price paid and to determine when and how the fish is going to be used in the production. This sensory analysis is different from the sensory analysis that is used by the processing plant quality staff to ensure that the final product is in accordance with the specification, for example, regarding fat content and texture. Both operations must be performed by highly professionally trained personnel, but in some instances the evaluations are more on an empiric basis, due to tradition.

A new area is now opening up for the use of sensory analysis in the fish chain. Starting January 1, 2005, the section of the "General Food Law" in the European Union (EU) Regulation 178/2002 dealing with traceability came into action. When introducing traceability in every link in the fish chain, it is necessary to have "tools" to control the validity of the information as part of traceability. Evaluation of the catching time in combination with registration of the actual time and temperature regime makes it possible to calculate the storage time and compare this result with specification of the batch.

Sensory Analysis of Fish

Several conditions are unique for sensory analysis of seafood. When selecting and training judges for sensory analysis, it is very important to be aware that some people cannot taste rancid flavor, iodine, or geosmin, and some have a very low response to cold-storage flavor and rancidity. Also some people are allergic or hypersensitive to different fish proteins, shellfish, or histamine.

Interpretors of the stimulus and response must be trained very carefully in order to receive objective responses. It is very easy, as an example, to give an objective answer to the question: Is the fish in rigor (completely stiff)?, but more training is needed if the assessor has to decide whether the fish is post- or prerigor.

Different Sensory Methods Used in the Fish Chain

Both objective and subjective sensory testing are used. The objective tests include discriminative (triangle test, forced choice) and descriptive (profiling, structured scaling) sensory tests. Both groups of tests are analytical measurements of the intrinsic quality of the product, whereas affective (subjective test) methods are used for consumer testing and measure the attitude and emotional response of the consumer toward the product. All of these methods are used in the fish chain (Figure 39.3). Each link in the chain has its own version of the tests as indicated by the numbers of the different methods (discriminative test 1, discriminative test 2, etc.) and coordination is needed.

When fish are landed in Europe today, the most commonly used method for quality assessment of raw fish in the inspection service is the EU scheme (Anonymous 1996). For cooked samples, the sensory methods that are used in some countries by fish industries and buyers of fish products are based on the Torry scale. The more objective and descriptive sensory methods such as sensory profiling and Quality Index Method (QIM) are described in the following chapters.

There are examples of subjective sensory tests in the chapter dealing with wild and farmed fish.

European Union Scheme

In the European Union (EU) scheme (Table 39.2), according to the Council Regulation (EC) No. 2406/96 November 26, 1996 (Anonymous 1996), there are three quality levels: E (Extra); A, B where E is the highest quality; and below B is the level where fish are discarded for human consumption. The EU scheme does not take into account the differences between species as it only uses general parameters and there are also problems with mixing subjective and objective sensory in the scheme. Several studies have shown that the QIM proved to be more reliable in assessing sensory changes of different fish species as compared to the EU grading scheme (Triqui and Bouchriti 2003).

Torry Scale

The Torry scale (Table 39.3) is a 10-point scale developed at the Torry Research Station (Howgate and others 1992). It is developed for cooked fish samples. Scores are given from 10 (very fresh in taste and odor) to 3 (spoiled). It is considered unnecessary to have descriptions below 3, as the fish is then not fit for human consumption. The average score of 5.5 may be used as the limit for consumption. The Torry scale has been developed for lean, medium fat, and fat fish species.

Translation of Sensory Results from One Part of the Chain to Another

Sensory analysis in the fish chain has principally been used to describe the intrinsic product qualities such as smell, taste, and texture, while consumer choice is based both on intrinsic and extrinsic qualities. Preference/acceptability tests have been widely used in both industry and research, but the tests do

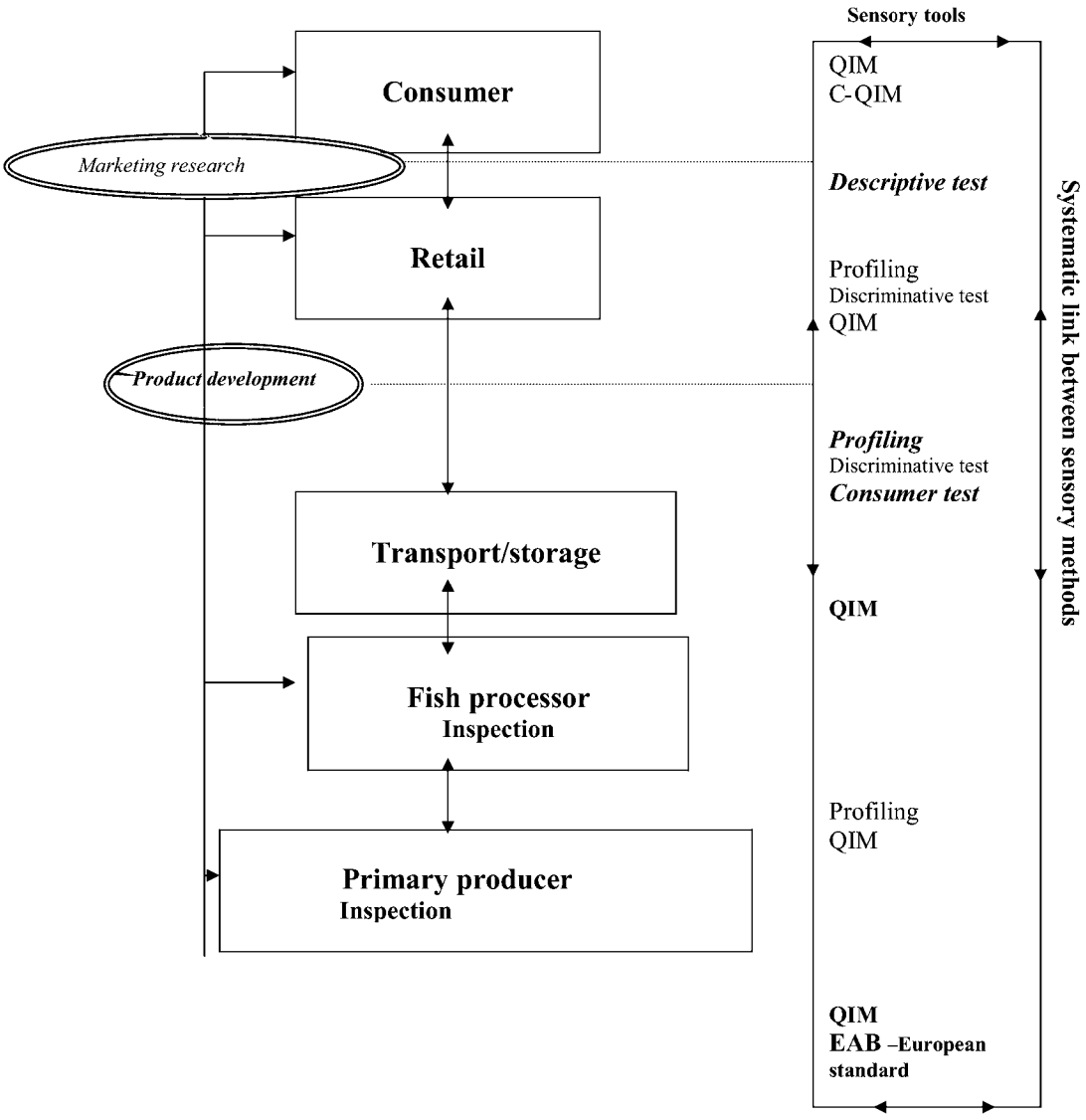

Figure 39.3. Sensory analysis in the chain from catch to consumer.

Table 39.2. White Fish: cod, saithe, haddock, whiting, plaice, redfish, ling, hake.

		E	A	B	Unfit (C)
Skin		Bright; shining; iridescent (not redfish) or opalescent; no bleaching	waxy; slight loss of bloom; very slight bleaching	dull; same bleaching	dull; gritty; marked bleaching and shrinkage
Outer slime		transparent; water white	milky	yellowish-gray; same clotting	yellow-brown; very clotted and thick
Eyes		convex; black pupil; translucent cornea	plane; slightly opaque pupil; slightly opalescent	slightly concave; grey pupil; opaque cornea	completely sunken; gray pupil; opaque discolored cornea
Gills		dark red or bright red; mucus translucent	red or pink; mucus slightly opaque	brown / grey and bleached; mucus opaque and thick	brown or bleached; mucus yellowish gray and clotted
Peritoneum (in gutted fish)		glossy; brilliant; difficult to tear from flesh	slightly dull; difficult to tear from flesh	gritty; fairly easy to tear from flesh	gritty; easily torn from flesh
Gill and internal odors	all except plaice	fresh; sea weedy; shell fishy	no odor; neutral odor; trace musty, mousy, milky, capryllic, garlic or peppery	definite musty, mousy, milky, capryllic, garlic or peppery; bready; malty; beery; lactic; slightly sour	acetic; butyric; fruity; turnipy; amines; sulphide; fecal
	plaice	fresh oil; metallic; fresh-cut grass; earthy; peppery	oily; sea weedy; aromatic; trace musty, mousy or citric	oily; definite musty, mousy or citric; bready; malty; beery; slightly rancid; painty	muddy; grassy; fruity; acetic; butyric; rancid; amines; sulphide; fecal

Table 39.3. Torry score sheet for freshness evaluation of cooked lean fish such as cod, haddock, and pollock.

Odor	Flavor	Score
Initially weak odor of sweet, boiled milk, starchy, followed by strengthening of these odors	Watery, metallic, starchy. Initially no sweetness but meaty flavors with slight sweetness may develop.	10
Shellfish, seaweed, boiled meat	Sweet, meaty characteristic	9
Loss of odor, neutral odor	Sweet and characteristic flavors but reduced in intensity	8
Wood shavings, wood sap, vanillin	Neutral	7
Condensed milk, boiled potato	Insipid	6
Milk jug odors, reminiscent of boiled clothes	Slight sourness, trace of off flavors	5
Lactic acid, sour milk, TMA	Slight bitterness, sour, off flavors, TMA	4
Lower fatty acids (e.g., acetic or butyric acids) decomposed grass, soapy, turnipy, tallowy	Strong bitterness, rubber, slight sulphide	3

not give good predictions of consumer behavior (Cardello and others 2000). There has, however, been very little integration between the descriptive/discriminative (objective methods) analysis and marketing tests (subjective methods). Further investigation in this area can perhaps make it easy to predict consumer behavior toward a product.

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40
Sensory Profiling of Fish, Fish Product, and Shellfish

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INTRODUCTION

Descriptive sensory analysis such as profiling is the most resourceful method to be used to characterize postmortem changes of all kind of seafood. Descriptive testing is capable of providing quantitative data and can both be very simple and used for assessment of a single attribute or more complex and give a total characterization of sensory quality. Profiling of seafood can vary considerably depending on species and storage method. The attribute to be assessed must be clearly defined and understood. Special care must be taken for a number of relations: the assessors must not be afraid of fish bones; some flavors are very special like iodine (from bromophenols) and muddy (from geosmin or 2-methyl-isoborneol) and must be known to the assessors; and there might be considerable differences between the individual fish and it can be a challenge to have homogeneous samples and even more complicated to get replicates for a panel of 12. The assessors require in all cases intensive training and a detailed briefing before each session.

PREPARATION OF FISH SAMPLES

Sampling for sensory analysis must be as representative as possible and therefore consideration must be given not only to how and where the samples are

cut, but also to the effect of the chosen method for heat treatment. The preparation must have a minimal sensory impact of the "innate" characteristics of the samples.

Sample Cut

Samples can be cut into portions before or after heat treatment. Samples from fish species such as cod, salmon, and saithe (or pollack) are cut from the loin part in 8 × 4 × 2 centimeter (cm) pieces corresponding to approximately 75 grams (g). Smaller fish species such as rainbow trout, herring, and plaice require a whole fillet, which gives samples of approximately 40 to 50 g for plaice and herring and approximately 75 to 100 g for rainbow trout.

For fat fish species, it is important to consider if the samples should be provided with or without skin. As Figure 40.1 shows, the dark muscle is right under the skin and if the fillets are skinned, some of the dark muscle will be removed. This can have influence on rancid flavor due to the high lipid content in the dark muscle.

Loin samples are cut from the dorsal muscle and cutlet samples across the whole fish as shown in Figure 40.1.

Replicates

For replicates each assessor has to evaluate samples from the same position of each fillet (Figure 40.1). In this way it is possible to have real replicates, and it is possible to identify if there is any difference between assessors or between fillets from different parts of the fish.

If the texture is of no importance, it is possible to make minced fish samples to eliminate the differences between individuals.

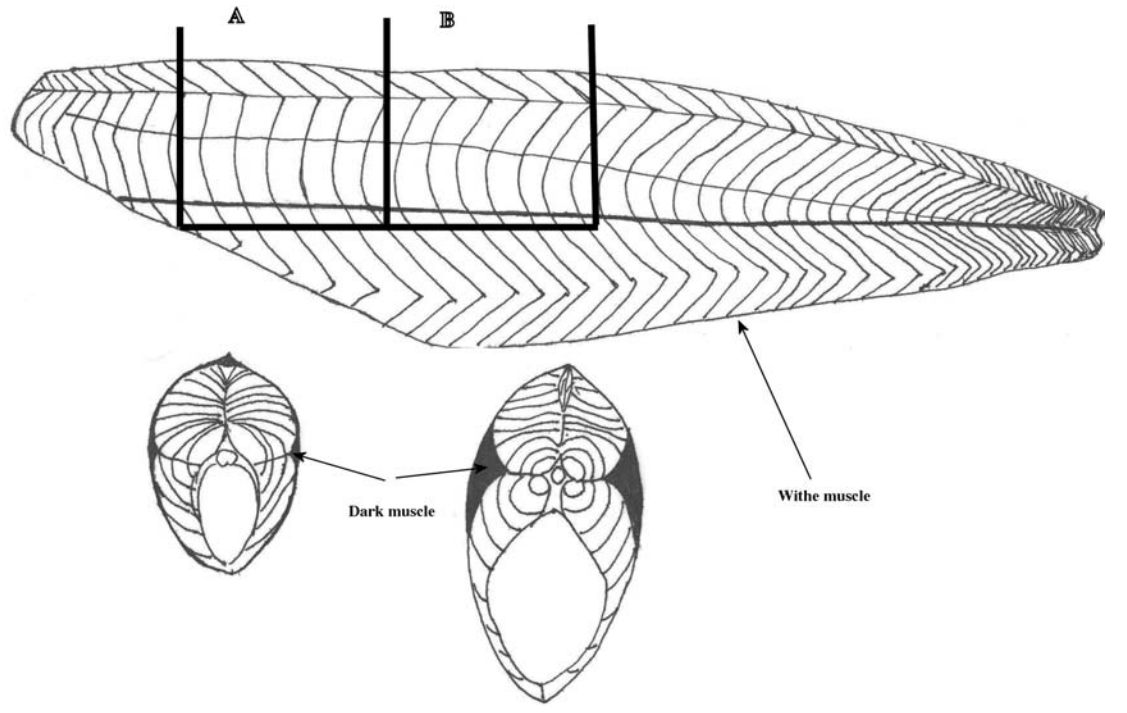

Figure 40.1. The dark and white muscle of fish. The muscle is constructed of adjacent muscle blocks (myotomes), separated from each other by sheets of collagenous tissue (myocommata). A and B are the two replicates for one assessor.

Sample Preparation of Heat-treated Samples

The optimal amount of fish prepared for each assessor is a portion size of 40 to 100 g. All samples must be marked individually with three-digit or four-digit codes. The samples must be placed in individual porcelain bowls and covered with porcelain lids (Figure 40.2a,b). The samples are heated (e.g., in a convection oven) in their own juices at 100°C to an internal temperature of 70°C.

Cooked in Water Bath

Fish can also be cooked in a water bath as suggested by Bjerkeng and others (1997), Nortvedt and Tuene (1998), Sivertsvik and others (1999), and Hemre and others (2004). Salmon or cod cutlets 1.5 cm thick or the loin part of Atlantic halibut fillets were packaged in diffusion-tight plastic bags under vacuum and cooked in a water bath at 85°C for 15 minutes or at 70–72°C for 1 hour without salt or other spices.

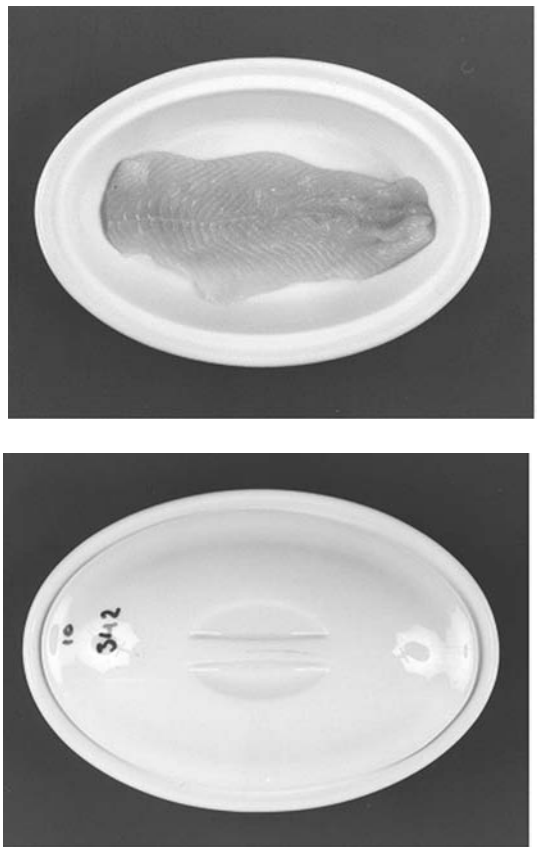

Figure 40.2. A trout fillet in a porcelain bowl with lid and with a three-digit code.

Different Types of Products

Different types of fish products demand different sample preparation. The following examples describe processed fish and fish broth.

Fish Broth

For very different fish species, it can be an advantage to have a sample preparation where the fish are cut into small pieces and boiled to a broth. This was done in the study by Morita and others (2003). All the species except loach were gutted, and tuna and swordfish were also skinned. Small fish species such as banded blue sprat, sardine, loach, pond smelt, and goby were boiled whole, while the others were cut into 3- to -4-cm lengths before being boiled as in ordinary domestic cooking. Each sample (200 g) was placed in a three-necked separable 31 round flask along with 200 milliliters (mL) of boiling water, then heated with refluxing for 30 minutes by a mantle heater. The fish broth (350 mL) obtained after filtering the boiled mixture through three layers of cotton gauze was divided into 35-mL aliquots in 50-mL glass vials. Fish broth (35 mL) prepared from 20 g fish was placed in a 260-mL disposable plastic cup, covered with a plastic petri dish, and served to the panelists at 40°C.

Smoked Samples

When profiling smoked samples, it is necessary to decide at which temperature the samples should be assessed and how to cut the samples. Smoked salmon can be cut into ½-cm-thick slices at a right angle to the fillet surface. This gives a part of the dorsal muscle and also a part of the belly flap. The samples are then served in petri dishes at room temperature.

Other methods suggested in the literature follow. The packages of smoked salmon were opened the day of the sensory analysis and kept open for 15 minutes at ambient temperature; thereafter each slice was individually repacked in aluminum foil and served to the assessors (Cardinal and others 2004).

Slices of smoked salmon 1-cm thick were cut at a right angle to the fillet surface and were served (Einen and Skrede 1998, Rørå and others 1998).

Hot smoked rainbow trout was assessed by serving a fraction from anterior, middorsal part of the right fillet at 20°C (Rasmussen and others 2000).

Marinated Fish

It is difficult to assess marinated fish due to the intensive flavor. The following procedure is suggested by Nielsen and others (2004a, 2004b, 2004c). The left fillets of herring (*Clupea harengus*) were marinated in plastic containers in a solution of 7% acetic acid and 16% salt (1.5 parts herring to 1 part brine) and stored at 2°C for a minimum of 4 weeks. Before sensory analysis, the samples were dewatered in a 1% salt solution for 2 days at 2°C (1 part herring to 1 part brine). The middle part of the fillet was cut into two pieces (approximately 10–15 g each) and served in replicate to the same assessor. The samples were served (skin side facing up) in porcelain trays with lids.

PREPARATION OF SHELLFISH SAMPLES

For shellfish there can be some special conditions because of sample size. For example shrimp can be very different in size; some are large enough to be assessed in one "mouthful," but others are so small that several shrimps are needed for one "mouthful." Concerning the variation among individuals, it can be an advance to ask the assessors to take three or four shrimps, depending on the size, at a time.

Prawns and Shrimps

Prawns and shrimps must either be thawed for 1 hour at room temperature before cooking or added directly to the boiling water, depending on the size. Cooking time in boiling fresh water is 3 minutes. After cooking the prawns/shrimps are drained, cooled with cold water, peeled, and held at 7–10°C until evaluation (Edmunds and Lillard 1979, Papadopoulos and Finne 1986, Whitfield and others 1997).

Prawns Broth

If a sensory profiling of whole prawn is wanted, it is possible to make a broth where both the shell and the meat are assessed at the same time.

Frozen prawns (kuruma prawns (*penaeus monodon*) and black-tiger prawns (*Penaeus monodon*) were thawed with running water, 190 g meat and/or 30 g shell were cut into 1 cm^2 pieces and after adding 400 ml of deionized distilled water, the mixture boiled for 30 minutes. Broth was obtained by filtering the boiled mixture through three layers of cotton gauze. The broth was divided into 35-mL portions each and preserved in 50-mL glass vials at −50°C until performing sensory evaluation (Morita and others 2001).

SESSIONS

It is important to serve the heated samples hot and in containers with lids. In this way the assessors get the full impression of the odor just after opening the lid. When samples are cooked in plastic bags, odor attributes are assessed immediately after opening the vacuum bags, while flavor and texture are evaluated after removing the samples from the bag (Bjerkeng and others 1997, Nortvedt and Tuene 1998). Each assessor evaluated samples in replicate, and all samples must be served to each assessor in randomized order to minimize possible carry-over effects between samples.

In storage experiments, extra samples can be included in each sensory session in randomized order to prevent the assessors from guessing the sensory scores of samples due to the relative time of chilled storage. The degree of freshness of the extra samples should differ between sensory sessions, and data from these samples must not be included in the study.

Number of Samples

The number of samples for each session depends on the number of attributes and type of sample. For smoked samples the assessors get tired after four samples, but for cooked samples without any spices added, the assessors can evaluate six samples within a session. If there are many attributes, it is necessary to cut down the number of samples in each session. It is important to give the assessors a short break—1 to 3 minutes—between each sample.

Between Samples

Depending on the samples, different cleaning items can be used to dissipate residual flavors and particles between evaluations.

Cooked Samples

Tap water can be used if is not too bitter, and it should be served at room temperature together with unsalted crackers or crisp bread.

The tap water can be filtered through a domestic water filter to remove any extraneous flavors (Farmer and others 2000), but also distilled water and unsalted crackers can be used (Cardello and others 1983).

Smoked Samples

To clean the palate between smoked samples, it can be necessary to have more than water. Also diluted apple juice combined with neutral bread can be used (Bech and others 1997, Rasmussen and others 2000) or mineral water and bread (Cardinal and others 2004).

Off Flavors and Taints

Depending on the off flavor or taint, some acidic solution can be used in combination with a longer break between samples. Bett and others (2000) used unsalted soda crackers and citric acid (0.03%) solution as "palate cleansers."

SCALES

Different types of scales can be used, but the most common are the line scale and the 9-point scale. For assessing color, it is helpful if standards are used, such as the natural color system (NCS®) or for smoked salmon, the *Salmo*Fan™ lineal from Roche.

Line Scale with Anchor Point

The most commonly used scale is the 15-cm unstructured line scale, with two anchor points: "little" and "much" of attribute intensity, e.g., placed 1.5 cm and 13.5 cm from the end point of the scale (Papadopoulos and Finne 1986; Hong and others 1996; Warm and others 2000; Bøkness and others 2002; Sveinsdóttir and others 2003; Nielsen and others 2004a, 2004b).

Line Scale Without Anchor Point

An intensity scale from 0 to 100 is also suggested (Schubring and Oehlenschläger 1997, Rasmussen and others 2000, Ginés and others 2004). Here 0 is defined as the minimum mark or nothing of the attribute and 100 as the maximum mark of the attribute. Others are using a nonstructured continuous line scale of different length: 8 cm (Stohr and others 2001), 10 cm (Bech and others 1997, Morita and others 2001, Richards and Hultin 2001, Cardinal and others 2004) or 15 cm (Whitfield and others 1997, Rørå and others 1998, Whitfield and others 2002, Morita and others 2003).

Nine-point Scale

The nine-point scale can be either a continuous intensity scale going from 0 (low intensity) to 9 (high intensity) (Johansson and others 2000, Einen and Thomassen 1998) or a line scale where the responses are transformed into numbers after assessment, where 1 equals no intensity and 9 equals high intensity (Edmunds and Lillard 1979, Bjerkeng and others 1997, Einen and Skrede 1998, Nortvedt and Tuene 1998, Rørå and others 1998).

Category Scale

Category scale is most often used for assessors with only a little training, but it can also be used for simple profiling where only one flavor is assessed.

McGill and others (1984) used an intensity scale ranging from 0 = absent to 5 = very strong, and Cardello and others (1983) used a 7-point category scale of intensity, of which three of the points had verbal labels, i.e., 1 = slight, 4 = moderate, and 7 = extreme.

Bett and others (2000) used a simple category scale for detecting the off flavor muddy/earthy. They instructed the panelists to rate each sample as 0 if no muddy/earthy flavor was detected, 1 for slight or marginal, and 2 for obvious.

TRAINING OF ASSESSORS

Training is essential as it provides all assessors with a common vocabulary, thus decreasing the risk of different quantitative and qualitative interpretations of descriptors (ISO 8586-1, ISO 11035). Furthermore, continuous training during long-term projects, preferably using reference material, can reduce the risk of drifting. If the training is interrupted, the assessors might forget descriptor meanings and/or rating levels with time. Just as with any other instrument in the laboratory, the performance of a sensory

panel has to be evaluated and calibrated in order to give reliable results. If this is not the case, the risk of drawing wrong conclusions from the experiment is high. In a study of marinated herring, Nielsen and others (2004c) used a reference material that was served both as mince and fillet portions. The sensory evaluations took place in five trials over a period of 19 months. It was therefore important to prevent "drifting" of profiling assessors, and all sessions began with the evaluation of the reference. To exclude variation between fillets, 7 to 10 fillets were minced together and then served in 10- to 15-g portions in porcelain trays. The assessors evaluated the mince and "calibrated" their evaluations according to a completed list of assessments of odor and flavor of the herring mince performed by an in-house expert panel. The reference material was also served as a coded (unknown) fillet sample in order to check the performance of the panel. The samples were treated in all ways like the "real" sample material. The sensory properties were related to assessors and trials by Partial Least Squares Regression (PLSR) using The Unscrambler®, version 7.6 SR-1 (Camo ASA, Norway) (Martens and Næs 1989). Both Discriminant-PLSR (DPLSR) and Anova-PLSR (APLSR) models were calculated (Martens and Martens 1999, 2001). Data from the sensory profiling were corrected for the "level effect" (i.e., assessors using different parts of the line scale) by the method described by Thybo and Martens (2000).

By serving the same product as a reference before each session, assessors can recapitulate the descriptors easily and can recalibrate their evaluations to the same scale. The additional use of coded reference samples served together with the actual samples makes it possible to monitor the performance of the panel. Multivariate data analysis allows for quick calculations, and results are easily interpreted with the visual layout. No drifting was found during the evaluation period, and this is most likely an effect of the continuous use of the reference (Nielsen and others 2004c).

ATTRIBUTES

Criteria for selection of attributes, which can discriminate between samples, have to be relevant for the specific seafood, must discriminate clearly between the samples, must be nonredundant, and be cognitively clear to the assessors. To see if these demands are fulfilled, the sensory data can be analyzed with regard to signal to noise relation for each assessor and attribute. The signal to noise analysis can be evaluated with multivariate data analysis (Thybo and Martens 2000). For texture attributes, see Chapter 42, Texture of Fish, Fish Products, and Shellfish.

In Table 40.1, there is a long list of words used in the References section (Edmunds and Lillard 1979; Cardello and others 1983; Papadopoulos and Finne 1986; Hong and others 1996; Bech and others 1997; Bjerkeng and others 1997; Schubring and Oehlenschläger 1997; Einen and Skrede 1998; Einen and Thomassen 1998; Nortvedt and Tuene 1998; Rørå and others 1998; Sivertsvik and others 1999; Whitfield and others 1999b; Besteiro and others 2000; Bett and others 2000; Farmer and others 2000; Johansson and others 2000; Rasmussen and others 2000; Warm and others 2000; Morita and others 2001; Richards and Hultin 2001; Sérot and others 2001; Stohr and others 2001; Bøkness and others 2002; Fletcher and others 2002; Whitfield and others 2002; Morita and others 2003; Sveinsdóttir and others 2003; Cardinal and others 2004; Ginés and others 2004; Hemre and others 2004; Nielsen and others 2004a, 2004c) for describing different attributes, but unfortunately only a few of them are defined. To be cognitively clear and to make reference samples, it is good to have a definition of the sensory attribute. Reference samples for the different attributes can be found not only in seafood samples, but also in cucumber and boiled potato. Another example is the sensory attribute warm milk. It is important to know that milk should only be heated and not boiled, because when milk boils, a sulphurous odor is developed.

Appearance

Appearance can be color/discolor and structure properties such as flaky or gaping. Flaky is assessed by pressing on the sample with a fork; if the muscle separates in flakes, the sample has a high intensity of flakiness. The fish is gaping when the muscle splits up in factions or chaps. Gaping can be seen both in raw and heat-treated fish muscle. Color references are described under scales. A description of the attributes is given in Table 40.2.

If the appearance is unimportant, the best method is to assess the samples in red light, in order to camouflage color differences.

Table 40.1. A list of all the words (from the References in Chapter 40) used to describe appearance, odor, flavor, and after taste properties. The words are grouped according to their similarities.

Appearance

Coarseness
Crumbly crumbliness
Flaky
Muscle segments
Separation

Juicy appearance
Moist appearance

Cured meat
Iberian ham

Spotted
White stripes

Fatty aspect
Oily

Coagulated protein
Sheen
Shininess
Shiny
Translucent
Translucent appearance
Waxy sheen

Fat droplets in water
Tearing of the slices
Yellow water

Beige
Brown
Brownish
Cream
Gray
Gray/blue
Grayish
Ochre
Orange
Peach
Pink color
Red
Red-brown

Reddish brown
Rose
Rosé wine
Salmon color
Whiteness
Whitish
Yellow/green
Yellowish brown
Yellowish

Color intensity
Darkness
Discoloration
Homogeneity of color
Hue
Paleness

Odor

Anchovy
Herring
Marine
Salmon-like
Shellfish
Crustacean
Raw salmon
Tuna
Typical oyster
Fresh shrimp
Fresh crab
Boiled prawn
Characteristic salmon
Fatty fish

Bacon
Canned tuna
Cooked fish
Fried chicken
Grilled fish
Ham/cooked meat
Heavy or gamey fish
Iberian ham
Roasted shrimp
Roasted soy sauce
Salty fish
Meatiness

Cold ashes
Sharp

Sea
Sea bed
Sea breeze
Seaside
Seaweed
Salty sea air

Fish oil
Fish/herring oil
Linseed oil
Oily
Nutty

Fresh
Fresh fish
Fresh odor
Freshness

Sweet
Sweet/fresh
Sweetness

Cucumber
Cucumber-like
Grass
Green
Green aroma
Freshly cut grass
Hay/grass

Bland
Rawness

Boiled milk
Fresh milk
Hot milk
Milky
Milky/buttery
Butter/caramel
Buttery

Acid
Acidic
Sourish
Sourness
Vinegar

Ripened sheep's cheese
Ripeness
Blue cheese/musty

Amine
Fish (old fish)
Fish meal
Fishiness
Fishy

Metal
Metallic

Maltiness
Musty
Yeasty

Earthy
Mould
Muddy/earthy
Mushroom

Cardboardy
Frozen storage

Sour
Sour/fermented

Painty
Rancid
Rancidity
Train oil

Off-odor
Ammonia
Boiled egg
Cabbage/gas/garlic
Faecal
Farmyard
Hydrogen sulphide/egg
Pungent
Rotten seaweed
Rubber

Table 40.1. continued

		***Odor* (*continued*)**	
Smoke odor	Boiled potato	Iodine	Sewage
Wood fire	Cooked potato	Medicine	Stagnant
	Boiled corn	Plastic/citrus fruit	Wet dog
Algae			
Dry seaweed			
		Flavor	
Raw fish	Sea	Cooked potato	Earthy
Marine	Sea bed	Mushroom	Mould
Herring	Seaweed		
Salmon-like		Blandness	Sour milk
Raw salmon	Fish oil	Neutral	Musty
Fresh shrimp	Fresh oil		Yeasty
Shrimp	Fresh oily	Hot milk	
Shrimpiness	Oily	Milky	
Characteristic shrimp		Cheese	Cardboard
Fresh crab	Nutty	Cheesy	Frozen storage
Clam	Nuttiness	Creamy	
Characteristic clam	Nutty or buttery		Sour
		Acid	
Bacon	Fresh flavor/taste	Acidic	Acidulous rancid
Chicken-like		Sourish	Painty
Chicken liver-like	Sweet	Vinegar	Rancid
Liver-like	Sweet/fresh		Rancidity
Meaty (boiled)	Sweetness	Fishiness	Train oil
Meaty (roast)		Fishy flavor	Rancid oil
Meaty	Bitter	Amine	
Iberian ham	Bitterness	Boiled cabbage	Off flavor/taste
Dry salt cod		Sulphide	Curry
Salty fish	Salt		Farmyard
	Saltiness	Metal	Rotten seaweed
Cold ashes	Salty	Metallic	Rubber
Sharp		Chemical-metallic	Soapy
Smoke odor	Brine		Turnip
Wood fire	Salty ripened cheese	Ocean/iodine	
Smokiness		Iodoform-like	Astringent
Acid smokiness		Medicine	Hot
		Aftertaste	
Chicken-like aftertaste	Oily aftertaste	Earthy aftertaste	Fishy
Salmon	Oily	Metallic aftertaste	Sour

Table 40.2. Description of some appearance properties evaluated using sensory analysis. Only references where the evaluation of the property is described are included in the table.

Attribute	Definition	Preparation[1]	Reference
Hue	• From red = 1 to yellow = 9	S	Einen and Skrede 1998,
	• yellow = 1, red = 9		Rørå and others 1998
	• Red = 1 to yellow = 9	C	Herme and others 2004
Color intensity and shade	• 1 = with/yellow, 7 = strong red	C	Sivertsvik et al. 1999
Flaky	• Visible flakiness of steak when cut with a knife	C	Farmer and others 2000
	• Tissue parts into flakes by pressing with fork	C	Warm and others 2000
Coagulated protein	• Whitish form on top of and beside sample	C	Warm and others 2000
	• Amount of coagulation that appears on the surface of the salmon steak	C	Farmer and others 2000
Fat droplets in water	• Quantity and size of fat droplets in the liquid	C	Ginés and others 2004
Separation	• Oiliness of the juice seeping out of the salmon	C	Farmer and others 2000
Juicy appearance	• Amount of juice that has seeped onto the plate from the salmon	C	Farmer and others 2000
Moist appearance	• Visible moistness of steak when cut with a knife	C	Farmer and others 2000
Shiny	• Gloss of tissue caused by oil	C	Warm and others 2000
Crumbly	• Crumbliness of steak when cut with a knife	C	Farmer and others 2000
Discoloration	• Color distribution uneven	C	Ginés and others 2004
Paleness	• Intensity of paleness in the uncut steak	C	Farmer and others 2000
Yellow water	• Degree of yellow water liquid present	C	Ginés and others 2004
Beige	• Intensity of beige color close to the skin	C	Farmer and others 2000
Brownish	• Light brown	C	Warm and others 2000
Grayish	• Light gray	C	Warm and others 2000
Orange	• Intensity of orange color in the uncut steak	C	Farmer and others 2000
Peach	• Intensity of peach color in the uncut steak	C	Farmer and others 2000
Pink	• Intensity of pink color in the uncut steak	C	Farmer and others 2000
Whiteness	• Intensity of white color in the uncut steak	C	Farmer and others 2000
Whitish	• Not totally white	C	Warm and others 2000

[1]C = cooked, S = smoked.

Odor

The odor often has a higher intensity than flavor in seafood. A description of the odor attributes is given in Table 40.3.

Taste/Flavor

A description of the attributes for taste/flavor and aftertaste is given in Table 40.4.

SENSORY CHARACTERIZATION OF FISH, FISH PRODUCTS, AND SHELLFISH

Different Fish Species

There can be large variation in the sensory characteristic of different fish species, but also similarities. Cardello and others (1983) characterized 17 fish species by sensory profiling, and by using cluster analyzing of the data, they found that the 17 species could be classified in three major groups. The first group is characterized by primarily low-fat, low flavor intensity, white-fleshed fish and consists of tilefish (*Lopholatilus chamaeleonticeps*), pollock (*Pollachius virens*), haddock (*Malanogrammus aeglefinus*), Wolffish (*Anarhichas lups*), Atlantic cod (*Gadus morhua*), cusk (*Brosme brosme*), white hake (*Urophycis tenuis*), whiting (*Merluccius bilinearis*), blackback flounder (*Pseudopleuronectes americanus*), Atlantic halibut (*Hippoglossus hippoglossus*), monkfish (*Lophius americanus*), and grouper (*Mycteroperca microlepis*). The second major group consists of the high fat, high flavor intensity, dark-fleshed fish, including bluefish (*Pomotomus saltatrix*), Atlantic mackerel (*Scomber scombrus*), weakfish (*Cynoscion regalis*) and striped bass (*Morone saxatilis*). The third group consists solely of swordfish (*Xiphias gladius*), but none of the sensory parameters described the swordfish very good. The swordfish was included in another study by Morita and others (2003). In this study different saltwater fish, migratory coastal fish, coastal bottom fish, pelagic fish, deep-sea fish, freshwater fish, anadromous fish and brackish water fish, in total 16 species were sensory characterized. By multivariate data analysis, four groups were observed in a biplot using PCA (principal component analysis). Red sea bream snapper (*Pagrus major*), common Japanese conger (*Conger myiaster*), Japanese eel (*Anguilla japonica*), and freshwater species, i.e., loach (*Misgurmus anguillicaudatus*), pond smelt (*Hypomesus nipponensis*), and carp (*Cyprinus carpio*) were grouped around the flavor "green." Migratory coastal fish species (i.e., sardine (*Sardinops melanosticta*), banded blue-sprat (*Spratelloides gracilis*), and chub mackerel (*Scomber japonicus*), were located close to "fish oil," "grilled fish," "sea breeze," and "fishy." Swordfish (*Xiphias gladius*), sablefish (*Anoplopoma fimbria*), and chum salmon (*Oncorhynchus keta*) were located close to "fried chicken." Slime flounder (*Microstmus achne*), pacific cod (*Gadus macrocephalus*), bluefin tuna (*Thunnus thynnus*), and yellowfin goby (*Acanthogobius flavimanus*) were grouped around "cooked fish," "sweet," "canned tuna," and "roasted soy sauce."

Storage and Shelf Life

During ice storage of fish, the intensity of the sensory attributes will change. Sensory profiling of ice storage farmed salmon showed that sensory attributes characterizing the salmon on the first day were seaweed, cucumber, and sourish odor, and sweetish, sourish, fish oil, and mushroom flavor. After 22–24 days of storage, the salmon were characterized by rancid, sour, and amine odor, and rancid flavor. The sensory attributes used in another storage experiment with farmed salmon stored in ice were grouped into "positive sensory parameters" and "negative sensory parameters." Samples from every second storage day were analyzed. The changes in the sensory attributes indicate that the salmon was approaching the end of acceptable flavor after 20–21 days, when the salmon was characterized by increasing intensity of sour, amine, and rancid odor and flavor. All positive attributes had a high intensity and were very characteristic for the salmon at the beginning of the storage time, but after 21–22 days of storage, they were hardly detectable. It was concluded from the result of the sensory profiling that shelf life, where the fish is no longer fit for human consumption, was 20 days in ice (Sveinsdóttir and others 2003).

In storage studies with modified atmosphere packing (MAP), the development of sensory attributes is different than for ice storage of the same fish species. Hong and others (1996) found that the odor of Atlantic mackerel (*Scomber scombrus* L.) during MAP storage changed from day 0 (seaweed, fishy, and rancid) to 7

Table 40.3. Description of some odor properties evaluated using sensory analysis. Only references where the evaluation of the property is described are included in the table.

Attribute	Definition	Preparation[1]	Reference
Acid	• Vinegar	S	Stohr and others 2001
Amine	• A solution of trimethylamine	S	Cardinal and others 2004
	• Urine	S	Stohr and others 2001
Bacon	• Odor corresponding to the product	S	Cardinal and others 2004
Blue cheese	• Musty	S	Stohr and others 2001
Boiled corn	• Sweet aroma in boiled corn	SH	Morita and others 2001
Boiled egg	• Smell of boiled egg	SH	Morita and others 2001
Boiled prawn	• Typical aroma in boiled prawn	SH	Morita and others 2001
Butter	• Caramel	S	Stohr and others 2001
Cabbage	• Gas/garlic	S	Stohr and others 2001
Canned tuna	• Aroma of canned tuna	C	Morita and others 2003
Cardboardy	• Marine fish off odor notes	C	Hong and others 1996
Cheese	• Feet	S	Stohr and others 2001
Cold ashes	• Odor of ashes once the fire is out	S	Cardinal and others 2004
Cooked fish	• Aroma of cooked fish	SH	Morita and others 2001
	• Typical aroma of cooked fish	C	Morita and others 2003
Cucumber-like	• Grated cucumber	C	Hong and others 1996
Earthy	• Intensity of any earthy/peaty odor	C	Farmer and others 2000
Farmyard	• Intensity of manure/cow dung odor	C	Farmer and others 2000
Fish oil	• Smell of canned mackerel or sardine	C	Morita and others 2003
	• "Fatty" odor, herring oil, typical smell of fresh herring	M	Nielsen and others 2004
	• Intensity of oily (fish oil) odor	C	Farmer and others 2000
Fishy	• Smell of raw fish	C	Morita and others 2003
	• Trimethylamine crystals (marine fish off odor notes)	C	Hong and others 1996
Fresh odor	• Typical odor of rainbow trout, an element of fresh, slightly acidulous odor intensifies the freshness	C	Johansson and others 2000
Fried chicken	• Aroma of fried chicken	C	Morita and others 2003
Green	• Fresh green aroma of soybean milk	C	Morita and others 2003
Green aroma	• Freshly cut grass	S	Stohr and others 2001
Grilled fish	• Aroma of grilled fish, roasted fish oil	C	Morita and others 2003
Ham	• Cooked meat	S	Stohr and others 2001
Herring	• Odor corresponding to the product	S	Cardinal and others 2004
Hydrogen sulphide	• Egg	S	Stohr and others 2001
Irritate	• Ammonia-like	SH	Morita and others 2001

[1]C = cooked, M = marinated, S = smoked, SH = shellfish.

Table 40.3. continued

Attribute	Definition	Preparation[1]	Reference
Marine	• Fresh (not processed) fish	C	Warm and others 2000
Medicine	• Dentist, chemical	C	Warm and others 2000
Metallic	• Warm metal, blood	M	Nielsen and others 2004
Muddy	• Earthy odorant 2-methylisoborneol	C	Bett and others 2000
Plastic	• Citrus fruit	S	Stohr and others 2001
Rancid	• Fresh oil kept at 65°C for 16 days (marine fish off odor notes)	C	Hong and others 1996
	• Rancid fish, paint, varnish	M	Nielsen and others 2004
	• Oxidized fish oil	S	Cardinal and others 2004
	• Linseed oil	S	Stohr and others 2001
	• Fresh oil kept at 65°C for 19 days (marine fish off odor notes)	C	Hong and others 1996
	• Painty odors	C	Richards and Hultin 2001
Raw salmon	• Odor corresponding to the product	S	Cardinal and others 2004
Roasted shrimp	• Aroma of roasted shrimp	SH	Morita and others 2001
Roasted soy sauce	• Aroma of roasted soy sauce	C	Morita and others 2003
Rotten seaweed	• Rotten seaweed	C	Warm and others 2000
Rubber	• Odor of burnt tire	S	Cardinal and others 2004
Salmon-like	• Intensity of distinctive salmon-like odor	C	Farmer and others 2000
Sea breeze	• Smell of sea	SH	Morita and others 2001
	• Smell of sea	C	Morita and others 2003
Seaweed	• Soaking water of 1 leaf of dulse (Atlantic Mariculture LTD, Grand Manan, NB.) In 50 ml of distilled H2O	C	Hong and others 1996
Sewage	• Smell of sewage	SH	Morita and others 2001
Sour	• Tomato sauce-like	SH	Morita and others 2001
	• Fermented	S	Stohr and others 2001
	• Marine fish off odor notes	C	Hong and others 1996
	• Sourish, dish rag	C	Warm and others 2000
Sourish	• Acidic, acetic acid, citric acid	M	Nielsen and others 2004
Stagnant	• Intensity of stagnant water odor	C	Farmer and others 2000
Sweet	• Sweet aroma in cooked seafood	SH	Morita and others 2001
	• Sucrose like	C	Warm and others 2000
	• Sweet aroma of cooked seafood	C	Morita and others 2003
Tuna	• Odor corresponding to the product	S	Cardinal and others 2004
Wood fire	• Odor of a wood fire	S	Cardinal and others 2004

[1]C = cooked, M = marinated, S = smoked, SH = shellfish.

Table 40.4. Description of some flavor and aftertaste properties evaluated using sensory analysis. Only references where the evaluation of the property is described are included in the table.

Attribute	Definition	Preparation[1]	Reference
Acidulous	• Fruit acid like flavor	C	Einen and Thomassen 1998
Bitter	• Quinine/or caffeine like	C	Warm and others 2000
Earthy flavor	• Intensity of any earthy/peaty flavor	C	Farmer and others 2000
Farmyard flavor	• Intensity of manure/cow dung flavor	C	Farmer and others 2000
Fishy flavor	• Intensity of any other fish-like flavors	C	Farmer and others 2000
Fresh taste	• The typical taste of rainbow trout. In this investigation the typical taste was scored after the sample had been masticated five times. As element of fresh, slightly acidulous taste intensifies the freshness	C	Johansson and others 2000
Herring	• Typical taste of fresh herring	M	Nielsen and others 2004
Marine	• Fresh marine	C	Warm and others 2000
Metallic	• Warm metal, blood	M	Nielsen and others 2004
	• Flavor associated with metal	C	Ginés and others 2004
Musty	• Flavor associated with damp earth	C	Ginés and others 2004
Oily	• Flavor associated with fish oil	C	Ginés and others 2004
	• Intensity of fish oil flavor	C	Farmer and others 2000
Rancid	• Rancid fish, paint, varnish	M	Nielsen and others 2004
	• The atypical taste associated with oxidized fat	C	Johansson and others 2000
Salmon-like flavor	• Intensity of distinctive salmon-like flavor	C	Farmer and others 2000
Salty	• Typical taste of salt, seawater	M	Nielsen and others 2004
	• Intensity of salt-like flavor	C	Farmer and others 2000
Smokiness	• Intensity of smoky flavor	C	Farmer and others 2000
Sourish	• Acidic, acetic acid, citric acid	M	Nielsen and others 2004
Sweet	• Sucrose like	C	Warm and others 2000
Sweet/fresh	• Characteristic flavor of cooked Arctic char fillets	C	Ginés and others 2004
Chicken-like aftertaste	Intensity of chicken-like aftertaste	C	Farmer and others 2000
Earthy aftertaste	Intensity of earthy aftertaste	C	Farmer and others 2000
Metallic aftertaste	Intensity of metallic aftertaste	C	Farmer and others 2000
Oily aftertaste	Intensity of fish oil aftertaste	C	Farmer and others 2000
Time	Time when aftertaste starts	C	Farmer and others 2000

[1]C = cooked, M = marinated.

days of storage (seaweed, cucumber-like, sour, fishy, painty, and rancid) and again from 14 days of storage (seaweed, sour, fishy, rancid) to 21 days of storage (seaweed, sour, fishy, metallic, and rancid).

The rapid development of rancidity in stored muscle from fish such as mackerel, herring, capelin, and bluefish is often attributed to the fact that these species contain high levels of lipid. However, studies by Richards and Hultin (2001) indicate that blood-mediated oxidation of washed cod lipids required ≤ 0.1% phospholipid to cause rancidity. They also found with long-term, frozen storage of mackerel muscle, sensory quality deterioration occurred in spite of no measurable change in fatty acid composition.

It is not always the highest intensity that is found when smelling the sample; sometimes the odor/flavor is first recognized when the structures are broken during chewing of the sample. The most pronounced sensory changes of salmon during frozen storage were first recognized by assessors when the salmon samples were in the oral cavity. For train oil, metal, and bitter taste significant time-temperature effects were determined. The intensities of train oil, metal, and bitter taste increased during storage at −10 and −20°C. The intensity of earthy and fish oil flavor decreased in salmon stored at high temperatures (Refsgaard and others 1998). They also found that both cooked and raw samples showed significant color changes during the storage time. The salmon color intensity decreased during frozen storage, and this change was not dependent on the storage temperature.

Bromophenol Compounds in Seafood

Simple bromophenol compounds are found in seafood and give an ocean-like flavor in seafood, which is desirable, but at high concentrations of bromophenols, the flavor becomes an off flavor, iodoform. In water, the most strongly flavored bromophenols are 2,6-dibromophenol, 2-bromophenol, and 2,4,6-tribromophenol, and they have flavor threshold concentrations of 5×10^{-4}, and 3×10^{-2}, and 0.6 nanograms per gram (ng/g), respectively (Whitfield and others 1997). At levels below their flavor threshold concentrations in water, these bromophenols contribute recognizable taste to marine- or ocean-like flavors and enhance the intensities of existing seafood flavors (Whitfield and others 1997).

Whitfield and others (1999a, 1999b) found that many species of Australian marine algae contain relatively high concentrations of bromophenols (433 to 2,590 ng/g). Other marine organisms that contain large concentrations of bromophenols include marine worms of the phyla Hemichordata and Phoronida. Accordingly, marine algae could be regarded as a major source of bromophenols in those fish that feed predominantly on marine plants. Species of Australian ocean prawns and bottom-feeding fish are known to feed largely on marine algae. The intensity of flavors caused by the presence of bromophenols in such seafood will depend on the quality and species of polychaetes consumed by the individual prawns and fish (Whitfield and others 1999a, 1999b). Whole wild ocean prawns have a total bromophenol content that ranges from 9.5 to 1,114 ng/g, depending on species, location, and time of year of catch (Whitfield and others 2002). Whitfield and others (1997) suggested that the flavor of farmed fish and prawns could be modified to have a "marine-like" flavor by the use of a feed containing a natural supplement high in bromophenol. Experiments with farmed prawns (*Penaeus monodon*) showed that three prawns given the experimental feed containing bromophenols all had flavors described as briny or iodine/ocean-like. The flavor of the prawns, fed with a commercial feed was also described as slightly briny and iodine-like. In these prawns, the concentrations of 2-bromophenol and 2,6-dibromophenol in the tails were 0.4 and 0.7 ng/g, respectively (Whitfield and others 2002). Feed with controlled concentration of bromophenols might be used in farming of seafood for selected species to give a wanted ocean-like flavor.

Influence of Fed and Starvation

The length of starvation before slaughtering can have a big influence on the sensory attributes. In farmed Atlantic salmon (*Salmo salar*), fresh flavor was significantly reduced in groups starved from 30 to 86 days, whereas no significant differences were found among groups starved for 0 to 30 days. The sensory test was performed on day 13 to 16 postmortem fish (Einen and Thomassen 1998).

The content of the feed can influence not only the growth rate, but also the sensory characteristics of the fish. For Atlantic halibut (*Hippoglossus hippoglossus*) fed with a diet containing 20 or 39% fat, the sensory test showed that larger fish (2.1–2.7 kilogram [kg]) were characterized by a fresher and more acidic flavor and a more juicy consistency,

compared to smaller fish (1.4 kg). The growth of the 20 halibut used in the sensory test showed that high growth was associated with small fish, which achieved higher scores for off flavor and rancid flavor than the larger halibut with lower growth rate. This does not imply that a high growth rate is a contributing factor to the off flavor or the rancid flavor of the small fish, but shows that these characteristics are typical for the small fish (Nortvedt and Tuene 1998). The odor for turbot (*Psetta maxima*) fed with three experimental diets (with 9% by weight [w/w] added lipid: fish oil, soybean oil, or linseed oil) were described as having a fatty fish note, even though the lipid content of turbot muscle was very low. Grass and hay notes were also found to characterize the odor of flesh (Sérot and others 2001).

Taint or Off Flavors

According to ISO 5492 1992, the term off flavor is defined as atypical flavor often associated with deterioration or transformation of the product, whereas taint is taste or odor foreign to the product. In this definition, geosmin and MIB are taints.

Microbial production of the muddy metabolites geosmin and 2-methyl-iso-borneol (MIB) can be a problem in freshwater fish such as trout, bream, and pike. Based on the values presented in the literature, Howgate (2004) estimated the odor detection threshold in water at a normal ambient temperature of natural geosmin to be 0.015 $\mu g\ 1^{-1}$ and 0.035 $\mu g\ 1^{-1}$ for MIB. Because geosmin and MIB are much more soluble in lipid than in water, the concentration of the chemical in the lipid phase will be much greater than in the water phase. This will influence the threshold in fish with different lipid content.

Bett and others (2000) found in channel catfish (*Ictalurus punctatus*) fillet that MIB is perceived less or not perceived at all in the presence of the masking agent, lemon-pepper. It is possible to mask some unwanted attributes, but it needs to be investigated if the same masking can be used during the storage period and how it will be perceived when the fish are used in a dish.

Smoked Fish

The content of the raw material will influence the smoked product. Einen and Skrede (1998) found that increased astaxanthin levels in raw salmon changed the appearance of the smoked fillets toward less whiteness, higher color intensity, and a redder hue. Higher fat levels increased the yellow hue of both smoked and raw fillet color. The sensory analysis in the study by Einen and Skrede (1998), revealed that increasing smoke odor and decreasing off flavor in the sensory analysis of smoked fillets were related to increasing astaxanthin content of the fillet.

Rørå and others (1998) found in an investigation of smoked Atlantic salmon (*Salmo salar*) that the total intensity of taste and the intensity of smoked, rancid, and off tastes increased and the intensity of fresh taste decreased with increasing fatness and decreasing juiciness. They also found that fillets perceived as fattiest had more taste and odor than fillets perceived as being less fatty. Smoked taste correlated positively with evaluated fatness, indicating that the main flavor compounds in the smoked salmon were fat soluble, although the measured fat content of the raw material did not show any significant relationship to either smoke taste or odor. Values for rancid taste and of taste/odor were, however, low compared with those of the other tastes and odors.

Einen and Skrede (1998) found that for smoked Atlantic salmon (*Salmo salar*) fillets, both rancid flavor and smoked flavor were significantly correlated with the fat level of the raw fillets. The observed increase in rancid flavor with increasing fat content of the fillets may be related to greater amounts of oxidizable fat in fillets with higher fat contents. At increasing fat levels of the raw fillets, the intensity in smoke odor decreased. They explained the weakening of the smoke odor with increasing fat levels by a higher solubility of the smoke odor-forming components in the fat phase of the muscle, resulting in less odor being given off in the fillets with the higher fat levels. For hot smoked farmed rainbow trout (*Oncorhynchus mykiss*), Rasmussen and others (2000) found that dry matter was negatively correlated with fresh oily taste.

Also the smoking method will influence the sensory quality of the smoked product. For farmed smoked eel, the taste attributes were related to the method of smoking but not to the feed (Bech and others 1997). Smokiness and acid smokiness were related to stone cabinet smoking and size of the eels. Off flavor was related to the smoking in an automatic cabinet.

Marinated Herring

Results from a recent study of herring (*Clupea harengus*) marinated immediately postmortem showed that fishing ground and season did not influence the

odor, flavor, and texture of herring, but they found a subtle variation was encountered in the appearance. The sensory properties were also influenced by body weight and age, but not by sex and gonad maturity. The influence of varying lipid content, water content, and liquid-holding capacity resulted in similar effects showing the high correlation between these properties. The liquid-holding capacity was related to the sensory profile with the significant descriptors metallic odor and flavor, herring and salty flavor, sourish odor, and gritty texture (Nielsen and others 2004a). An increased weight, length, or age introduced an increase in juiciness, herring odor and flavor, and sourish flavor and a decrease in rancid and metallic odors and flavors. There was an apparent effect of spawning type on the sensory profile. Autumn spawners were characterized with rancid and metallic odors and flavors, while spring spawners had higher intensities of herring and sourish odors and flavors, salty flavor, and juicy texture (Nielsen and others 2004b).

SHELLFISH

Manipulation of the environmental salinity affected the flavor of frozen white shrimp (*Penaeus vannamei*). Since free amino acids are major osmoeffectors in shrimp and also primary flavor producers in marine products, more flavorful shrimp can be produced by acclimation to high environmental salinities (Papadopoulos and Finne 1986).

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Quality Index Methods

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INTRODUCTION

The Quality Index Method (QIM), as it has come to be known, was born out of necessity. During the late 1970s and early 1980s, the Tasmanian Food Research Unit (TFRU) of the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia, was conducting a series of trials on different post-catch handling and storage methods for fish and on different packaging and storage methods for fish and for fillets. These trials involved comparisons of the product and it was evident that no satisfactory method existed that allowed differentiation between the various materials over the course of the storage period. Furthermore, no method existed that clearly evaluated the nature of the starting material so that comparisons could be drawn between the different trials, since start material that was exactly similar could not always be obtained. This is a situation that is faced daily by fish buyers and agents as well as by experimenters seeking to determine storage properties.

Although changes occurring in stored fish had been recorded many times, the majority of the schemes used were descriptive and comparisons were difficult. Furthermore the TFRU trials were (naturally) being conducted on species from the Southern Hemisphere, which had scarcely been fished previously on a commercial basis, and about which very little was known. There was thus no body of standard information about the nature of the changes and no experienced personnel to train assessors. In Australia no standard schemes were in use at the time and those available, for example, the Torry schemes (Shewan and others 1953) and the European Union (EU) grading scheme (Anonymous 1996) had been developed on Northern Hemisphere species most of which were unrelated, to those in Australia.

At that time the concept of the relative rate of spoilage had been published (Olley and Ratkowsky 1973a, 1973b). This states that most protein foods, such as fish, meats, and milk, all spoil at similar rates expressed as a ratio to the rate they spoil at the reference temperature of zero degrees Celsius (0°C). This means that spoilage at a variety of temperatures

can be reexpressed in terms of days equivalent storage at 0°C and that the integrated effects of storage at different temperatures can be taken into account. Because much recently caught fish is chilled in ice, this provides a uniform method for expressing spoilage and shelf life. It also follows from the nature of this relationship that fish spoil four times faster at 10°C than at 0°C, and twice as fast at 4°C as at 0°C. No schemes proposed up to that point had considered this aspect. It was therefore obvious that a new approach was necessary that could be used on a variety of species, did not require extensive training, was robust, easy to understand, and capable of integrating the effects of time and temperature.

When a scheme is developed, consideration must be given to why it is required, what advantages it has over existing schemes, its accuracy and precision, whether it fits a need, what it will be used for, the circumstances in which it will be used, its robustness in different hands, its adaptability to changing circumstances, its potential in meeting future requirements, its communication value, its ease of use, its cost, its likelihood of adoption, its consistency with known theory, and thus its predictive capacities.

It is fair to say that the developed scheme meets all these criteria and is probably the only scheme yet devised that does so, or in which all these criteria have been considered in its development.

The terminology used in the scheme has been deliberately chosen to avoid conveying the impression that the scheme measures quality itself or freshness or even spoilage since these are vague concepts about which different opinions are common (Bremner 2000, Bremner and Sakaguchi 2000). What the scheme measures is the degree and rate of change in important criteria and in the sum total of these changes, which can be interpreted into equivalent days of storage and remaining shelf life. These are very factual, very practical considerations. The later coining of the term Quality Index Method (QIM) shows that the result obtained in the scheme can be used as an index of what the material may be like for an appropriate end use. This may be a judgment of its ability to withstand a process, or a selection of it for a particular grade of product, or it may foreshadow what the product is anticipated to be like when it is cooked and eaten by the consumer. There are now also QIM schemes developed for cod and plaice fillet (Larsen and others 1998–1999) and for frozen cod (Hyldig and Nielsen 1997, Warm and others 1998, Herrero and others 2003; also see Chapter 44, Quality of Frozen Fish). The QIM scheme for frozen cod measures the storage history. It has already been stated that the total score can be used to assess storage history and to estimate remaining shelf life, but this is not its only use. The output score is a small number, generally below a value of 20, and this makes labeling and assessment of changes very easy. Although schemes can be readily expressed and constructed in different languages, a numerical value as an output is universal and can be understood by all. The score can be used to judge handling and storage procedures, efficiencies of personnel, and duration of transport, and it is particularly useful in troubleshooting.

The original scheme was a general scheme used with slight modifications to assess some southern temperate species (Statham and Bremner 1983, 1985; Statham and others 1985; Fitzgerald and Bremner 1998) and some tropical species (Bremner and others 1984). With suitable modification, scampi (Bremner 1988) and shrimp (Chinivasagam and others 1995) were also assessed using the same general principles.

The technique was adopted at the Danish Institute for Fisheries Research, Department for Seafood Research and during the late 1980s, a number of more specific schemes suited to Northern Hemisphere species were developed in a Nordic project (Jespersen and Helba 1991, Larsen and others 1992). In the Nordic collaboration project between fish research institutes in Iceland, Norway, Denmark, and the Faroe Islands, QIM schemes were developed for some of the most important fish species in the Nordic countries. These were published in Danish (Anonymous 1992a, 1992b). The name Quality Index Method was coined as a concise term for the method.

An EU-funded Concerted Action (CA) project on fish freshness, recommended QIM as a general reference method (Martinsdóttir 1997). Later, several EU-funded projects have had the objective to develop QIM schemes for the various fish species and to study the possibility of incorporating QIM into normal practice, specifications, and standards.

On the basis of these projects, a major development has recently been announced in the formation of the QIM-Eurofish Foundation (www.qim-eurofish.com), which is an alliance among the three major fish research institutes in Holland, Iceland, and Denmark that have been most concerned with the development and exploitation of QIM. The aim

of this alliance is not merely to promote the use of QIM but to safeguard the manner in which it is used. In this respect, training and audit of schemes has been done on a needs basis. Needs from the industry are based on new technological developments such as auctioning through the internet (i.e., PEFA.com) and remote trade. Borders of EU countries have no trade barriers any more, and these days it is common practice to buy fish sight unseen in different European countries. The industry therefore needs a reliable, universal, and useful tool to assess the quality of fish. In practice the EU scheme (Anonymous 1996) is found to be inaccurate and not practical and is mainly used for detecting fish that are unfit for human consumption at the point of landing. For quality grading, the current EU scheme is not adequate. In a grading scheme, sensory attributes of three to six different criteria may be used. Each of them has to be evaluated according to the specifications of the grade standards being used, which often consists of three to six subdescriptions for each of the different grades. Thereafter, a final grade is determined. It often happens that the sensory characteristics of the criterion being evaluated do not agree with all of the other subdescriptions. This will increase the time used to evaluate the fish and can cause confusion for the assessor (Botta 1995). The EU scheme is not species related and the sensory characteristics among the various fish species are very different. QIM Eurofish will continue to stimulate the implementation of QIM within the fish sector and its recognition and adoption by official bodies like the European Commission (Luten and Martinsdóttir 1997). Now there are QIM manuals in 11 European languages (Danish, Dutch, English, French, German, Greek, Icelandic, Italian, Norwegian, Portuguese, and Spanish) covering 13 QIM schemes for commercially important species (Martinsdóttir and others 2001, 2004).

Principle

The scheme is based on the proposition that evaluators cannot judge degrees of perfection but can very readily detect deviations or changes from it. A simple illustration of this would be a crack in an otherwise perfect wall. Thus defects in the product were allotted demerit points, which were summed to a total to provide an overall evaluation. The higher the number of demerit points, the more defects the product had. This approach was derived from the understanding that during storage of fish, changes occur in it that are readily detectable and often measurable. This is also in keeping with the fact that the vast majority of chemical, biochemical, and microbiological tests on fish products start from either zero or a low value and increase with both temperature and period of storage.

Setting the start point at zero is a fundamentally different view from many other evaluative schemes in which a perfect product starts at a high score, often arbitrarily chosen at 10 or 100, and deviations from it are subtracted to result in a lower total. The approach where the start value is a high score requires a priori knowledge of the stages of change in order to set the scaling system and, although it is commonly used in many fields, it is counter intuitive. Only its familiarity makes it seem comfortable to users. It is valid to score a product on a single criterion but differentiation among products is difficult unless accuracy and precision are high and the scale is sufficiently large. These factors are rarely ever the case when evaluating fish and if a single characteristic such as the shape of the eyes was used, there are few descriptive categories available and judges would have reservations about differentiating among the descriptors flat and slightly flat.

Conversely, scoring large numbers of criteria is laborious, tedious, time consuming, and hence prone to error due to operator fatigue; many may be irrelevant and may not result in any better differentiation than a smaller number of criteria. In addition, the scoring allotted to each criterion was such that no single criterion could dominate and so the score values were easy to judge. Using the example for shape of eyes, for fish species such as cod, only three scores may be necessary: 0 = convex, 1 = flat, 2 = sunken. All of these are very easy for an untrained assessor to judge. A fourth score 3 = very sunken may be included in a QIM scheme if it is necessary. (See Table 41.1).

The resultant scheme became a list of attributes each of which was scored on a restricted scale (0 to 3), and the scores were then added to provide a total, a quality index (QI).

With further experience, the attributes and the scores were adjusted slightly to result in a scoring system that gave a straight-line relationship with period of storage of the fish (Bremner 1985; Larsen and others 1992; Huidobro and others 2000; Sveinsdóttir and others 2002, 2003; Nielsen and others 2003;

Table 41.1. The part of the QIM schemes with the quality parameter for the eyes for cod (*Gadus morhua*) and salmon (*Salmo salar*) (Martinsdóttir and others 2001).

Quality parameters		Description	Score
		cod (*Gadus morhua*)	
Eyes	Cornea	Clear	0
		Opalescent	1
		Milky	2
	Form	Convex	0
		Flat, slightly sunken	1
		Sunken, concave	2
	Color of pupil	Black	0
		Opaque	1
		Gray	2
		Salmon (*Salmo salar*)	
Eyes	Pupils	Clear and black, metal shiny	0
		Dark gray	1
		Mat, gray	2
	Form	Convex	0
		Flat	1
		Sunken	2

Hyldig and Nielsen 2004; Hyldig and Green-Petersen 2004). Further trials and theoretical investigations underpinned the validity of the scheme and demonstrated that it was capable of integrating the effects of time and temperature during storage (Bremner and others 1987; Sveinsdóttir and others 2002, 2003). The slope of the line is the rate of demerit point accumulation per day of storage, and a simple calculation can indicate the equivalent of the number of days at 0°C that the product has been stored. If the decision had been made at which value the product should no longer be sold, or where it crosses some arbitrary set boundary between product grades, then the remaining shelf life can be calculated for the appropriate end use.

One Scheme for Each Species

The recent activity has been in the development of QIM schemes suited to individual species. As already mentioned, some of the problems among earlier schemes, such as the EU scheme (Anonymous 1996) are that they do not take into account the differences among species. To do that, it is necessary to develop one scheme for each species, and the aim when developing QIM for various species is, also, to have the QI increase linearly with storage period of the fish expressed in equivalent days in ice. QIM does take the inherent differences among fish species into account and therefore it is necessary to develop QIM schemes for each fish species. To illustrate this, parts of different QIM schemes are shown in Tables 41.1 and 41.2. Table 41.1 shows the part with the eyes from the QIM schemes for cod (*Gadus morhua*) and salmon (*Salmo salar*). There are three quality parameters concerning the eyes in the scheme for cod (cornea, form of the eyes, and the pupil) and only two in the scheme for salmon (pupil and form of the eyes).

Table 41.2 shows that there are large differences in appearance between plaice and salmon. The texture is

Table 41.2. The part of the QIM scheme with the quality parameter for the appearance for plaice (*Pleuronectes platessa*) and salmon (*Salmo salar*) (Martinsdóttir and others 2001).

Quality parameters		Description	Score
		Salmon (*Salmo salar*)	
Skin: Salmon	Color/ appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow and clotted	2
	Odor	Fresh seaweedy, neutral	0
		Cucumber, metal, hay	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 seconds	2
		Plaice (*Pleuronectes platessa*)	
Skin: Plaice	Skin (both dark and white side)	Fresh, bright, metallic, no discoloration	0
		Bright, but without shine	1
		Matte, rather dull, slight green/blue or purple discoloration	2
		Dull, green/blue, purple discoloration	3
	Mucus	Clear, not clotted	0
		Slightly clotted and milky	1
		Clotted and slightly yellow	2
		Yellow and clotted	3

not in the scheme for plaice, because here texture is not easy to measure and it does not change much during storage.

DEVELOPMENT OF SCHEMES

As described in the Introduction, there are several considerations to take into account to develop any new scheme. It is necessary to have some specific knowledge about the fish species, to have on hand two tested and trained sensory panels, a facility to conduct storage experiments under standardized conditions, and to be able to make a statistical validation of the developed QIM scheme. In the following paragraphs, this standardized development is described in detail.

The Raw Material

The selection of fish species is based on practical considerations and economic value. The fishing gear used to catch them and the fishing grounds where they are caught correspond with this selection and are fixed for QIM schemes. The handling of the fish should be according to Good Manufacturing Practice (GMP) (Codex 1976). GMP can mean various standards or technical specifications, such as that the fish is gutted at sea (if gutting is the normal procedure) and washed. The fish is directly cooled down to 0°C in melting ice or equivalent cooling media. The fish is stored in fish boxes, with sufficient ice, which may be replenished, during the whole storage period. The storage trials begin with homogeneous batches of fish, preferably from one haul, with known history such as date of catch, storage condition, etc. For the complete development of a new QIM scheme, at least three storage trials are needed and the experiment must begin at the time of catch and continue till after the end of shelf life. The QIM schemes developed with these batches are valid for these conditions.

The Draft Scheme

For the development of a draft QIM scheme, the first storage experiment is needed. In this stage, the fish is described in detail during the complete ice storage until the end of an expected shelf life. This is done at fixed time intervals of 12 to 48 hours, depending on the expected shelf life of the species. The shorter the expected shelf life is, the shorter the time intervals required. A group of a maximum of five experienced panelists, including a panel leader, is put together. This group is selected, besides the usual criteria for sensory panelists, for their good use of vocabulary and knowledge of the fish species. The panel leader is experienced in controlling the discussion and enables everyone to express their own meaning. Approximately five fishes are used at the time, and they are discarded after each session. During the sessions, the fishes are placed on chill plates or ice. Further sessions are continued until the fish is completely spoiled. All attributes are listed and described in detail. The attributes to be assessed vary per species; for example, for flat fish species, the appearance of both sides might be relevant which is, obviously, not the case for round fish species. The appearance for shrimp will have a completely different description than for cod. All descriptions are written down, preferably in terms the whole group agrees on. A description is written on how the assessment was done—for example, the assessment of the texture attribute for cod is done by pressing the finger (firmly but not too hard) on the spine muscle and observing how fast the flesh recovers.

After this first storage trial, the panel leader selects those attributes that change over the storage period, the descriptions are grouped together per attribute, and major changes are scored with "demerit" points from 0 to 3. If, for example, the maximum of three demerit points are scored within the first 5 days, but it is generally known that then the shelf life is, in total, about 14 days, the description per demerit point needs to be changed in such a way that the scoring covers more of the complete shelf life. This results in the draft QIM scheme that will be used in the next storage trial.

Testing the Draft Scheme

The testing of the draft QIM scheme is done with a second batch of fish during a new storage trial. For this test, a new panel of approximately 10 panelists (experts in QIM) is organized, and the panelists who participated in the development of the scheme are included.

During the storage trial at fixed time intervals, again depending on the shelf life but less frequently than in the first storage trial, five fishes are assessed individually by using the draft QIM scheme. Each expert scores every attribute and comments are written down. After each session the scores, comments, and questions on how to assess the attributes are discussed among the panelists. The results are analyzed and the attributes are selected for being discriminative and for ease of assessment. Statistical analyses used to assist these decisions are Principal Component Analyses (PCA) or calculation of linear regression lines per attribute (Sveinsdóttir and others 2002, 2003). These decisions on attributes and scores are of major importance for meeting several of the above-mentioned considerations like the balance between the strength of the scheme, reliability of the results, and practical use of the scheme, and cannot be made without knowledge of the fish species, spoilage pattern, practical importance of the sensory attributes, and QIM principles in general.

At the same time when the draft scheme is tested, the end of shelf life needs to be determined. The shelf life is defined as the number of days that a whole, fresh (gutted) fish can be stored in ice until it becomes unfit for human consumption. It needs to be emphasized that the estimated shelf life is based upon optimal catching and storage conditions (Sveinsdóttir and others 2002, 2003; Martinsdóttir and others 2001).

Spoilage due to microbial activity is the main limitation of the shelf life. Another cause of spoilage may be rancidity, especially in fatty fish species. The flesh of newly caught fish is free of bacteria. However, considerable amounts of bacteria may be in the viscera, on the gills, and on the skin, which can give contamination during processing. When the fish is stored whole in ice, the deterioration caused by bacteria is minimal for the first days of storage and then it will increase.

In general, when fish is stored in ice, the flavor and odor compounds that characterize newly caught fish decrease and disappear in the first few days during storage, and the fish flesh becomes almost flavorless and odorless for a while. Then an increase in bad-smelling sulphur and nitrogenous volatiles will

result in rejection of the fish for human consumption. This can be measured by descriptive sensory profiling or by using the Torry scale. A tested and trained sensory panel evaluates cooked samples from the storage experiment. The sensory panel must be a second panel of different composition in order to overcome bias. The descriptive sensory profiling and the Torry scale are described in the previous chapter. From the results of the descriptive sensory analysis, the shelf life is defined.

For future use of the QIM scheme, such as training of QIM inspectors and illustration of the descriptions of the different attributes, pictures are of utmost importance. During the development of QIM, pictures need to be taken of all the changes of the different attributes. These pictures are preferably taken by a professional photographer in order to be clear and with the right use of flashlights. In practice, the importance of the quality of the pictures and the difficulties in taking them are highly underestimated. This can result in low-quality pictures that are not suitable for printing in reference manuals.

Calibration Curve

The next step in the development of a QIM scheme is the so-called calibration curve. As mentioned in the Introduction of this chapter, the QIM resulted in a scoring system that gave a linear relationship with the storage period and the slope of the line is the rate of demerit point accumulation per day of storage. A simple calculation can indicate the equivalent of the number of days at 0°C that the product has been stored (i.e., predicted storage time). To calculate the calibration curve, a simple linear regression analysis is performed. The data for this calculation is obtained from a third more complex storage trial. Before this third trial, the QIM panel is trained for the newly developed QIM scheme. The QIM panel will assess according to good sensory practice. They will evaluate unknown coded samples in random order. This implies that, during the storage trial several new batches, which have been stored for different equivalent days in ice, are randomly allocated so that fish of different QI are assessed in one session. From each batch, five fishes are assessed within each trial and a maximum of five assessments are performed per fish. If more than five panelists assess the fish, a double batch is needed. The storage trial is finished when the fish is spoiled, meaning some days after the determined end of shelf life, in order to get a complete calibration curve for the QIM scheme.

The average results are used to calculate the linear regression. The equation is calculated as well as the variation around the curve. The intercept at the y-axis is of major importance and should be close to zero. This follows the QIM principle of "deviations from a perfect product." The correlation coefficient between the QI and the days in ice should be close to 1.0. With this result, the calibration curves give reliable prediction of the storage time. The calculated Standard Error of Prediction (SEP) results in the reliability of the QIM assessments. Sveinsdóttir and others (2002, 2003) published results with the assessment of three salmon per batch. The reliability was 2 days, and with assessment of five salmon per batch the reliability was 1.5 days (Sveinsdóttir and others 2002, 2003).

The combination of the calibration curve and the determined end of shelf life makes it possible to calculate the remaining shelf life for the appropriate end use. Figure 41.1 shows a batch of fish reaching a sum of 10 points—corresponding to 12 days in ice—and having a remaining shelf life of 8 days in ice.

Validity of the QIM Scheme

To finalize the QIM scheme, the foregoing steps need to be combined and will result in a QIM scheme that is more than only a table with attributes, descriptions, and scores. The scheme is developed under standardized conditions that need to be described in order to know the validity of the scheme under different circumstances.

A fish caught by handline/longline will generally show fewer signs of deterioration than one caught by trawling, and its starting characteristics when stored in ice will be different. A trawled fish stored in refrigerated seawater will have a different appearance than those stored in ice (Nielsen and Hyldig 2004). In both these instances, the QIM scores at the start of storage will be different, but later during the storage period, any differences may be insignificant. Either a general scheme that does not differentiate on the basis of catch or storage medium should be used, or particular schemes should be developed that allow for this difference in properties. It depends on circumstances, anticipated storage life, and the use of the scheme. Huidobro and others (2001) reported

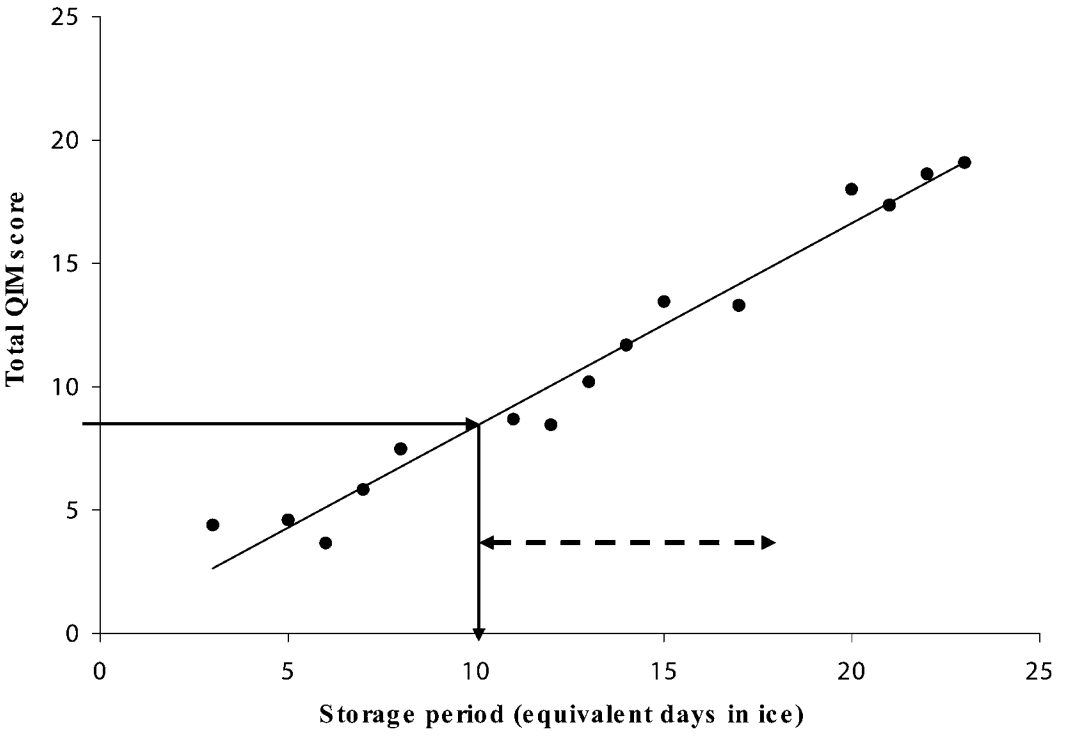

Figure 41.1. The calibration curve for salmon (y = 0.692x + 1.57). When a batch of fish reaches a sum of 10 points it corresponds to 12 days in ice and the remaining shelf life is 8 days in ice.

that the QIM of washed sea bream developed similarly to that of unwashed sea bream for up to 10 days' storage in ice. After that, less slime and lower trimethylamine (TMA) formation in the washed fish retarded the normal increase in score, in comparison to unwashed fish. This illustrates another instance where the process may influence the score. But anticipated shelf life should also be taken into account since these were aquacultured sea bream destined for sale as fresh (i.e., unfrozen) fish. Clearly there would be a failure in the market and distribution system if such a prime product was not sold in much less than 10 days, and a scheme should not necessarily be required to cover all treatment options beyond this time.

To a large extent, all changes in stored fish are governed by time and temperature with the proviso, as we have seen above, that species and catch and handling practices, and maybe seasonal factors, are similar. If the commercial fish handling system maintains fish at a steady temperature of 0°C then any lack of ability to integrate is not of concern. However, it is a matter of common and recorded observation that temperature in the fish chain is not always well controlled. It may be that some schemes provide an incorrect assessment depending on how long the fish has been at elevated temperatures. However, most other instruments and schemes have not been tested in this regard either. The Torrymeter for example cannot integrate time and temperature at the correct rate (Bremner and others 1987). The original scheme, on which QIM is based, was shown to be capable of integrating the effects of time and temperature (Bremner and others 1984). Experiments at the Danish Institute for Fisheries Research in 2003 were set up to check whether the developed QIM schemes could integrate the time/temperature effects. The results from experiments with cod and plaice showed that if the fish have been stored at elevated temperatures (between 0 and 5°C) and then stored at 0°C, the development in QI would follow the calibration curve for the fish species.

Some tropical reef fish have inordinately long shelf lives of more than 3 weeks when they are iced soon after catch and kept cold during storage (Bremner and others 1984). This seems to be due to the absence of spoilage psychrotrophic bacteria, unless they are introduced, and to a stable level of inosine monophosphate (IMP) in the flesh. Consequently, the flesh remains acceptable in flavor for considerable periods of time when virtually all of the external indicators that would be used in a QIM scheme are at or near their maxima. However, it is still obvious that the fish have been stored for a considerable period and their remaining commercial life must be extremely limited. The use of a QIM approach for these species is still valid but care must be taken in formulating a scheme in which the maximum QIM score does not undershoot shelf life by too great a margin.

To validate the newly developed QIM scheme, a storage experiment at another location, season, or catching ground can be conducted (Sveinsdóttir and others 2002, 2003).

QIM Schemes for Fish Kept in Ice

In a reference manual for the fish industry (Martinsdóttir and others 2001, 2004) QIM-schemes for the following fish species are published: brill (*Rhombus laevis*), cod (*Gadus morhua*), deep water shrimp (*Pandalus borealis*), farmed salmon (*Salmo salar*), whole fjord shrimp (*Pandalus borealis*), haddock (*Melanogrammus aeglefinus*), herring (*Clupea harengus*), peeled shrimp (*Pandalus borealis*), plaice (*Pleuronectes platessa*), pollock (*Pollachius virens*), redfish (*Sebastes mentella/marinus*), sole (*Solea vulgaris*), and turbot (*Scophthalmus maximus*).

Moreover Andrade and others (1997) have published schemes for Atlantic mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*), and European sardine (*Sardina pilchardus*), and Barbosa and Vaz-Pires (2004) published a QIM scheme for common octopus (*Octopus vulgaris*).

Tables 41.3, 41.4, and 41.5 show the schemes for salmon, cod, and plaice.

TRAINING OF QIM ASSESSORS FOR THE INDUSTRY

Training QIM assessors for the industry implies both training for being a sensory panelist, including the standard sensory procedures, as well as training for implementation of QIM in practice. The sampling system, methods, and procedures for sensory evaluation must be very well defined to serve its purpose in quality management.

QIM is an objective method and, compared to other sensory methods, it is easy to work with, since it includes instructions and easily understood illustrational material.

Test, Selection, and Numbers of Assessors

QIM assessors must be selected on their ability to evaluate appearance, color, odor, and texture. Assessors must also be healthy and possess normally sensitive taste and odor senses (ISO 8586-1 1993, ISO 11035 1994). Personal characteristics are also very important such as conscientiousness and accuracy, and they must be able to work in a group without disturbing the other assessors with noise, talk, and making faces. Depending on the regular duties of the individual, he or she must be readily available.

For a company, it is necessary to have a panel leader and a group of tested and trained assessors. The assessors for the QIM evaluation are then picked out from the group depending on availability, but it must be emphasized that all assessors in the sensory group are used frequently. A company could have a panel leader and six tested and trained assessors for the sensory group, even though all do not participate in all sensory evaluation sessions. Table 41.6 shows an example with four assessors in each session.

Standardized Training of QIM Assessors

QIM is well suited to train assessors and monitor performance of the panel. The QIM sessions must take place without any disturbance among the assessors. Assessors should know the nature and limits of the sense organs and learn how to recognize and evaluate appearance, taste, odor, and texture of fish after different periods of storage.

The training of the sensory panel should begin by describing the procedures of the sensory evaluation, what is expected of the assessors, etc. The nature and limits of the sense organs are described, such as the importance of breathing deeply and resting

Table 41.3. QIM scheme for whole farmed salmon (*Salmo salar*) containing description for each parameter and the given scores in succession from 0 to 3.

Quality parameters		Description	Score
Skin	Color/appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow and clotted	2
	Odor	Fresh seaweedy, neutral	0
		Cucumber, metal, hay	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 seconds	2
Eyes	Pupils	Clear and black, metal shiny	0
		Dark gray	1
		Matte, gray	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills	Color/ appearance	Red/dark brown	0
		Light red, pink/hazel	1
		Gray-brown, brown, gray, green	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odor	Fresh, seaweed	0
		Metal, cucumber	1
		Sour, moldy	2
		Rotten	3
Abdomen	Blood in abdomen	Blood red/not present	0
		Blood more brown, yellowish	1
	Odor	Neutral	0
		Cucumber, melon	1
		Sour, reminds of fermentation	2
		Rotten/rotten kale	3
Quality Index (0–24)			

between samples during odor evaluation. The schemes intended for use must be carefully explained. The general descriptions of the parameters are shown in Table 41.7. It should be emphasized to the assessors that they must not let their hedonic personal judgment interfere with the evaluation.

For training, three to four samples of fish of different known storage periods in ice and treatment are used. The storage time of the fish is introduced to the assessors before they evaluate the fish and they are asked if they can agree on the scores that should be given for each sample. The samples are number coded. All panelists should become very familiar with fish of all freshness stages (i.e., not only raw material that is on the borderline of production). Training results should be evaluated. Average and standard deviations of each sample are calculated and a comparison is made between the assessors,

Table 41.4. QIM scheme for whole cod (*Gadus morhua*) containing descriptions for each parameter and the given scores in succession from 0 to 3.

Quality parameter		Description	Score
Appearance	Skin	Bright, iridescent pigmentation	0
		Rather dull, becoming discolored	1
		Dull	2
	Stiffness	In rigor	0
		Firm, elastic	1
		Soft	2
		Very soft	3
Eyes	Cornea	Clear	0
		Opalescent	1
		Milky	2
	Form	Convex	0
		Flat, slightly sunken	1
		Sunken, concave	2
	Color of pupil	Black	0
		Opaque	1
		Gray	2
Gills	Color	Bright	0
		Less colored, becoming discolored	1
		Discolored, brown spots	2
		Brown, discolored	3
	Smell	Fresh, seaweedy, metallic	0
		Neutral, grassy, musty	1
		Yeast, bread, beer, sour milk	2
		Acetic acid, sulphuric, very sour	3
	Mucus	Clear	0
		Milky	1
		Milky, dark, opaque	2
Blood	Color	Red	0
		Dark red	1
		Brown	2
Fillets	Color	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
Quality Index (0–24)			

that is, by performing statistical analysis (analysis of variance, for example). The ability of the assessors can be examined during repeated evaluation of the same samples. Repetition of the training will show the capabilities of the assessors. Regular training of the sensory panel should be done and performance of the assessors monitored. It is also important to keep the assessors motivated. Finally, the assessors must be able to perform QIM in a fast and accurate way and be in agreement with other QIM panels in proficiency tests.

APPLICATION OF QIM IN PRACTICE

The application of QIM in practice must be tailor-made. The desire for standard sensory evaluation procedures must be balanced with practical purpose

Table 41.5. QIM scheme for whole plaice (*Pleuronectes platessa*) containing descriptions for each parameter and the given scores in succession from 0 to 3.

Quality parameter		Description	Score
Appearance	Skin (both dark and white side)	Fresh, bright, metallic, no discoloration	0
		Bright, but without shine	1
		Matte, rather dull, slight green/blue or purple discoloration	2
		Dull, green/blue, purple discoloration	3
	Mucus	Clear, not clotted	0
		Slightly clotted and milky	1
		Clotted and slightly yellow	2
		Yellow and clotted	3
Eyes	Form	Convex	0
		Convex but slightly sunken	1
		Flat or swollen (like a balloon)	2
		Flat, sunken in the middle	3
	Brightness	Clear, black shining pupil	0
		Rather matte, black pupil	1
		Matte, opaque pupil	2
		Milky, gray pupil	3
Gills	Odor	Fresh oil, sea weedy, metallic, peppery	0
		Neutral, oily, grassy, slightly musty	1
		Musty, bread, beer, malt, slightly rancid	2
		Rancid, sour, rotten, sulphurous	3
	Color	Bright, light red	0
		Slightly discolored, especially at the end of the gill filaments	1
		Discolored	2
		Yellowish, brown, gray	3
	Mucus	No mucus	0
		Clear	1
		Yellowish, slightly clotted	2
		Yellow, brown, clotted	3
Flesh, fillets	Color	Fresh, translucent, bluish	0
		Waxy, milky	1
		Dull, slightly discolored, yellowish	2
		Opaque, discolored, yellow, brown	3
Quality Index (0–24)			

Table 41.6. Distribution of assessors in a 3-week period, with a panel leader and four assessors in each session.

		Tested and trained assessors					
		A	B	C	D	E	F
Week 1	Panel leader		X		X	X	X
Week 2		X		X		X	X
Week 3		X	X	X	X		

Table 41.7. General description of parameters.

Appearance	
It is important that the fish do not lie for such a long time that the skin dries out.	
Skin	The whole fish is inspected for the appearance of the skin and fins.
Mucus	The appearance of mucus on the skin is assessed. Mucus can be difficult to find on fish such as salmon, but it is often located around the dorsal fin.
Odor	The odor of the skin is assessed by smelling the spine. If the fish has been lying more than 15 minutes on the table, it should be turned over and smelled on the other side.
Texture	
Texture/firmness: The texture is assessed by pressing a finger (firmly, but not too hard) on the spine muscle and observing if/how fast the flesh recovers. Only fish in rigor is given a score of 0. Prerigor fish is soft/very soft and therefore given a high score, but if it is known that it is a prerigor fish, the texture should be 0.	
Belly	The consistency of the belly is assessed by pinching it between fingers or by stroking it with the fingertips.
Eyes	
Avoid touching the eyes with your fingers. If one eye is damaged, assess the other one. Eyes where the cornea is swollen are often difficult to assess, but the membrane may be stung or cut for easier assessment of the eye.	
Cornea	Color and clearness of the cornea is assessed.
Form	The form of the eyes is assessed by looking at the eye directly or from the side.
Gills	
The gills are assessed by lifting the opercula. If the gills have been cut on one side of the fish, assess the gills that have not been cut. Avoid touching the gills since the appearance and mucus of gills can easily be destroyed.	
Gill Color	The color of the gills is assessed.
Gill Odor	Odor of the gills is assessed by lifting the opercula and smelling by the gill bow.
Mucus in gills	Color and appearance of the mucus is assessed.
Viscera	
Fish kept in ice with the viscera (ungutted) must be opened. The appearance of the viscera is assessed.	
Color of blood in abdomen	
Usually, remains of blood in abdomen are visible in gutted fish. Blood may also be assessed in the cut wound (near the gills), if no remaining blood is left in the abdomen the score should be 0.	
Odor in abdomen	
Odor in the abdomen is assessed by smelling inside the abdomen.	
Fillets/cut surface	
Color of fillets is assessed by the cut surface at the flaps or by assessing the fillets. Some fish such as redfish must be filleted from one side to be able to see the fillets and viscera.	

and possibilities. These standard considerations must be taken into account when QIM is to be implemented in a particular company. An experienced consultant is likely to be needed.

Test Room or Testing Areas

Sensory evaluation of whole fish is generally carried out by trained assessors in the reception or processing halls of fish factories or at auction sites. In quality control procedures, special facilities or rooms are preferred for sensory evaluation, but it is not always possible. In industry and auctions, the testing area should be located giving consideration to what is practical.

- Noise level shall be kept to a minimum. During a session, the assessors must be able to work without any interruption.

- Lighting is very important. It is preferable that the light is either real daylight according to ISO standard (ISO 8589 1988) but as a minimum be an intensity of 600—1,500 lux/square meter (m^2).
- The area must be free of any foreign odor. At a minimum, there must not be any waste or other matter, or operation with a strong smell nearby.
- No eating, drinking, or smoking shall be allowed in the testing area.
- The testing area must be easy to clean and disinfect. Regular cleaning and disinfecting shall take place. It must be ensured that the cleaning agents used do not leave odors in the testing area.
- Keep the temperature low and constant.

Sampling

The aim of non-biased sampling is to obtain a representative random sample from a lot. It is vital that the sample is selected randomly to ensure that it is representative. The number of fish to be sampled is determined by the accepted uncertainty, the characteristic of the lot, and economy (NMKL procedure No. 12 2002, Codex XOT 13-1969).

From a defined homogeneous lot, preferably 3 to 5 fish (10 for small fish species) should be assessed according to QIM schemes. A homogeneous lot of fish should be assessed (i.e., from the same catching day). Number the boxes in a standard way, for example, always from left to right and from top to bottom and generate 3 to 10 random numbers. Take one fish out of each of the 3 to 10 numbered boxes as decided. Make sure the fishes are taken from different places in the boxes (not always from the top layer). Evaluate all the 3 to 10 fish using the QIM schemes as provided.

Preparation

The panel leader prepares the evaluation by giving the fish samples three-digit codes and places the fish in random order on the table. They are kept cool either by placing them on a cooling plate or on ice. To avoid bias, samples should always be coded with two- to three-digit numbers that provide no information about the samples. The samples must be kept cool under evaluation and the panelists should not see the samples being placed. The boxes where the samples have been taken from must also be removed from the testing area, because this might enhance expectation error. Order of presentation should be random and the order should be balanced. The panelists must be told in which order they should evaluate the samples.

Evaluation

Hunger or satiation can influence the performance of the assessors. The assessors must not eat or smoke for an hour before the sensory evaluation. The assessors must be quiet and concentrate during the evaluation. Trained assessors can evaluate 40 fish with QIM in 20 minutes, and the method is nondestructive.

During continuous assessment of odor, assessors become insensitive to odors after some time. People become desensitized to odors as the receptors in the olfactory senses become saturated. Therefore, it is necessary to rest and breathe fresh air between samples when evaluating odor. Also, by taking a deep breath, the airflow through the olfactory senses increases and the odor becomes easier to detect.

When applying the QIM schemes, the outer appearance of the fish, eyes, gills, and texture are evaluated. The odor of gills is evaluated, and for some species, the odor and mucus of the skin is also evaluated. The color of blood and fillets (or the cut surface at the flaps) are evaluated in gutted fish. All attributes are to be assessed in the same order for each fish. For some fish species that are not gutted, such as redfish, dissolution of viscera is evaluated as well.

The assessor must evaluate all the parameters involved in the scheme (he/she cannot determine which parameters are most important). The assessors write down the scores given. For (quality) control purposes, it is important to write down the information about the batch, date of assessment, and name of the assessors prior to assessing the fish. To make the scheme uniform and easy to use and to ensure all criteria were scored, it was programmed into a handheld computer (Branch and Vail 1985, Helbo 1990, Jónsdóttir and others 1999) and a prototype dedicated handheld device was developed (Bremner and others 1987). An Icelandic company has developed software that can be connected to a handheld computer. The software includes both QIM schemes and photographs of fish at different spoilage stages (Luten 2000).

RESULTS OF QIM ASSESSMENTS

The scores for all the characteristics are summarized to give an overall sensory score, the so-called Quality Index. If a score for one of the parameters is

missing, it is not possible to calculate the total sum and thereby the QI for the assessed fish. If this situation is an incident (for example, damaged eyes makes it impossible to assess this attribute), the best way to deal with it is to leave that particular fish out and take another sample. If it occurs on a regular basis (for example, because of washing procedures at a company the mucus is always removed), the scheme should be adapted and a new calibration curve constructed in which the particular attribute (e.g., mucus) is removed.

Having the QI on an electronic basis means the data can be rapidly communicated from a boat, the quayside, or an auction and that it can be used in management systems to plan supply and production and to allocate product to different grades or to end uses according to production or market requirements. The full assessment data can be stored in databases. This ability to transmit a meaningful quality index along with identity and traceability information over the Internet represents a major advantage and a progressive step in electronic marketing of fish products. It further enhances opportunities for quality chain management to ensure product of known properties is handled correctly along the supply chain.

Prediction of Remaining Shelf Life of Fish Stored in Ice

As the Quality Index increases linearly with storage time in ice, the information may be used in production management. From the QI results, an estimate can be calculated for the remaining shelf life, which equals the shelf life minus the predicted storage time. In the following equations, the calibration curves for cod, salmon, and plaice are shown.

Cod: QI = 1.20 × days in ice − 0.04 (R^2 = 0.966)
Salmon: QI = 0.692 × days in ice + 1.57
(R^2 = 0.953)
Plaice: QI = 1.28 × days in ice (R^2 = 0.89)

It is emphasized that the remaining shelf life should be used with some caution due to the uncertainty in the estimation. Various factors can affect the remaining shelf life. It depends on the handling of the fish. Rapid cooling after the catch and an uninterrupted cold storage, different fishing gear, and bleeding and gutting methods are important. The season and catching ground can also have an effect. In the literature, several storage studies are reported and the estimated shelf life of different species is recorded (Howgate 1985, Martinsdóttir and Blomsterberg 1987, Magnússon and others 1990, Rehbein and others 1994, Larsen and others 1998–1999, Martinsdóttir and others 2000, Sveinsdóttir and others 2001). These are summarized in Table 41.8.

Quality Grading Relation EU System

When industry professionals use the QIM as a quality grading scheme, they need to refer the scores to the current EU classifications of E, A, and B. For

Table 41.8. The estimated shelf life for some fish species.

Species	Estimated shelf life in ice
Brill (*Rhombus laevis*)	14 days
Cod (*Gadus morhua*)	15 days
Deep water shrimp (*Pandalus borealis*)	6 days
Farmed salmon (*Salmo salar*)	20 days
Fjord shrimp (*Pandalus borealis*)	6 days
Haddock (*Melanogrammus aeglefinus*)	15 days
Herring (*Clupea harengus*)	8 days
Peeled shrimp (*Pandalus borealis*)	6 days*
Plaice (*Pleuronectes platessa*)	13 days
Pollock (*Pollachius virens*)	18 days
Redfish (*Sebastes mentella/marinus*)	18 days
Sole (*Solea vulgaris*)	15 days
Turbot (*Scophthalmus maximus*)	15 days

*The storage life before peeling

Table 41.9. The relation between the EU classification and the QI score for four fish species.

EU classification	Plaice (*Pleuronectes platessa*)	Cod (*Gadus morhua*)	Sole (*Solea vulgaris*)	Turbot (*Scophthalmus maximus*)
	QI score	QI score	QI score	QI score
E	0–5	0–4	0–5	0–5
A	6–16	5–13	6–19	6–19
B	17–21	14–16	20–27	20–26
Rejected	> 22	> 17	> 28	> 27

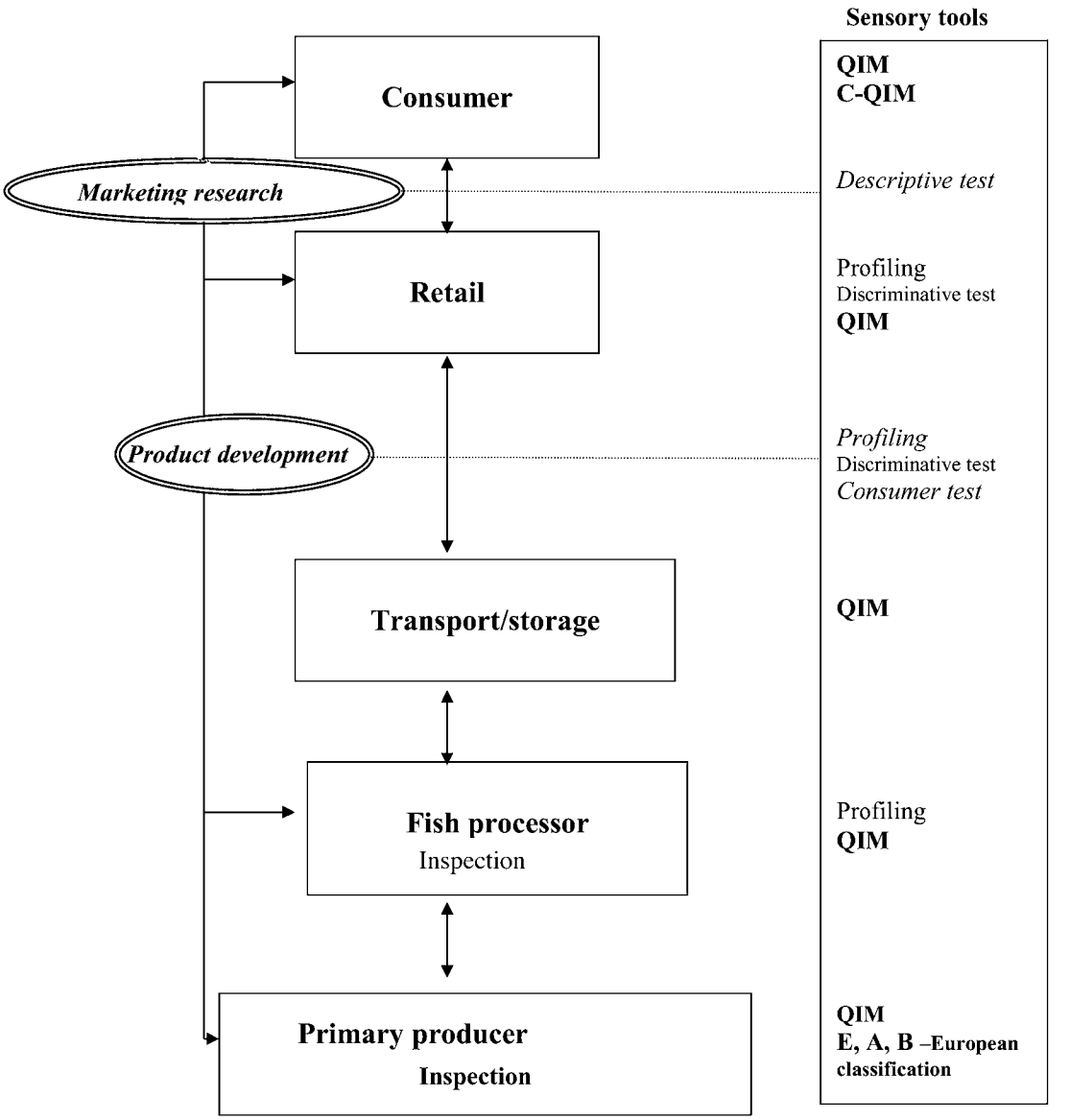

Figure 41.2. Sensory methods to be used in the fishery chain.

this purpose, QIM Eurofish advises the following relation between the QI scores and EU classification.

In case the average score of the assessed fish samples (3 to 5) per batch is between the mentioned QIM ranges, the results should be rounded off to the nearby QIM range.

Table 41.9 clearly shows that A, after the EU classification, is too broad and not detailed enough to use in electronic auctions and in production management.

C-QIM

The QIM schemes have been developed from the viewpoint of the industry and from technical research, and the obvious question arose as to how this relates to the consumer. This question can now be answered since a first version of a so-called consumer (C) QIM (C-QIM) has been developed (Warm 2000). This work used an external consumer panel, which developed and tested its own vocabulary in comparison with the standard laboratory terms for five species. The important characteristics for both consumer and sensory scientist were appearance, odor, and texture. These selected key parameters described intensity and gave an overall evaluation of fish quality and thereby fulfils the need for a method that does not mix analytical and hedonic terms.

C-QIM is not an acceptance test, but a tool for decision making for the consumer buying fish in a market or at a fishmonger (Nielsen and others 2002). The work developing the first C-QIM was based on five fish species only and therefore further work developing a C-QIM is ongoing at the Danish Institute for Fisheries Research, Department for Seafood Research (Hyldig and Larsen 2003).

FUTURE USE OF QIM IN THE WHOLE FISHERY CHAIN

A list of where and which sensory methods can be used in the whole fishery chain from catch to consumer is provided in Figure 41.2. The different sensory methods like profiling, descriptive, and discriminative tests have been described in previous chapters. It has been shown that the Quality Index Method can be used at each key stage. This is a major advance and a validation of the general applicability of the QIM approach. There is a need for models to be developed that link consumer's perceptions of sensory quality with sensory characteristics perceived and measured in the different stages of the fish handling chain. Consideration should be given to effects of different environmental factors (i.e., fish species, packaging, processing, and transport) on the sensory properties. Knowledge of these influences will open up opportunities for greater control of sensory quality and differentiation of products. This would also stimulate improvements in production of seafood of enhanced quality to meet consumers' preferences.

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Texture of Fish, Fish Products, and Shellfish

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INTRODUCTION

Texture is one of the most important quality parameters of fish for producers, processors, and consumers. Processors want a texture that makes the fish easy to process and gives high quality products with high yields. Market research indicates that 75% of buyers (smoke houses, supermarkets, etc.) of Norwegian salmon rate texture as one of the most important quality parameters (Koteng 1992). Many fish species do not have a strong flavor and texture therefore it becomes very important for consumer acceptability. Both intrinsic and extrinsic factors influence the texture of fish (Barroso and others 1998, Sigurgisladottir and others 1997, Mackie 1993, Love 1983) and, thus, the importance of reliable texture evaluation methods in product management is evident. The sensory evaluation often carried out in the industry is the so-called "finger method"; this method can to some extent be imitated by the instrumental compression methods. In plants, personnel press their finger on the fish or the fillet and so evaluate the firmness (Sigurgisladottir and others 1997). A more comprehensive description of the texture of fish is obtained using the Texture Profile Method (Johnson and others 1981, Bourne 1978, Breene 1975, Friedman and others 1963). This sensory method requires a highly trained panel and can be time consuming.

DEFINITIONS OF TEXTURE

The literature provides several definitions of texture, and most of these are very broad. This indicates that texture is a very complex sensory phenomenon, covering all impressions when food comes into contact with human surfaces (e.g., finger, tongue, or teeth). Furthermore, foods can be regarded as very complex physiochemical systems.

Jowitt (1974) defined texture as the attribute of a substance resulting from a combination of physical properties as perceived by the senses of touch (including kinaesthesis and mouthfeel), sight, and hearing. This definition is quite different from the classical definition of texture given by, for example Szczesniak (1963), who stated that texture is a combination of (1) the physical structure of the food and (2) the characteristics of the food during mechanical treatment. Bourne (1982) concluded from different definitions that the texture of food has several characteristics: (1) It is a group of physical properties that derive from the structure of the food. (2) It belongs under the mechanical or rheological subheading of physical properties. (3) It consists of a group of properties, not a single property. (4) Texture is

sensed by touch, usually in the mouth, but other parts of the body may be involved (frequently the hands). It is not related to the chemical senses of taste or smell. (5) Objective measurements of texture are by means of functions of mass, distance, and time only.

Guinard and Mazzucchelli (1996) summarized texture and mouthfeel of foods and beverages as multiparameter qualities. Evaluation in the mouth is a highly dynamic process in which the physicochemical properties of the food are continuously altered by chewing, salivation, and, possibly, body temperature. A variety of mechanoreceptors embedded in the tongue, palate, gums, and periodontal membrane, as well as the muscles and tendons of the jaws, are involved in the perception of texture and mouthfeel.

Meilgaard and others (1999) define texture as the sensory manifestation of the structure or inner makeup of products in terms of their reaction to stress and tactile properties. The reaction to stress is measured as mechanical properties such as hardness/firmness, adhesiveness, cohesiveness, gumminess, springiness/resilience, and viscosity by the kinesthetic sense in the muscles of the hand, fingers, tongue, jaw, or lips. The tactile feel properties are measured in terms of geometrical particles (e.g., grainy, gritty, crystalline, and flaky), or moisture properties (e.g., wetness, oiliness, moistness, and dryness) by the tactile nerves in the surface of the skin of the hand, lips, or tongue.

Regarding the complexity of food texture, it is of great importance to be aware of what physical parameter is being measured. This can be even more important when the aim is to correlate instrumental and sensory measurements.

Muscle Structure

In fish, the flesh is constructed of adjacent muscle blocks (Figure 42.1), called myotomes, separated from each other by sheets of collagenous tissue called myocommata. Within each myotome, the muscle fibers run approximately parallel to each other. The myocommata are connected internally to the skin and to the skeletal system (Bremner 1992).

The muscle consists of two major components: (1) the connective tissues of the myocommata and the extracellular matrix, and (2) the intracellular contractile proteins, mainly actomyosin. The muscle cells of fish are very short (barely 1 centimeter [cm] in large species) in comparison to mammalian muscle. This means that any sample invariably contains these two main components, which have very different effects on the overall texture. Furthermore, their relative effect on fracture changes with heating. The collagen shrinks and softens, whereas the actomyosin complex changes from a soft gel to a firmer denatured complex (Dunajski 1979). This makes it very difficult to relate the textural attributes of raw flesh to the attributes of the same material after it is heated.

SENSORY EVALUATION OF TEXTURE

Texture is by definition a sensory parameter, and only a human being can perceive, describe, and

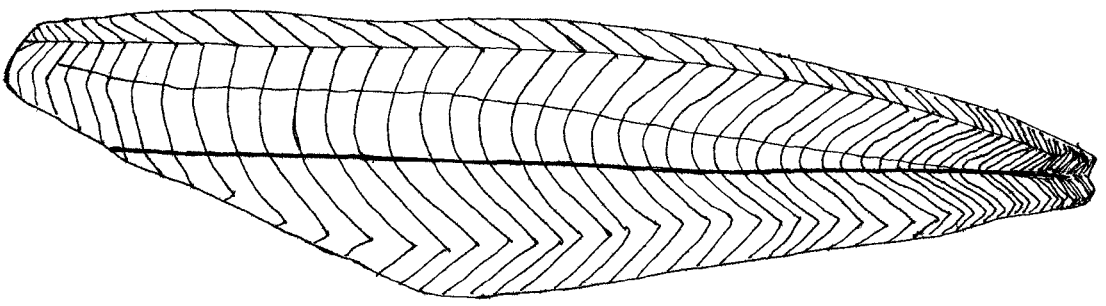

Figure 42.1. The structure of fish muscle. The muscle is constructed of adjacent muscle blocks (myotomes), separated from each other by sheets of collagenous tissue (myocommata).

quantify texture adequately. However, texture is very difficult to evaluate due to its complexity. In many cases, several terms are used to describe the same characteristics. In other cases, the same term is used to describe several characteristics. Also the same word may have different meanings to different people (Szczesniak 1963). The use of sensory texture profiling therefore requires highly trained assessors (Meilgaard and others 1999, Barroso and others 1998). Sensory perception of texture has been thoroughly reviewed by Jack and others (1995). The best way to get a "full picture" of the texture is by the Texture Profile Method. A texture profile is defined as the sensory analysis of the texture complex of a food in terms of its mechanical, geometrical, and compositional (fat and moisture) characteristics, the degree of each trait present, and the order in which they appear from the first bite through complete mastication (Brandt and others 1963). The profiling method establishes different parameters in the sensory perception of food and generates extensive data matrices that preferentially should be evaluated by multivariate data analysis (Cardello and Maller 1987).

Measurements have been performed on whole fillets, different fillet cuts, and minces. Mincing the fish results in lower coefficients of variation, but also destroys the texture in a way that makes it impossible to say anything about the texture of the intact fillet. Moreover, in cooked fish, the muscle segments tend to slide upon compression. Fish has a flaky structure, and during heating, the connective tissue that holds the flakes together disintegrates. This makes the fish muscle very fragile to any handling after cooking and it separates easily into flakes. Hatae and others (1990, 1984) found that muscle fibers of species having firm texture slid or shifted over one another to a lesser extent than those of species with soft texture. The heat-coagulating material seemed to obstruct the displacement of the fibers.

A review of the literature reveals that many attempts have been made to correlate instrumental measurements with sensory evaluation of fish texture, but with varying results (Hyldig and Nielsen 2001). One of the reasons for lack of correlation is that the fish muscle is very heterogeneous making sampling and, hence, measurements, difficult to reproduce. Another important reason is that sensory and instrumental evaluations are basically very different in the way they measure texture.

Preparation of Fish Samples

The texture of whole fish muscle is difficult to assess due to the lack of uniform structure (Figure 42.1). The segmentation and orientation of the fillet structure makes it difficult to prepare samples of a standard size and dimension. If the measurement location is not clearly defined, and is not representative of the whole sample, the variations within one fillet can be higher than between fillets. Mixing samples from different locations on the fillet should be avoided. Several investigators have found the texture of the middle section of the fillet to be most representative of the fillet (Botta 1991, Andersen and others 1997, Einen and Thomassen 1998, Sigurgisladottir and others 1999, Hansen and others 2000).

With instrumental measurements, Veland and Torrissen (1999) found significant effects from temperature. When the temperature was raised from 0 to 20°C, the force and work decreased by 20–25%. These results show that it is important to keep the temperature constant.

Sample Preparation of Heat-treated Samples

When measuring cooked samples, the cooking procedure (temperature and time) can influence the results. Studies using light microscopy have shown a relationship between the water-holding capacity of the muscle of cod and salmon, and the tissue-specific structural changes, which occur during heating. In the temperature range of 5 to 70°C, there was an initial delay in the change in water-holding capacity. Water loss increased rapidly and reached a maximum at 45–50°C, after which water loss for both fish species seemed to decrease. The increasing water loss was due to changes in the connective tissue, and the maximum water loss was accompanied by a transverse shrinkage of the muscle cells, intercellular gaps, and widening of the extracellular spaces (Ofstad and others 1993). These changes in muscle structure may account for some of the effects of temperature on texture measurements.

Consideration must be given to the effect that the methods of heat treatment might have on judgments of the sensory characteristics of the fish. The major criterion for choice of a suitable method is that it has a minimal sensory impact on the innate characteristics of the sample. As an example, if salt is added or

the sample is dipped into a salt solution for some time before heat treatment, the added salt will have influence on the texture characteristics and of course also on the taste. It is important to decide if it is the innate characteristics of the fish species or the sample that are to be measured.

Samples can either be cut into portions before heat treatment or after. The optimal amount of fish prepared for each assessor is a portion size of 40–100 gram (g). Samples from fish species such as cod, salmon, and saithe are cut from the loin part in 8 × 4 × 2 cm pieces corresponding to approximately 75 g. Smaller fish species such as rainbow trout, herring, and plaice require a whole fillet, which give samples of approximately 40–50 g for plaice and herring and approximately 75–100 g for rainbow trout. All samples must be marked individually with three-digit or four-digit codes. The samples are placed in individual porcelain bowls and covered with a porcelain lid. The fish samples are heated for example, in a convection oven in their own juice at 100°C to an internal temperature of 70°C.

Other Methods of Heat Treatment

Cooked—Samples are packaged in cook-plastic pouches or aluminum foil pouches, without any salt or spice added, heated in a water-bath at 80°C until a center temperature of 75°C is reached (approximately 30 minutes), and removed from the bag before serving to the assessors (Cardello et al. 1983, Hurling and McArthur 1996, Sivertsvik and others 1999).

Baked in oven—The samples are wrapped in foil/aluminum foil without any seasoning added and baked at 170–180°C to an internal temperature of 65–70°C (Orban and others 1997, Fletcher and others 2002).

Fried—The samples are cut into steaks (76 millimeter [mm] × 38 mm × 12 mm) then breaded and fried at 180°C ± 2°C in 2 mm vegetable oil for 4 minutes per side (Weddle 1980).

Different Types of Products

Model products of minced fish flesh—The model products are composed of minced fish flesh, water (added as ice), and 3% salt with a moisture-to-muscle protein ratio of 5:1. The model product is then cooked in a pan at 71 or 85°C. Portions are cut into cubes, approximately 1.5 cm on each side, and kept on ice prior to evaluation (Hamann and Webb 1979).

Smoked fish—Required samples are cut manually, at right angles to the long axis, into thin slices of 2-mm thickness and approximately 4 square centimeters (cm^2) area on the day of the evaluation. For each sample, two slices are presented to the assessors in a clear glass or plastic container. The containers are removed from the refrigerator 30 minutes before evaluation (Morzel and others 1999).

Dried fish meat—Samples from dried fish meat must be cut so the fibers are in the same direction for all samples and the dimension must also be the same (Iseya and others 1996).

Marinated fish products—Samples from marinated herring were cut from the loin and middle section of the skinned fillets. Each section was divided into two pieces (approximately 10–15 g each) and served in replicate with the skin side facing up in porcelain trays at room temperature (Nielsen and others 2004).

Preparation of Shellfish Samples

Shrimp was cooked in a microwave oven at 240 watts for 140 seconds and with a 120-second holding time before serving to the sensory panel (Gundavarapu and others 1998). The shrimp were cooked and frozen, and before the sensory evaluation the shrimps were thawed at 5°C for 16 hours and tempered for about 2 hours at room temperature (Bak and others 1999a).

Blue crab (*Callinectes sapidus*) was steam cooked whole at 116°C for 12 minutes, air cooled at 4°C overnight. A portion of 12 g of crabmeat was served in a plastic cup (Henry and others 1995).

The canned retorted samples were drained for 30 minutes and separated into claw, tail, and piece of meat; only the claw and tail muscle were sensory evaluated. The samples were served at room temperature in portions of 112 g in white paper cups (Leblanc and Leblanc 1990).

ATTRIBUTES

In Table 42.1, there is a long list of words used in the literature for describing texture properties, but unfortunately only a few of them are defined.

Definitions of Attributes

A description of the attributes is given in Table 42.2.

Table 42.1 A list of all of the words (from the References section) used to describe texture properties. The words are grouped according to their similarities.

Resistance	Brittle	Fat
Firmness	Brittleness	Fattiness
Hardness	Crisp	Fatty mouthfeel
Soft	Crispness	Fatty texture
Softness	Crunchy texture	Greasiness
		Oily mouth coating
Melting texture	Broken surface mouth feel	Oiliness
Meaty	Chalkiness	
Tender	Coarseness	Flaky
Tenderness	Graininess	Flakiness
Tough	Particles	Roughness of fiber
Toughness	Roughness	Fiber size
		Fibrosity
Adhesiveness	Cohesiveness	Fibrousness
Stickiness	Denseness	
		Ease of swallowing
Resilience	Dryness	Remainder left to chew
Elasticity	Juiciness	
Gumminess	Juicy	Apparent flake thickness
Springiness	Moistness	Flaky
Chewiness	Moisture release	
Chewy	Mouth dryness	
Pastiness	Succulence	
Pasty texture	Watery	
	Wateriness	

Instruction to the Assessors

It is very important to be sure that all assessors have understood the definition of the texture attribute. Written instructions can be helpful for the assessors during the evaluation. Such instructions must contain all that the assessors must know about how to assess the individual attribute such as how to take a piece with the fork, which teeth to use, number of chews before assessing (e.g., hardness), and if they have to take a new piece for assessing juiciness, etc. Some of the texture properties need a lot more training than others depending also on the different types of sample.

TEXTURE OF FISH, FISH PRODUCTS, AND SHELLFISH

Many factors affect the texture of fish, for example, species, age, size, and nutritional state of the fish. Postmortem factors influencing texture include glycolysis, pH, and rigor mortis. The accompanying contraction of the muscle often leads to the separation of muscle segments (gaping). External factors include the temperature profile during storage, temperature of cooking, and the presence of sodium chloride (NaCl) (Johnston 1999, Dunajski 1979). As the fish grows, the diameter and length of the muscle fibers increase and this makes the muscle coarser (Dunajski 1979). Hurling and others (1996) found a high correlation between fiber cross-sectional area and sensory perception of firmness in seven fish species. Sensory firmness decreased with an increase in average cross-sectional area of muscle fibers of the different species. This may seem to be contradictory, but there exists no clear relationship between fish size and firmness. There are differences among species and among fish belonging to the same species (Dunajski 1979).

Table 42.2 Description of some texture properties evaluated using sensory analysis. Only references where the evaluation of the property is described are included in the table.

Property	Definition	Preparation[1]	Reference
Firmness	• The effort to bite through the fish sample with the front teeth	C	Hurling et al. (1996)
	• The force required to compress the sample between the molar teeth	C	Hamann and Webb (1979)
	• The force required to compress the material between the molars, or between the tongue and the palate	C	Borderias et al. (1983)
	• Biting with front teeth	D	Iseya et al. (1996)
Soft	• Resistance to a very slight opening and shutting of the jaws	S	Schubring and Oehlenschläger (1997)
	• Force required to compress samples	C	Warm et al. (2000)
Hardness	• Resistance to breakdown on chewing to a state suitable for swallowing	C	Borderias et al. (1983)
	• Force required to compress a substance between molar teeth (in the case of solids) or between tongue and palate (semisolids)	X	Sánchez (1996)
	• The perceived force required to compress the sample using the molar teeth	C	Cardello et al. (1982)
	• Chewing with molars	D	Iseya et al. (1996)
	• Force required to bite the shrimp between second and third segment with incisors	SH	Gundavarapu et al. (1998)
	• Compress sample between molar teeth and release pressure	SH	Leblanc and Leblanc (1990)
Tenderness	• Resistance to breakdown in substructures when compressing between tongue and palate	S	Schubring and Oehlenschläger (1997)
Elasticity	• The ability of the material to return to its original shape after deformation. Judged by compressing the substance slightly between the molars, or between the tongue and palate, and noting to what extent the material returns to its original shape	C	Borderias et al. (1983)
Springiness	• Degree to which a product returns to its original shape once it has been compressed between the teeth	X	Sánchez (1996)
	• Degree to which the sample rapidly returns to its original shape after a partial deformation between the molar teeth	C	Hamann & Webb (1979)
Cohesiveness	• The extent to which a material can be deformed before it ruptures	C	Borderias et al. (1983)
	• Degree to which the sample deforms before it ruptures during a bite between the molar teeth	C	Hamann & Webb (1979)
	• Degree to which a substance is compressed between the teeth before it breaks	X	Sánchez (1996)
Adhesiveness	• Degree to which the sample sticks to the mouth surface	C	Hamann & Webb (1979)
	• Force required to remove the material that adheres to the mouth during the normal eating process	X	Sánchez (1996)
Chewiness	• Number of chews required to prepare the sample for swallowing	C	Hamann and Webb (1979)

Property	Definition	Preparation[1]	Reference
	• Length of the time (in seconds) required to masticate the sample at a constant rate of force application, to reduce it to a consistency suitable for swallowing	X	Sánchez (1996)
	• The total perceived effort required to prepare the sample to a state ready for swallowing	C	Cardello et al. (1982)
	• Measured as the difference in intensity of chewiness from standard	SH	Gundavarapu et al. (1998)
	• Length of time to prepare sample for swallowing	SH	Leblanc and Leblanc (1990)
Chewy	• Number of chewings (0 to 20) before swallowing	C	Warm et al. (2000)
Tough	• The tissue makes lump after several chewings	C	Warm et al. (2000)
Juiciness	• The sensation of a progressive increase of free fluids in the oral cavity during mastication	C	Borderias et al. (1983)
	• Sensation of wetness and juiciness	C	Beilken et al. (1991)
	• Amount of juice released during mastication of shrimp with molars	SH	Gundavarapu et al. (1998)
	• Increase in free liquids in mouth during mastication	SH	Leblanc and Leblanc (1990)
Juiciness 1	• The initial impression of moistness (free water, flavor juices, liquid fat, oil, and saliva) of the sample on initial chewing. (1, 2, or 3 chews)	F	Weddle (1980)
Juiciness 2	• The total impression of succulence in the mouth just prior to swallowing	F	Weddle (1980)
Moisture release	• Degree to which the sample feels moist during chewing	C	Hamann and Webb (1979)
Moistness	• The perceived degree of oil and/or water in the sample during chewing	X	Cardello et al. (1982)
	• Overall impression of moisture content of sample	SH	Leblanc and Leblanc (1990)
Wateriness	• The release of water on compression	C	Borderias et al. (1983)
Succulence	• The sensation of juiciness in the mouth after prolonged chewing, an increase of moisture in the mouth; the remaining water in the disintegrated sample	S	Schubring and Oehlenschläger (1997)
Greasiness	• Sensation of fattiness in the mouth	C	Beilken et al. (1991)
Oiliness	• Degree to which oil is perceived after chewing	C	Hamann and Webb (1979)
Oily mouth coating	• The perceived degree of oil left on the teeth, tongue, and palate after swallowing	C	Cardello et al. (1982)
Fat	• Amount of fat left on mouth surface and teeth	C	Warm et al. (2000)
Flakiness	• The perceived degree of separation of the sample into individual flakes when manipulated with the tongue against the palate	C	Cardello et al. (1982)
Flaky	• Tissue parts into flakes by pressing with fork	C	Warm et al. (2000)
Fibrousness	• Impression of fibers in the sample during chewing	F	Weddle (1980)
	• The perceived degree (number × size) of fibers evident during mastication	C	Cardello et al. (1982)

[1]C = cooked, D = dried fish meat, F = fried in oil, S = salted, X = not described in the reference, SH = shellfish.

Different fish species have different texture properties. In a survey Cardello and others (1982) evaluated 17 species of North Atlantic fin fish for their sensory texture attributes of hardness, flakiness, chewiness, fibrousness, moistness, and oily mouthcoating, and appearance, using a 7-point category scale and found a distinct grouping of fish species according to similarities and dissimilarities in their sensory characteristics.

Hong and others (1996) evaluated the sensory texture of Atlantic mackerel fillet, which was stored in carbon dioxide (CO_2)-modified atmosphere packaging at −2°C for 21 days. The trained sensory panel found no significant changes in firmness and elasticity of the raw flesh or in degree of gaping of the cooked fillet during the storage period.

The diet that is fed to farmed fish affects the composition of the fish muscle. Research shows that fillets from farmed salmon (Andersen and others 1997, Sheehan and others 1996), rainbow trout (Færgemand and others 1995), and sea bream (Orban and others 1997) that have been given a high-fat diet have significantly higher fat content than fillets from the same fish species given a diet with a lower fat content. In some research projects, fat content did not influence firmness (Einen and Skrede 1998, Rørå and others 1998, Færgemand and others 1995), while in others, a positive correlation between softness and increased dietary fat content was shown (Andersen and others 1997). Increased dietary fat content results in a greasier mouthfeel (Einen and Skrede 1998) and increased fatness (Rørå and others 1998) in farmed salmon, and juicier and greasier texture in farmed sea bream (Orban and others 1997). Sheehan and others (1996) found that the softer texture and more pronounced gaping in smoked farmed salmon fillets was the result of increased fat content in the diet. Farmed Atlantic halibut raised on a high-fat diet are found to have a juicier texture, but the fat does not affect hardness, fattiness, or roughness (Nortvedt and Tuene 1998). The protein content in the diet has not been shown to influence the texture of farmed rainbow trout (Færgemand and others 1995). The content of fat and protein in many species of wild fish depends on the season. Cod caught late in the winter or early in the spring are thin, have a low protein content and a high water content, and are subsequently not as firm as cod caught at other times of the year (Howgate 1977). The seasonal change in meat quality of red sea bream depends mainly on the change in the muscle firmness caused by sexual maturation and spawning (Touhata and others 1998). The breaking strength of the muscle is higher in the winter in both male and female fish, and decreases immediately after spawning. Also in herring, an increase in softness, fatty mouthfeel, and decrease in grittiness is found, when the lipid content increases (Nielsen and others 2004).

Starvation prior to slaughter was found to be a weak tool for changing salmon fillets as only small changes were found in texture (Einen and Thomassen 1998). Færgemand and others (1995) found that the texture changes in farmed rainbow trout could partly be ascribed to the slaughtering procedure used, and Sigholt and others (1997) found that stress prior to slaughter of farmed salmon resulted in softer fillets. On the other hand, Azam and others (1988) found no relationship between method of slaughter and texture in rainbow trout. However, the fish in their research had a pH of 6.2 when the analysis began, which may mean that the fish were already stressed, and that texture changes had probably already taken place in the fish muscle. Bleeding removes enzymes from the fish and consequently delays the softening of fish flesh (Ando and others 1999).

One of the most dramatic changes in fish muscle postmortem occurs when it passes through rigor mortis. Rigor mortis has a major effect on texture, especially in fish frozen at sea. Sea-frozen fish is often filleted and frozen prerigor and this can lead to texture damage (Howgate 1977). A slight or more dramatic decrease in pH can be observed during rigor mortis in most species, because lactic acid is formed from glycogen. Variations in pH depend on many factors, such as the species and physiological state of the individuals. Love (1983) reported a relationship between texture and pH in cooked cod. He found that the cod became more soft or sloppy when the pH increased to above 7. The texture of fish with a low pH is described as firm, dry, and a little tough, while the texture of fish with a higher pH is softer, juicier, and very tender. This correlates well with the fact that fish with a high pH often contain more water than fish with a low pH and that the higher water content in lean fish correlates with a lower protein content (Howgate 1977). Love and others (1974) found a relationship between pH and fish size in well-fed cod. The pH was lower in large cod and

these were slightly tougher than the small cod. Sigholt and others (1997) found the pH to be lower in larger farmed salmon than in smaller fish. In an experiment where sardines were stored for up to 10 days at 4°C, the firmness measured with a Kramer compression test was found to decrease, while pH increased during the storage period (Gökodlu and others 1998).

Fish muscle generally becomes softer during chilled storage after catch. Færgemand and others (1995) found that storage time on ice was the single most important factor affecting the fillet texture of farmed salmon. The fillets became softer after storage on ice for 10 days. Sato and others (1991) and Ando and others (1992a, 1992b) also observed a softening of rainbow trout during chilled storage.

There is no general agreement about the exact mechanisms involved in the texture changes observed during ice storage of fish. In a review Verrez-Bagnis (1997) concluded that during storage in ice some myofibrillar proteins degrade, but no changes occur in the structure of the contractile elements. This was also found in a study by Busconi and others (1989). Transmission electron microscopy showed no change in the structure of contractile elements in white croaker stored for 7 days on ice, but there was a marked degradation of nebulin.

There is not much information to be found about the influence of structure on the texture of fish muscle. Hatae and others (1990, 1984) related observations from optical and scanning microscopy with data from compression tests on five fish species. Species with firm texture had thin muscle fibers with considerable heat-coagulating material between them, and species having soft texture had thick muscle fibers with little heat-coagulating material. With the use of a modified scanning electron microscope method, Ando and others (1992b) found that the difference in firmness among three fish species was related to both the density and the arrangement of collagen fibrils in the connective tissue. Ando and others (1992a, 1991b) showed with light and transmission electron microscopy that the softening of rainbow trout postmortem is caused by a disintegration of collagen fibers. Sato and others (1991) showed that in rainbow trout, the solubility of type V collagen increases significantly during storage of the fish on ice. According to Eckhoff and others (1998), collagen fibers in farmed salmon contain few cross-links, and some cleavage of intermolecular cross-links seems to occur during storage on ice. This can explain why some of the collagen becomes more soluble during ice storage of fish.

Several mechanisms are involved in the texture changes that take place in fish muscle during frozen storage and there are several reviews on the subject (Barroso and others 1998, Mackie 1993, Bremner 1992). During frozen storage both the myofibrillar proteins and the collagens aggregate inducing a toughening of the muscle (Montero and Borderías 1992, 1990). Proteins are known to denature during freezing, but this alone is not believed to cause the toughening. In particular, the sarcoplasmic reticulum degrades and then appears to act like cement to hold the individual myofibrils together (Howgate 1977). An investigation of cell fragility using a homogenization technique followed by measurement of optical density in the cell dispersion showed that if cod was first frozen and then thawed, the cells became progressively more resistant to mechanical breakdown with increasing time in the frozen state. The deterioration proceeded more slowly as the temperature of frozen storage was reduced (Love 1983). Frozen storage temperature has an influence on the rate at which the muscle changes. In an investigation of frozen cod fillets stored at −20 or −30°C, the results showed that −20° gave the largest increase in firmness. This was correlated to an aggregation of myosin and actin involving nondisulphide covalent bonds, which occurred faster in the samples stored at −20°C (Careche and others 1998). The gadoid fish differ from other fish species, because they contain the enzyme trimethylamineoxide-dimethylase (TMAO-ase) that converts trimethylamineoxide (TMAO) into dimethylamine (DMA) and formaldehyde. The enzyme has been found in a few non-gadoid species, but it is primarily found in gadoid species such as saithe, red hake, and cod (Nielsen and Jørgensen 2004). Formaldehyde induces cross-linking of the muscle proteins making the muscle tough. The enzyme is most active, when the tissue membranes are disrupted (e.g., during frozen storage at temperatures over −30°C or by temperature fluctuations (Mackie 1993).

Bak and others (1999b) found that the sensory evaluation of shrimp meat toughness revealed a clear effect of the packaging method and storage conditions. The sensory scores for toughness were significantly higher for samples packed in atmospheric air compared to samples packed in modified

air. In addition, storage in light resulted in significantly tougher shrimp meat. The sensory scores for toughness were almost constant for samples packed in modified air for the first 9 months of frozen storage, whereas a significant increase was observed between 9 and 12 months of frozen storage.

Among other processing conditions known to affect texture are salting and smoking. Because of dehydration, these processes increase the firmness of the fish muscle. In catfish, a longer salting time before smoking results in a drier and harder texture, and the smoking time also influences the texture, because most of the dehydration takes place in the first 3 hours (Tomé and others 1999). The firmness of smoked Greenland halibut is influenced by water content, and can be increased if the fish is frozen before smoking. This can be explained by the faster penetration of sodium into the thawing musculature during curing, enhancing the dehydration of the fish muscle (Priebe and Reichstein 1975). In salt curing of mackerel fillets, firmness increases in parallel with the decrease in water content and the increase in the solubility of sarcoplasmic proteins (Toyohara and others 1999).

Færgemand and others (1995) found that the texture of the farmed fish depended on the method of slaughter and storage time on ice. The texture differences correlated with the differences in dietary fat content. Einen and Thomassen (1998) investigated starvation prior to slaughter in Atlantic salmon. The sensory analysis on a scale from 1 to 9 indicated that long-term starvation (86 days) reduced the hardness of cooked fillets, whereas, the instrumental texture analysis using a single compression method indicated that long-term starvation (58 days or more) can increase the hardness of raw fillets after starvation.

RECOMMENDATIONS AND FURTHER WORK

Results from texture measurements are highly influenced by sample treatment and temperature, both for instrumental and sensory analysis. When publishing results, it is very important to give detailed descriptions of the methods used and the material studied. The physical condition of the sample should also be carefully described. There can be large differences in the definitions of the same attribute obtained by different methods, and this must be taken into consideration while evaluating and trying to correlate results from different methods.

In many scientific papers, results are presented where the sensory evaluations are performed on cooked or processed fish samples, whereas the instrumental measurements are performed on raw muscle. All process alters the muscle structure, and therefore, it is not surprising that the correlation between results from the two methods often is very small. Ideally, if the objective is to correlate sensory and instrumental evaluations of texture, instrumental measurements must also be performed on samples prepared in the same way as for the sensory evaluation (e.g., cooked). More research is needed to define the differences between raw and processed fish muscle, and how this influences the texture. One possibility could be to perform the sensory evaluations of hot cooked fish and the instrumental measurements of cold cooked fish, but this requires knowledge of the changes that occur in the cooked muscle during cooling. There is also a need for more panels tasting raw fish, because interest in sushi is increasing outside Japan.

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Perception of Sensory Quality of Wild and Farmed Fish by Experts, Consumers, and Chefs or Cooks in the Restaurant Sector

Gunn Berit Olsson, Mats Carlehög, Morten Heide, and Joop Luten

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INTRODUCTION

In this chapter we present an overview of the sensory quality properties of wild versus farmed fish as observed by expert panels and consumer panels. Some consumer studies and studies involving chefs or cooks from high-end restaurants evaluating the quality of wild or farmed fish are presented.

Expert panels normally consist of 5 to 20 panelists screened for sensory acuity and motivation, oriented to different sensory test methods, and trained (Lawless and Heymann 1998). The sensory methods used include difference tests and a descriptive analysis (Meilgaard and others 1991).

The evaluation of products by consumers can be performed under controlled conditions. Products are prepared in the same way for all consumers and offered under similar conditions (studio tests). In-home product testing is a further option, where consumers prepare the products in their own kitchen according to their own procedures, or following guidelines or instructions. Analytical, holistic, and credence attributes are evaluated on hedonic intensity scales ranging from "not at all" to "very much."

Sensory evaluation in the restaurant sector can be performed by presenting products for preparation to chefs in the restaurant kitchen. The method used is an evaluation of the received products on a semantic differential scale by the chefs followed by in-depth interviews.

Food consumption can be influenced by many variables, including the food itself, the individual, and the eating location and situation (Edwards and others 2003). There are several theoretical approaches to studying food consumption with the focus on sensory evaluation, psychology, or sociology and consumption environment or context.

A well-known problem with wild-caught fish is the variation in quality during the cause of season. The most important explanation for this problem is that throughout the year, wild fish are subjected to considerable environmental change and fluctuations in the availability and composition of feed that will affect the proximate composition of the muscle. In traditional fisheries, the fishermen can, to a limited

extent, control the quality of the final product by choosing appropriate catching methods, handling, and processing. Harvesting and storage conditions are known to affect postmortem changes in quality (Hultin 1985, Esaiassen and others 2004).

In addition to food safety, the most important attributes of fish products from a consumer's point of view are sensory attributes such as appearance, color, texture, flavor, and odor. The opportunity for controlling these attributes is one of the most attractive features of aquaculture. Product quality of farmed fish in general depends on a wide range of biological, environmental, and dietary factors. Important factors are age/size, growth rate, water temperature, photoperiod, feed composition, feeding regime, specific dietary components, preslaughter handling, and finally, slaughter procedures. Each of these will, if compromised, affect the quality of the product.

One important determinant for success in fish farming in general is that consumers regard the farmed fish as equivalent or superior to the wild form. Taking into account the different life situations of farmed fish and their wild counterparts, especially with respect to feeding possibilities, it is of great importance to know which attributes vary and how they can be influenced. The composition of farmed fish may be much more stable than that of their wild counterparts because farmed fish are provided with feed throughout the year. Furthermore, farmed fish are not subject to the vagaries of the annual production cycles of their natural prey. Nevertheless, there may be a marked seasonal variation in the composition of farmed fish because some species undergo periods of voluntary anorexia prior to spawning (Kadri and others 1995, Tveiten and others 1996, Hernández and others 2003). Variation in appetite due to environmental factors like day length and water temperature may also influence the composition and quality (Mallekh and others 1998).

SENSORY EVALUATION OF FARMED VERSUS WILD FISH BY EXPERT PANELS

Unheated Samples

Results reported from sensory assessments of farmed versus wild fish are contradictory and no generalizations can be made about differences. This can, to some extent, be explained by differences in the experimental design of the studies, different species, and differences in feed/feeding regimes. Furthermore, publications referring to farmed/cultivated fish may refer to both caught wild fish that have been raised in captivity and to fish hatched in captivity.

The external appearance of cultured gilthead sea bream (*Sparus aurata*) fed a commercial diet has been reported to be different from that of wild gilthead sea bream (Grigorakis and others 2002). The skin color of the wild gilthead sea bream had a more bleached appearance and species-characteristic iridescent colors (a golden band between the eyes, and a red patch on the side of the head on the gill cover). The cultured group was evaluated as having much darker dorsal and head areas and fewer iridescent colors (species-characteristic), if present at all.

Similar results have been observed in other studies on gilthead sea bream. Flos and others (2002) studied farmed sea bream raised under various land-based regimes and compared them with wild sea bream. Sea bream raised under superintensive conditions (automatic feeding, high density of fish) were more compact and without the characteristic color pattern of the species. Sea bream raised under semi-intensive conditions (hand feeding or automatic feeding, low density of fish) were not significantly different from the wild group.

Alasalvar and others (2002a) have shown fundamental differences in morphology between farmed and wild sea bream. The appearance of wild sea bream was described to be more bleached greenish, have sharper dorsal fins, more scales, sharper teeth with greater height and conical edge, and smaller bellies and shorter tails compared to their cultured counterparts. Furthermore, they also had a golden tape between the eyes and a reddish patch on the surface of the gill cover. This is in accordance with both Aoki and others (1991) and Hatae and others (1989) who also showed that wild red sea bream (*Chrysophrys major*) were more reddish than cultured ones. Furthermore, Alasalvar and others (2002b) showed by using the Tasmanian Food Research Unit Scheme that wild sea bass (*Dicentarchus labrax*) were evaluated as significantly better (lower sensory scores) at the end of the shelf life period when compared to cultured sea bass.

One of the most important quality criteria of salmonids is the red color of the muscle. It has been shown that the color intensity varies between farmed

and wild Atlantic salmon (*Salmo salar*) (Skrede and Storebakken 1986). In the sensory analysis, the wild and farmed salmon were all judged to be significantly different raw, baked, and smoked. The panelists described the farmed fish as less dark, less colored, and more yellowish in hue than wild fish. However, instrumental color analysis directly on the raw flesh did not show any significant differences in color between the two groups of salmon.

The opposite has been observed for Coho salmon (*Oncorhynchus kisutch*). Farmed Coho salmon from two different locations were compared with wild Coho salmon. The flesh color of the wild fish exhibited a consistently deep red color whereas farmed fish displayed less intense redness and more yellowness in the flesh (Higgs and others 1989).

Heated Samples

Results published regarding the sensory evaluation, by expert panels, of heated samples of wild versus farmed fish are contradictory.

In a study by Prescott and Bell (1992), two types of farmed Australian snapper (*Pagrus auratus*), one group fed with fish and the other with pellets, were compared with wild Australian snapper. No significant differences in the attributes of flavor, color, off flavors, fresh taste, and oiliness were observed by an experienced panel of 30 members. However, the texture of wild snapper was evaluated as significantly softer than either of the two groups of captive fish.

A comparison between intensively farmed sea bream, fed artificial feed in tanks and extensively farmed sea bream, raised on a natural diet in a brackish water lagoon, showed that differences were mainly observed in muscle fat content (Orban and others 1997). This affected the perceived texture as evaluated by descriptive testing by a sensory panel of 10 assessors. The intensively farmed fish group received higher scores for juiciness and greasiness as well as freshness while the extensively farmed group was given a higher score for fibrousness.

In a study performed by Alasalvar and others (2002a), a panel of five to six assessors evaluated farmed versus wild sea bream. The sensory panel did not observe any significant differences between the cultured and the wild fish over the storage period of 23 days.

Grigorakis and others (2003) described the opposite using an expanded forced-choice triangular test. A panel consisting of 15 assessors evaluated wild gilthead sea bream to be significantly different in their sensory attributes compared to their cultured counterparts. Descriptors from the panelists were used to illustrate which of the two groups was preferred. Wild was mainly described by the attributes pleasant taste and delicious. The cultured fish was described as having a poorer taste.

Freshness quality of cultured and wild sea bass stored up to 22 days was evaluated by a sensory panel consisting of five to six panelists (Alasalvar and others 2002b). No significant differences existed between the cultured and wild sea bass during the entire storage period.

Wild and farmed Atlantic salmon have been compared with respect to flavor and odor attributes (Farmer and others 1995), and no differences between the groups were observed in this study. A later study by Farmer and others (2000) showed that differences in texture existed between farmed and wild salmon. Some sources of farmed salmon consistently received the highest scores for moist, light, and tender textures, and the wild fish received the highest scores for firmness and chewiness. The flesh color of the wild salmon was described as lighter compared to the farmed fish. However, the farmed fish did come out at least as acceptable to the panelists as their wild counterparts.

In contrast to the above profiling results, a sensory panel of eight experienced assessors found no differences in odor, flavor, or texture when comparing farmed Coho salmon from two different locations with wild Coho salmon (Higgs and others 1989).

Even though farmed fish are provided with a well-formulated diet throughout the year, there may be seasonal variations in the sensory quality. Olsson and others (2003) showed by descriptive profiling that the textural attributes of Atlantic halibut (*Hippoglossus hippoglossus*) changed during the cause of the season. In the warm summer months (July and August), the farmed halibut were given higher scores for fibrousness and chewiness than the wild halibut caught during the same period. In May and August the farmed halibut was described as being whiter than the wild halibut. For the rest of the year there was no significant difference in whiteness between the two groups. Individual variation in the wild halibut quality was shown to be considerable. However, no significant variation with respect to season was detected.

Carlehög and others (2001) have investigated possible differences between farmed and wild spotted wolffish (*Anarhichas minor* O.). The farmed spotted wolffish was evaluated by descriptive profiling by seven trained assessors with higher intensities for the attributes, fresh smell, fresh taste, sweet taste, elasticity, firmness, and chewiness. Lower intensities were given for old/stale smell and taste, rancid smell and taste, yellow color, and wateriness. On the basis of these sensory evaluations, it was concluded that the farmed spotted wolffish was as good as, or even better than, the wild wolffish.

Results, from the beginning of the 1990s, from three different trials showed significant differences between farmed and wild Atlantic cod (*Gadus morhua*), mainly for texture attributes (Nyvold 1989, 1990; Solberg 1990). A sensory panel of 7 to 10 assessors evaluated wild cod as having lower intensities of hardness, fibrousness, and chewiness and higher intensities for juiciness compared to the farmed cod. Recently similar results were obtained in two different trials with wild and farmed cod (Luten and others 2002, Otterå and others 2004). The sensory panel gave wild cod lower scores for fibrousness, whiteness, and dullness and higher scores for juiciness compared to the farmed cod.

SENSORY EVALUATION OF FARMED VERSUS WILD FISH BY CONSUMER PANELS

Farmed Versus Wild Fish

Sylvia and others (1995) conducted a consumer study of three types of fresh salmon (wild and farmed Chinook (*Oncorhynchus tshawytscha*) and farmed Atlantic salmon) in the United States. Eleven sensory attributes related to flavor, texture, and color were selected for analysis. The results show that both taste and texture attributes are critical for the consumer enjoyment of salmon. Statistical tests showed that delicate/fresh fish flavor had the strongest effect on overall enjoyment. For overall enjoyment, the ranked scores for wild Chinook were significantly higher than those for farmed Chinook and farmed Atlantic salmon. Furthermore, the results suggested a difference in the sensory evaluation of farmed and wild salmon. Wild Chinook was found to have a more delicate/fresh flavor than both farmed Chinook and farmed Atlantic salmon. No significant difference in delicate/fresh flavor was found between farmed Chinook and farmed Atlantic salmon.

In a prestudy by Luten and others (2002) with approximately 1,000 consumers in 403 households from the Netherlands, wild and wild-caught, farmed-raised cod were evaluated. The expected and experienced quality of both products was measured in relation to information given about the origin of the cod. In addition, the effect of freshness of the farmed cod was measured. The quality profile of farmed and wild cod, based upon the evaluated product properties was similar. Expected and experienced quality in both products differed very little. Farmed cod seemed to be appreciated as much, after consumption, as wild cod. Higher scores were given on some quality attributes where information was given about the origin of the cod (wild or farmed).

In a major study, Kole and others (2003) investigated the influence of product information on the consumer perception of fillets of wild and farmed cod examined in a real in-home environment. The aim of the study was to establish the external validity of these influences. In several randomized, full factorial experimental designs, modified atmosphere packed cod fillets were presented to approximately 1,440 consumers of the Dutch TasteNet consumer panel to be assessed in their normal household situation. The products were presented over several sessions, one at a time. Product information was included on the package covering production type (wild or farmed), quality control (independent or retailer controlled), price (high or low), capture date (recent or long ago), remaining shelf life (short or long), and information on the advantages of fish farming (present or absent). A control with no information at all except that the product was cod, was included in this study. Consumers gave their preconsumption expectations and postconsumption experiences of the cod for overall and analytic (sensory) quality attributes. Close attention was paid in this study to the effects of information and price. In contrast to the uninformed condition, cod that was believed to be farmed was judged less favorably than cod that was believed to be captured in the wild. Higher priced cod was generally rated more favorably than lower priced cod. Additional (positive) information about fish farming showed no additional effect on product judgment, neither did capture date, shelf life information nor the quality control.

Interaction effects of information with product perception prove to be robust. Even in a real life household situation, there are significant interactions. Apparently, farming of fish is associated with less favorable characteristics and perceived as such. This has to be taken into account in the market focus.

Hahn (2003) described a blind test of three wild and farmed fish species (turbot, sea bass, and salmon) by an "expert consumers" panel. Each fish species was prepared in two ways by a chef and afterward 14 sensory attributes were rated on a 9-point scale by nine food experts. The assessors were also asked to indicate the origin (wild or farmed) of the fish they consumed. The differences between wild and farmed turbot (grilled or steamed) were minimal. The wild turbot was less firm and was rated higher on chewiness. Only minimal differences were observed for fried sea bass. In the case of salmon (smoked and steamed), the assessors gave in general a higher rating for wild salmon than for farmed salmon. Although the origin of the salmon species is not mentioned in the paper, the differences may be due to the fact that probably sockeye salmon (*Oncorhynchus nerka*) was used as wild salmon and Atlantic salmon as farmed salmon in this trial.

Wild Fish

In 1988 Sawyer and others (1988) investigated the relationship between consumer and trained panel evaluations of fish. In a fish usage and acceptance survey, 290 consumers were asked about the frequency of eating fish as well as their degree of liking or disliking on a 9-point hedonic scale. Also a list of descriptive terms considered relevant and important for describing sensory properties were asked. Flavor was cited as the main reason for their liking or disliking much more than texture alone. There was some evidence that texture was more important for those consumers who disliked the fish.

For the development of sensory attributes to describe and differentiate species sensory testing, consumer interviews and discriminant analyses were carried out. A consumer panel with approximately 40 participants and an expert panel trained in flavor and texture was used. After an initial test, a list of 27 attributes could be established, which were submitted to a step-wise linear discriminant analysis resulting in 13 attributes that were found to discriminate most efficiently among test species. These attributes were applied to the evaluation of 18 common Atlantic species (e.g., cod, haddock, halibut, mackerel, and monkfish). Significant positive correlations between the expert and consumer panel judgments of flavor, texture, and appearance were obtained. One interesting aspect of the flavor data is that all of the flavor notes for which there were strong associations were heavy notes for example, those that differentiate "strong-tasting" fish such as Atlantic mackerel from "mild-tasting" fish such as Atlantic cod and haddock. The "brininess/saltiness" and "shellfish" notes, for which there were poor correlations, were more delicate notes. Thus, it seems that while consumer judgments of fish flavor were in good agreement with trained panel judgments for salient notes, they were in poor agreement for subtle flavor.

Regression analysis showed that trained profile panelists used a wider range of intensity scale than consumers did.

A consumer preference study of fresh cod and plaice (*Pleuronectes platessa*) fillets was carried out in Denmark in 1998. That study showed no significant effect from 2 to 12 days of ice storage on consumer preferences (Poulsen and Juhl 1999). However, a trained sensory panel clearly detected the effect of storage on ice (Larsen and others 1999). Based upon these results a new study with cod fillets was set up in 2001, with the aim of comparing the consumer's preference with the results obtained from a trained sensory panel. This study documented that consumer responses to fresh cod fillets correspond to those obtained by a trained sensory panel (Larsen and others 2003).

In a consumer study in France by Honkanen (2000), Norwegian saithe (*Pollachius virens*) was compared with French saithe as well as with Alaska pollack (*Theragra chalcogrammus*). Expected quality before preparation and experienced quality after consumption were measured in a real in-home setting. The total number of consumers involved in this study was approximately 400.

The expected quality of the French saithe fillets was higher on all 18 attributes compared to the Norwegian saithe. The largest differences were found in the attributes color and appearance, and the least differences were found in juiciness and taste. No significant differences were found for the attribute smell. The evaluation after preparation (experienced quality) gave similar results, but there

were fewer significant differences. After consumption there were no significant differences between the two saithe species for the attributes texture, taste, fat content, and calorie content. The largest differences in evaluation after preparation were found for the attributes color and appearance.

The perceived differences between Norwegian saithe and Alaskan pollack were relatively large. The consumers preferred Alaskan pollack both before and after preparation. For the expected quality, a significant difference was found for the attributes texture, color, smell, and taste. After preparation a significant difference was found for color, appearance, and fat and calorie content.

Preference mapping was used by Sveinsdottir and others (2003) to compare consumer preferences for fresh and thawed cod packed in air and modified atmosphere. The results were compared with the results of sensory evaluation by a trained sensory panel. The packed samples were stored for 2 and 10 days (0–1°C) before evaluation. Fresh cod in air kept for 2 days received the highest sensory scores for freshness, juiciness, and tenderness according to the sensory panel. The study showed that consumers found differences between different storage times of cod, 2 and 10 days, preferring the freshest cod. The unfrozen, modified atmosphere packed sample was preferred over the air packed samples, thawed samples, and the modified air packed, thawed samples.

Farmed Fish

Olsen (personal communication, unpublished results) tested smoked Norwegian farmed salmon among 660 consumers in France and 660 in Germany. A correlation between the expected and experienced quality was observed for color. It was shown that only the texture and fat content had a significant influence on consumers' evaluation of the experienced quality of farmed salmon.

In a consumer test with 400 participants, Rødbotten (2001) studied the response to cooked female or male, farmed Atlantic halibut. On a liking scale from 1 to 9, the male halibut received an average score of 5.83, which was slightly higher then the average score 5.60 for the female halibut. In response to a question regarding the origin of the fish, approximately 12% of the consumers responded that none of the samples were farmed and the remainder were of the opinion that both or one of the samples was farmed.

In a study by Richardsen and Østli (2003) conducted in Japan, consumer preferences were tested for seven farmed salmon products (fresh Tasmanian Atlantic salmon, frozen Chilean Atlantic salmon, frozen Atlantic salmon, and fresh Atlantic salmon) and trout (*Salmo trutta*) (frozen Chilean trout, fresh Norwegian trout, frozen Norwegian trout). Two different product categories, "smoked" and "sashimi," were evaluated by 240 consumers (married women) in central-location tests. The consumers evaluated four different product attributes for each individual product in addition to a total evaluation regarding what degree they liked the product.

The results for the smoked products showed that Norwegian trout, both fresh and frozen, was the most preferred product. Trout had a preferred red color to that of the salmon products. High levels of fishy flavor and oily flavor were perceived as favorable in terms of consumer satisfaction with the smoked products.

The results for the sashimi products showed that Norwegian trout (fresh and frozen) and fresh Norwegian salmon had the highest preference. These products had a natural red color and received high scores because of the fat content. Preference mapping indicated that a high fat content, oily aftertaste, sweetness, melting texture/elastic texture, and distinct/visible white (fat) stripes are favorable "consumer" attributes for sashimi salmonid products.

Cardinal and others (2003) have undertaken a preference mapping of the consumers in some European countries (Belgium, France, Germany, Italy, and the United Kingdom) for 30 smoked salmon products currently on the market. The results show that the smoked salmon products can be classified into different groups according to sensory properties and composition. The European consumers showed specific preferences according to product characteristics, and five groups of consumers could be identified. The first group of consumers looked for smoked smell and taste. The second group appreciated low salt products and seemed to have little sensitivity to spoilage. A definite preference was found among the consumers in the third group with respect to little salt and little smoked taste and "fish" note. A preference for salted products with a wood smoke smell was observed in the fourth group of consumers. In the final consumer group, a preference was observed for appearance (homogeneous) and the color (orange) of the salmon slices.

Restaurant Sector

The catering sector represents an environment in which many different seafood products are prepared and consumed. Several food trends first appear in high-end restaurants as a result of chefs experimenting with new ingredients, recipes, and products. The chefs hold a unique position in product development because they are expert evaluators of both product quality and use. They must evaluate product quality both before and after preparation. Furthermore, chefs must have a commercial perspective on new products and product development. They depend on selling new products to their customers for success. Introducing products to a chef for preparation in his own kitchen provides an opportunity to test new products in the chefs' natural environment.

In a study by Johansen and Heide (2001), the quality perception of fresh farmed spotted wolffish was evaluated by 32 chefs in the high-end restaurant sector in Norway, France, and Germany. They evaluated the wolffish as a whole fish, fillet, and after preparation on a 7-point semantic differential scale. The results showed that the Norwegian chefs were most satisfied with the sensory quality, in comparison with their German and French colleagues. The freshness, texture, skin color, flesh color, smell, and juiciness of the wolffish generally received high scores. Many of the chefs found the taste too mild and the fish too fatty.

Heide and others (2003) studied the sensory quality of farmed cod as evaluated by 70 chefs in the high-end restaurant sector in Norway and the United Kingdom. A similar study was conducted in Spain with approximately 30 chefs (Østli and Heide 2004). The chefs filled out a questionnaire during the evaluation of the cod. All chefs were interviewed 1 to 2 weeks after they had evaluated the cod.

The results showed that English and Norwegian chefs generally had long experience in using fresh wild cod, and the chefs used cod on their menu on a regular basis. The Spanish chefs had some experience in using fresh wild cod, but on a less regular basis.

To establish how the chefs were to evaluate the quality of fresh cod, they were asked to describe a whole cod, a fillet, and a prepared cod of good quality on a 7-point (1 to 7) semantic differential scale.

The words anchoring the scale were different for the attributes. For example the scale for taste was "bad taste"—"good taste" and color "unappealing color"—"appealing color." The whole cod was evaluated on the basis of freshness, smell, texture, and skin color. The raw fillet was evaluated on the basis of color, smell, texture, and appearance and after preparation evaluation was conducted on the basis of attributes of taste, juiciness, color, texture, and appearance. In addition, an overall liking was measured for the different product types (scale of "do not like"—"like very much"). Results from the sensory evaluation of the farmed cod showed small differences between the chefs in the different countries. All sensory attributes received an average score of approximately 5 or higher, which indicated that the overall quality of the farmed cod was good. The chefs confirmed this during the interviews. Freshness was the attribute, which received the highest scores, with an average rating between 5.8 (Norway) and 6.5 (Spain). Some of the chefs found the taste of the cod neutral. This can be explained by the higher degree of freshness. Cod stored on ice for 2 to 6 days naturally has a neutral taste. As shown in Table 43.1, the chefs from the different countries generally used the same attributes when evaluating the quality of a fresh cod.

FUTURE

In the future, aquaculture must increasingly meet the consumers' demands and generate products of consistently high quality. The consumers' perceptions depend on attitudes and beliefs about the products and their production (Cardello 1995, Cantin and Dubé 1999, Alba and Hutchinson 2000, Zeelenberg and others 2000). As has been shown for seafood by Kole (2003), these attitudes are dynamic and can change depending on information and knowledge and independently of any apparent characteristics of the end product.

Another challenge for the aquaculture industry is the consumer attitude toward farmed fish. Several studies have found that consumers hold overall negative attitudes toward farmed fish. Gross (2001) found that consumers have a predominantly negative attitude toward farmed seafood. Farmed seafood is associated with the possible presence of diseases in fish farms, loss of flavor, mass production, and animal welfare.

Døving (1997) found that Norwegian consumers have a negative attitude to farmed fish both as a food

Table 43.1. Attributes most frequently cited by chefs in Norway, the United Kingdom, and Spain during the evaluation of farmed cod.

Norway	United Kingdom	Spain
Whole fish	**Whole fish**	**Whole fish**
1. Bright skin color	1. Firm texture	1. Firm texture
2. Firm texture	2. Bright eyes	2. Bright eyes
3. Red gills	3. Bright skin color	3. Bright skin color
4. Bright eyes	4. Red gills	4. Red gills
Fillet	**Fillet**	**Fillet**
1. White color	1. Firm texture	1. Firm texture
2. Firm texture	2. White color	2. White color
3. Fresh smell	3. Size	3. Fresh smell
	4. Fresh smell	
Prepared	**Prepared**	**Prepared**
1. White color	1. White color	1. Flaky
2. Flaky	2. Taste	2. Taste
3. Taste	3. Firm texture	3. Firm texture
4. Juicy	4. Flaky	4. White color
5. Firm texture	5. Juicy	5. Juicy

and an industry. The main reason is that the consumers perceive farmed fish as something "wrong" and "unnatural." Results showed that 60% of consumers think that wild salmon tastes better than farmed salmon, whereas only 1% is of the opposite opinion.

In contrast to the above-mentioned studies, Kuznesof and Ritson (1996) found that British consumers perceived farmed fish as at least as acceptable as wild fish, if not more so. The British consumer perceived fish farming systems as caring, nonhostile, and occurring in a less polluted environment when compared to that of wild fish. They assumed that farmed fish would be healthier than wild fish, and the majority regarded the perceptible difference in taste as negligible.

No studies have so far addressed the possible discrepancy between attitude and behavior in the consumers' choice of farmed fish. Despite negative attitudes toward farmed fish, large amounts of farmed fish are consumed every day. A number of factors may influence the attitude-behavior relationship: attitude strength, ambivalence, etc. (Eagly and Chaiken 1993). Considerations like convenience, price, and availability might influence consumers in an actual purchase situation. Meiselman (1992) called for greater research on real foods in real environments as one strategy for better prediction of food-related behavior. This may be one method for studying what the determinants for buying farmed seafood really are. Also Gross (2001) emphasized that there might be a difference between attitudes and actual consumer behavior in the case of farmed fish. There could be a discrepancy between what consumers think ("bad attitudes toward farmed seafood") and what they actually do ("buying it despite the negative feelings they expressed"). Olsen (2003) provided an overview of some recent findings on consumer attitudes and other important antecedents of seafood consumption and buying behavior. Taste, distaste (negative effect), nutritional value, and freshness (quality) are suggested to be the most important factors in forming consumers' attitudes and preferences toward buying and consuming seafood. Social norms, moral obligations, and health issues may be among the more important motivational factors in explaining seafood consumption in relation to most other food products. Price/cost, convenience, knowledge, and availability of fresh products are suggested to be important factors in seafood consumption in certain sectors and age groups of consumers. The overall conclusion is that seafood is less driven by taste and preference and more by moral obligation and health issues, than is the case with other food products.

However, the quality attributes that can be evaluated by touching, tasting, smelling, and eating (sensory attributes) are useful guidelines for production. Both types of information (consumer attitudes and quality attributes) are essential for the development of future quality products corresponding to the consumers' quality evaluation. Reproducible methods of monitoring the different quality attributes of farmed fish will enable the industry to more fully meet the consumers' quality-related demands.

The most obvious factor is the feed composition. However, factors that are not necessarily related to the feed have an impact on quality attributes. The fish may have different genetic potential and thus use the feed to a different degree. Consequently, the feed consumption and growth rate may be influenced. Furthermore, the water temperature, photoperiod, ration levels (Johansson and others 2000), and season all affect the appetite of fish. Other important factors may be slaughter methods (Sigholt and others 1997, Tejada and Huidobro 2002, Sørensen and others 2004), starving periods before slaughtering (Johansson and Kiessling 1991, Einen and Thomassen 1998), and "feeding up" or "feeding down" (Rasmussen and others 2000), all used to "tune" to the required quality attributes.

Furthermore, specific consumer requirements may in the future have a greater impact on production methods of farmed fish. If new feed components are to be used in fish feed, the following factors must be addressed. The fish should remain safe and healthy for the consumer and not contain unwanted contaminants. The feed should not have any negative effect on fish health. Animal welfare should be maintained, negative effects on the environment should be minimized, and finally the feed conversion should be optimal.

The globally increasing production of farmed fish has already demonstrated the need for using alternative sources (of oil and protein) in feed production. The current production of marine oils and meal will not meet the predicted demands of aquaculture.

Several studies have been performed where fish oils or fish meal have been replaced by oils or proteins from vegetable sources. Vegetable oils contain lower levels of polyunsaturated fatty acids and are thus more stable with respect to oxidation. This factor may also exert the influence on the taste and odor components of the fish products as shown by Sérot and others (2001, 2002). Most of the odor components that developed in trout and turbot (*Psetta maxima*) were related to the oxidation of unsaturated fatty acids. If some marine oil is replaced by vegetable oils, salmonids may develop a more neutral odor (Skonberg and others 1993), which may be considered as favorable by some consumers.

Alternatively, it has been reported that high levels of vegetable oils may give rise to an unwanted taste in Atlantic salmon (Thomassen and Røsjø 1989, Waagbø and others 1993). From experiments where marine oils in fish feed have been partly or fully replaced by vegetable oils varying results have been reported. Rosenlund and others (2001) did not observe any differences in muscle color of Atlantic salmon. However, the opposite was shown by Thomassen and Røsjø (1989) as well as by Waagbø and others (1993). Oxidation has also been shown to indirectly influence the fillet color since astaxhantin, normally used in color production is used as an antioxidant (Lie 2001).

There are a few studies reporting textural effects of using vegetable oils. Both Atlantic salmon (Waagbø and others 1993) and Arctic char (*Salvelinus alpinus*) (Guillou and others 1995) fed with a vegetable diet have been evaluated as hard, less juicy, and firm. In contrast to this, Rosenlund and others (2001) and Bencze-Rørå and others (2003) did not observe any effects of using vegetable oils on the textural quality of salmon.

Vegetable proteins (soybean concentrate) used as a partial or total replacement for fish meal were tested in a study on rainbow trout (*Oncorhynchus mykiss*) by Kaushik and others (1995). Products from fish fed two diets, 100% fish meal versus 100% soy protein, were tested by a sensory panel. The sensory evaluation did not reveal many differences in terms of flesh textures. However, the panel distinguished the two groups based on two taste attributes. The fish fed 100% soy protein scored higher for both rancid and freshwater taste. However, a panel of 11 trained assessors did not observe any off odors or off flavors in the Atlantic salmon fed a diet containing approximately 10% full-fat soybean protein (Bjerkeng and others 1997).

In conclusion, there is an increasing need for safe and healthy seafood products with a high sensory quality. This demand needs to be met by increased seafood production from farming. Several (controllable) factors in fish farming may affect some important technical quality attributes (e.g., taste, texture) as judged by the consumer. In particular, the

impact of replacing fish oil with vegetable oils and the use of new protein sources in the fish feed requires careful attention.

Moreover, the attitudes, beliefs, and behavior of consumers toward fish farming as a process, alternative feed sources, and farmed products may have a significant impact on the perception of quality of seafood. Integrated research embracing both elements offers the option to fulfill consumers' demand for high quality seafood products.

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44
Quality of Frozen Fish

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INTRODUCTION

Freezing is the most widely used preservation method for fish—30% of all fish and fish products, globally, are frozen before being sold on the market. Nevertheless, the eating quality of fresh fish (not frozen before being sold on the market) is considered superior to that of frozen fish. It is, however, possible to produce frozen fish of high eating quality by freezing the fish quickly and subsequently storing it at low and stable temperatures. In this way, the physical and chemical processes causing the quality of the fish to deteriorate may be reduced. Quality deterioration is seen as for example in the development of ice crystals, oxidation of fat, and degradation of muscle protein. The effect of freezing and frozen storage has been studied for some years and is described in a number of reviews (Sikorsky and others 1976, Shenouda 1980, Haard 1992, Love 1992, Mackie 1993, Sikorsky and Kolakowska 1994). Part of this chapter has also been published in Danish by Jessen and Nielsen (2002).

Frozen fish is easily stored and distributed. Under ideal circumstances (low and stable storage temperatures), some fish species may retain a fair eating quality for over a year. Shelf life can be assessed either in terms of Practical Storage Life (PSL) or High Quality Life (HQL). PSL is defined as the time the product can be in cold storage before it loses its characteristic properties or becomes unsuitable for consumption. PSL is often determined between trade partners, and no legislative rules apply to this area. HQL is a target for how long the product can be in cold storage before taste panels are able to discern a clear difference from the original quality of the fish. HQL is normally two to three times shorter than PSL. PSL, moreover, is what is eventually declared on the product. The shelf life of fish in cold storage depends on time, temperature, and the species of fish (Table 44.1).

Quality Changes in Fresh Fish and in Frozen Fish

During chilling (i.e., temperatures between 0 and 5°C), the quality of the fish changes due to oxidation, especially of its fat (oxidative rancidity), self-digestion brought on by the fish's own enzymes (autolysis), and bacterial growth. Normally, the waste products of the bacteria are what cause the fish to smell bad, and bacterial decomposition is what causes its purification. Freezing and cold storage

Table 44.1. Storage time for PSL (Practical Storage Life) and storage time for HQL (High Quality Life) stated in months for lean fish (cod), big fatty fish (salmon), and small fatty fish (herring).

	Shelf life in months			
	−18°C		−30°C	
Fish species	PSL	HQL	PSL	HQL
Lean fish (e.g., cod)	7	3	12	6
Big fatty fish (e.g., salmon)	7	3	18	6
Small fatty fish (e.g., herring)	5	2	10	5

hinders bacterial growth whereas oxidation and autolysis continue, albeit these processes are slowed down when the temperature is below the freezing point. The quality of frozen fish moreover deteriorates because of changes in the structure of the proteins (protein denaturation).

THE FREEZE CHAIN

The route from catch to consumer is long (Figure 44.1)

Frozen fish products are usually processed in one of two ways: the fish is either frozen aboard the ship or on land. Aboard the ship the fish is either frozen whole or as fillets. The frozen fillet is often used directly for sale, whereas the whole fish is thawed on land, processed, and then frozen again (double freezing). There is no tradition for double freezing in Danish waters where the fish is brought on land in chilled condition (stored in ice) and subsequently processed and frozen.

Industrial freezing of fish products is done at various stages of production. On board trawlers, whole fish are frozen in vertical plate freezers and fillets in horizontal plate-freezers as interleaved fillets or standardized blocks (Bøknæs and others 2001). In shore facilities, the fish are either frozen in blocks for further processing as fish fingers and portions or in retail sizes. The modern fish industry is equipped with quick freezing facilities such as air blast freezers and plate freezers. Fluidized bed and cryogenic freezers are used for individual quick freezing of shrimps and other shellfish. Quick freezing means that the time to pass between 0°C and −5°C must not normally exceed 5 to 10 hours and the warmest part of the product must be the temperature of the following frozen storage room. Due to the flow in production, it is preferable that the total freezing period is no more than 1 to 2 hours. Slow freezing over several days (fish stacked in insulated containers in silent air in a frozen store) can be avoided if the modern equipment is used.

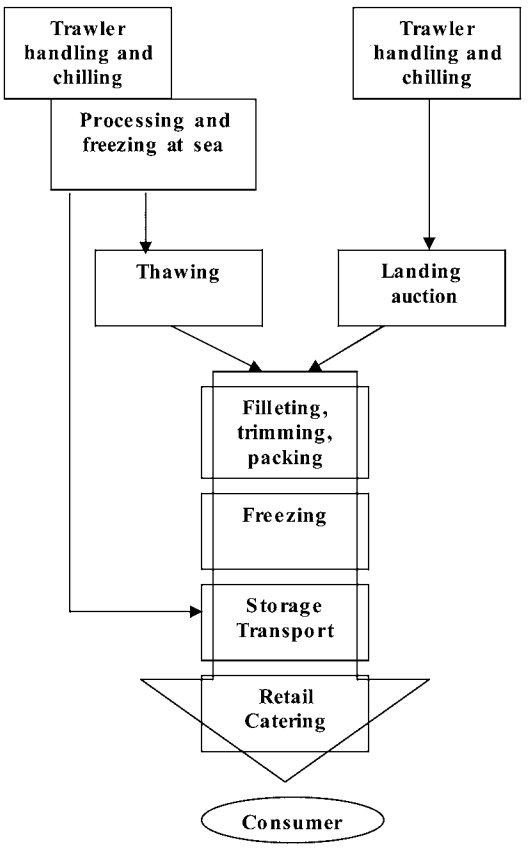

Figure 44.1. The freeze chain. The fish is either frozen aboard ship or on land.

HOW CAN CHANGES IN SENSORY QUALITY OF FROZEN FISH BE SEEN?

The quality of a frozen fish depends on its initial quality and on how it is handled. A white fish such as cod is cleaned and bled immediately after being killed. This means the guts are removed and the blood is allowed to drain from the muscles. In an entirely fresh cod, the blood has no effect on quality. However, when the fish is frozen and kept in cold storage, the blood may give the fish a metallic tinge and later contribute to its going rancid. When a lean fish such as cod goes rancid, it will often taste somewhat like wet cardboard (cold-storage flavor). Fatty fish such as salmon and herring do not acquire cold-storage flavor but will acquire a rancid flavor of cod liver oil if not properly protected in airtight packaging during storage.

Differences in Freezing Quality of Fish Populations Belonging to the Same Fish Species

Different batches of cold stored cod can be of very different quality even though the fish were handled identically and stored in the same period of time. This could be due to differences in the composition of the fish, which changes, for example, depending on time of season and spawning. Researchers believe the reason lies in both hereditary and environmental conditions.

- Hereditary differences can occur when cod in a certain location act like an individual population, not particularly interested in mixing with cod from another location (belonging to another population). Hereditary differences will be seen between such populations.
- Environmental differences can occur when cod from a population stay in two different locations where conditions differ so that the fish develop differently.

The interplay between the hereditary genes of the individual and its environment defines its "appearance" (size, shape, chemical composition, etc.), in other words it defines the phenotype of the individual fish. A fish product manufactured from several different phenotypes may vary in quality even though the fish were handled identically aboard the fishing boat and at the factory.

If cod is handled with care during and after catching, if it is frozen as quickly as possible and stored at a stable temperature of −30°C (or below), there will be no difference in taste between a thawed and a fresh fish for the first month. After 3 months, rancidity sets in, cold-storage flavor starts to develop, and the taste characteristics of the specific species gradually disappear completely. However, the frozen fish may be suitable for consumption for up to 1 year when kept in cold storage at a low and stable temperature.

Fatty fish, especially large fish like salmon, are more suitable for freezing than cod and at low temperatures may be suitable for consumption for up to 1.5 years (Sørensen and others 1996).

Also here, the taste of the fish becomes more and more neutral, however, off flavors will only develop after very long storage times, and rancidity occurs only if the fish has not been packed properly.

Numerous consumer studies show that retailed frozen fish taste significantly poorer than fresh fish. As a consumer you can never be sure of the quality of the fish you buy—there is nothing on the packet to indicate the quality of the fish. Packets are labeled with packaging date, but say nothing about when the fish was caught, the temperature at which it has been stored, for how long it has been stored as raw material, or if it has been frozen and thawed several times, etc.

The consumer evaluates the eating quality of a fish through the senses—by the look, smell, texture, and taste of the fish—using sensory assessment. As only the consumer can determine what he or she likes, such an evaluation will be subjective. Sensory assessment may also be applied professionally, by individuals who have been trained in the assessment of specific properties of fish. In this way sensory assessment becomes objective. To handle the quality of fish through the frozen fish chain, the quality index method has proven to be a good tool for frozen cod (Warm and others 1998, Jensen and others 2001).

The Quality Index Method (QIM) for frozen fish can be looked upon as a simple profile. Tables 44.2 through 44.4 show the schemes developed for frozen cod from raw material to product.

For frozen fish, the theoretical demerit line (see Chapter 41, Quality Index Methods) has been more difficult to determine than the one for fresh fish because of the extended storage time dependent on freezing method, temperature of frozen storage,

Table 44.2. QIM-scheme for thawed whole cod.

Quality parameter	Characteristics
Texture	0: Firm texture. Stiff and firm to finger touch.
	1: Elastic to finger touch. Marks disappear after a few seconds.
	2: Fairly soft and plastic flesh. Marks do not disappear.
	3: Very soft. The flesh is easily penetrated on pressure.
Remains of guts	0: No remains in the belly.
	1: Few remains in the belly.
	2: Many remains in the belly.
Shape of fish	0: Normal round shape as freshly caught cod.
	1: Fairly normal round shape with few marks after freezing.
	2: Very mechanically damaged during freezing. Very deformed.
Marks from fishing tackles/ catch handling	0: No marks.
	1: A few small marks.
	2: Many marks.
	3: Very many/big marks.
Odor	0: Fresh marine and seaweedy.
	1: Neutral.
	2: Slightly sour and metallic.
	3: Strong sour and metallic. Painty.
Appearance	0: Iridescent or opalescent. Bright, shining. No bleaching.
	1: Slight bleaching.
	2: Dull and very bleached, no iridescent. Freeze dried.
Flesh color in open spaces	0: Open surfaces white and blood in throat cut red.
	1: Open surfaces gray or slightly yellow.
	2: Open surfaces yellow or brown.
	3: Open surfaces very yellow and brown. Milky surfaces as freeze dried.

temperature fluctuations, etc. The approach for establishing a quality index for frozen fish (cod) has therefore been different from the method used for fresh fish. The decision of which parameters to choose in the QIM schemes for frozen cod was based upon parameters for fresh cod showing the largest variation over time. The goal with the development process was to describe each score in a way so that people with little training in sensory assessment of fish understood the description. This required a difference between each score. Ranking the descriptions for each parameter and giving numbers to the ranks in succession from 0 to 4, the indexing method has been established for whole fish, fillets, and cooked fillets. The grading schemes have been evaluated and further developed in a great number of trials with both trained and untrained panel members using frozen cod from the Baltic Sea, Iceland, and Russia. The different parameters have been assessed and the parameters selected to give a picture of the total sensory quality.

A manual containing the total plan for evaluation, explanation of the evaluation terms, and color slides illustrating the different levels of quality of frozen cod and cod fillet has been produced for industrial use.

The three QIM schemes can be used effectively through the chain from fisherman to consumer. It will be possible to check out critical points in a production line by the QIM. When the quality index differs significantly from whole fish to fillet, a decision can be made to change the production conditions.

A continuous problem about QIM is the set up of various quality categories. For thawed fillets, three categories were suggested (Warm 2001), as shown in Table 44.3.

An advantage of the QIM is that the limits for quality categories can be set individually depending on the product.

Table 44.3. QIM-scheme for thawed fillet.

Quality parameter	Characteristics
Texture	0: Firm and stiff texture. No wateriness.
	1: Slightly soft, initial wateriness.
	2: Soft, wateriness noticeable.
	3: Very soft and pronounced wateriness.
Odor	0: Neutral.
	1: Slightly sour off odor.
	2: Very sour off odor.
Color	0: Plain white.
	1: Grayish.
	2: Gray, starting yellow maybe slightly red.
	3: Either yellow or very red. Milky surfaces as freeze dried.
Blood stains	0: No stains.
	1: A single stain (diameter less than 3 mm).
	2: Single small stains (1–2 with a diameter under 5 mm).
	3: Very discolored from many stains or totally red.
Gapping	0: No gapping, coherent.
	1: Slight gapping.
	2: Gapping noticeable, disrupted.
	3: Gapping pronounced, disrupted.
Parasites	0: No parasites.
	1: One parasite.
	2: More than one parasite.

Table 44.4. QIM-scheme for cooked fillet from thawed cod.

Quality parameter	Characteristics
Odor	0: Sweet, marine, and seaweedy.
	1: Loss of odor.
	2: Neutral.
	3: Slightly cold storage odor (cardboard), slightly citric, amine.
	4: Cold storage odor (cardboard), citric, strong amine.
Color	0: White and opalescent.
	1: Loss of whiteness.
	2: Grayish, one small blood stain.
	3: Slightly yellow, a few small blood stains.
	4: Light brown. Discolored of blood.
Flavor	0: Sweet, marine, and seaweedy.
	1: Loss of taste, slightly sweet.
	2: Neutral.
	3: Slightly cold storage flavor (cardboard), insipid, slightly as soap, slightly citric, slightly amine.
	4: Cold storage flavor (cardboard), dry fish, soap, citric, strong amine.
Texture	0: Very succulent, flaky and coherent also succulent after chewing several times.
	1: Succulent, flaky and coherent. Succulent after chewing several times.
	2: Initial feeling is succulent, watery after first chewing. Dry and tough during following chewings.
	3: Dry, fibrous, and/or tough. Dry and tough from the first chewing.
	4: Very dry, fibrous and/or tough. Very tough from the first chewing.

Table 44.5. QIM score—use of product.

Category	Examples of characteristics	Use of product
High quality: Index 0–4	• Firm texture • Neutral odor • White color • No blood stains • Minimum gapping • No parasites	• Many possibilities for use • Luxury products (loins, tails, etc.)
Medium quality: Quality index 5–12	• A little soft and watery • Somewhat sour odor • Grayish color • Single blood stains • Medium gapping • One parasite	• Breaded products • Several cuttings • Block products
Low quality: Quality index 13–16	• Very soft, very dry • Very sour odor • Yellowish, reddish • Big blood stains • Much gapping • More than one parasite	• Very few possibilities for use • Block products

The Freezing Process—From Water to Ice

Fish muscle contains large amounts of water, often about 80%. When fish are frozen, the temperature in the center of the fish quickly falls to just below 0°C (Figure 44.2). At −1°C, ice crystals start to form in the water, that is, the water changes from liquid to solid form. At this stage, the temperature falls slowly as the heat from the fish is removed and most of the water in the muscle turns to ice. When about 75% of the water is frozen, the temperature once more starts to drop rapidly, and at −30°C, about 90% of the water will have turned into ice while the remainder is still liquid. The remaining liquid water is called "non-freezable water."

Water turning from liquid to solid form is a physical process, and the degree to which this process affects the quality of the thawed fish depends on where in the fish muscle the ice crystals develop and on their size (see Formation of Ice Crystals). As it is, a great part of the ice that has formed outside the muscle cells will drain from the fish muscle during thawing (thaw drip or drip loss) and during cooking (boiling loss). This means the fish will be drier in texture when prepared and eaten.

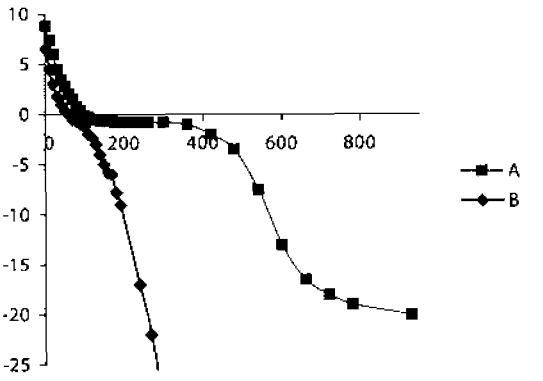

Figure 44.2. Temperature profiles during freezing of whole cod. A: Freezing at −20°C in still air (home freezer). B: Freezing at −45°C in air blast freezer (industrial freezer). The temperature was measured in the center of the fish (core temperature). It is apparent from the figure that the fish can be brought through the critical temperature interval, 0 to − 5°C, when quick freezing takes place at a low temperature and when a fan at the same time makes sure that the heat from the fish is removed quickly from the surface.

Formation of Ice Crystals

Where crystals are formed and their size depend on whether the fish is frozen before (pre), during (in), or after (post) rigor mortis (stiffening after death), as well as on how speedy the freezing process is.

In prerigor fish, the water is only inside the muscle cells, and when the fish is frozen, the ice crystals will therefore form inside the cells. The ice crystals will mainly be small. Large ice crystals will form only if the freezing process is slow (e.g., in still air above −18°C). In in-rigor and prerigor fish, a small amount of water, however, will be outside the cells and thus the speed of freezing becomes significant for the formation of ice crystals. If freezing is quick, small ice crystals will form both inside and outside the cells. However, if freezing is slow, ice crystals will first form outside the cells, resulting in an increase in the salt concentration. The higher concentration of salt outside the cells will extract more water from the cells and when this happens during freezing the ice crystals, which have already formed inside the cells, will grow in size rather than allowing for the formation of new ice crystals. In in-rigor and postrigor fish, the speed at which the fish are frozen can therefore determine how large the ice crystals are and where in the muscle they form. This will range from many small ice crystals both inside and outside the cells, to only very few large ice crystals outside the cells.

Ice crystals form not only during freezing but also during cold storage. Here, not many new crystals are formed, but a recrystallization process takes place in which the surface of crystals, small ice crystals in particular, melts. This melting water subsequently freezes to the larger ice crystals, thereby leading to more large crystals at the expense of smaller ones. Recrystallization is especially rapid at fluctuating storage temperatures, even when the temperature fluctuates by only a few degrees.

Ice crystals consist of pure water and therefore substances that are normally dissolved in the water in the muscle have less and less water to be dissolved in as more and more of the water is converted into ice. These substances are primarily salts and enzymes, the concentrations of which become very high in the remaining "nonfreezable water." Many of these substances take part in processes that deteriorate the quality of the frozen fish. High concentrations of salt, for instance, damage the proteins of the frozen fish (see page 584 on "protein changes"). The speed of chemical reactions slows in step with the lowering of the temperature, whereas it accelerates as the concentration of the substances in the reaction increases. Thus, there will still be chemical reactions in the "nonfreezable water" at the very low temperatures present in frozen fish due to the very high concentration levels. Reactions will take place even at −30°C, however, they will be very slow.

Rigor Mortis

Immediately after death, fish muscles are completely lax and the fish feels soft and elastic. When kept in ice, the fish will go into rigor mortis (stiffen) after a few hours. This is because the chemical energy present in the live fish is used up, which causes a large part of the proteins in the muscle to bind into a network, which stiffens and hardens the muscle and thus the whole fish. After a couple of days in ice, the fish will again soften and become elastic as certain proteins in the network are broken down by enzymes.

Apart from the physical formation of ice leading to higher concentrations of substances, which in turn speeds up the destructive chemical processes, the ice crystals can directly and physically damage the structure of the muscle. Here, large ice crystals may cause much more damage than small ice crystals. Damage to membranes of muscle cells is particularly serious. This has to do with the fact that some of the cell salts and protein-disintegrating enzymes are in areas confined by membranes. When the membranes are ruptured, the salts are allowed to penetrate to other areas of the cell and increase the chemical processes there.

CHEMICAL PROCESSES THAT DETERIORATE THE QUALITY OF FROZEN FISH

The physical changes in the fish muscle during freezing and cold storage, also, as mentioned above, greatly affect the chemical processes and thus the quality of the fish. The most significant of these processes—oxidative rancidity, protein changes (protein denaturation), and the formation of formaldehyde—are described in the following.

Fat and Fat Decomposition

Fish contain fat and changes in the fat fraction of fish greatly influence the taste of the frozen product. The amount of fat in the muscle of fish varies from species to species, and great variation may occur even within the same species as for example in herring where the content of fat may vary from 1% to 22% during 1 year. The fat content of fish can vary from 0.2% to around 25%.

The fat or lipid content of fish is found in the membranes of cells and as lipid depots just under the skin, in the belly flap, and in the connective tissue. Membrane lipids (primarily phospholipids) make up less than 1% of the total lipid content. Membrane lipids and the fat in lipid depots consist of long fatty acids (primarily triglycerides). During freezing, the fat decomposes and is converted into substances that taste unpleasant. The changes are due to oxidation or enzymatic degradation. Fish fat has a particularly high content of polyunsaturated fatty acids, especially susceptible to oxidation, which produces aldehydes and ketones that have a rancid flavor and odor. Enzymatic degradation produces free fatty acids (soapy flavor and odor).

Oxidative Rancidity

Oxidative rancidity shortens the shelf life of fatty fish in particular. Oxidation takes place on the surface of the fish, and smaller fish such as mackerel and herring with a relatively large surface go rancid more rapidly than other fish. The cleaning and cutting into filleting of fish increases the total surface area, and the fish will therefore become more rancid. Oxidation often takes place very soon after the fish has been killed, especially in the fat layer just under the skin. Moreover, the process is exacerbated by salt and metals such as iron from, for example, the blood of the fish. In codfish, oxidative lipid degradation may give poor flavor, namely the very characteristic cold-store flavor (due to the substance cis-4-heptenal).

Can Oxidative Rancidity Be Avoided?

Oxidative rancidity in frozen fish can be avoided, or at least counteracted, in several ways. First of all, the fish must be frozen as quickly as possible after death, however, allowing time for proper bleeding of the fish in ice water. As mentioned, oxidative rancidity is an oxygen-dependent surface process; glazing and vacuum packing, which reduce the degree of contact with oxygen, are therefore advantageous.

Glazing

Once frozen, the fish is sprayed with vaporized water, which immediately freezes to a thin layer on the surface of the fish. In this way, the entire surface of the fish is covered in an ice layer hindering air (oxygen) from getting in contact with the fish.

It is also possible to add substances that hinder/delay the oxidation process, so-called antioxidants. Antioxidants can be applied to products, to the surface of fish, or to glazing water. A very low storage temperature (−30°C or below) reduces the speed at which chemical oxidation takes place.

The enzymatic degradation of lipids does not depend on oxygen and is therefore not inhibited by antioxidants, vacuum packing, or glazing. The only way to reduce this degradation process is to maintain a very low storage temperature.

Protein Changes

In lean fish, protein changes have the greatest influence on the shelf life of the frozen and cold-stored fish. The proteins become less soluble, and the texture of the fish turns dry, tough, spongy, and/or grainy. Changes may also lead to alterations in the functional properties of the proteins, and this means that their ability to bind water and fat is lessened. When thawing, plenty of water drains from the fish (drip loss) and the fish becomes less juicy and less suitable for minced fish meat products. Protein changes also affect flavor. Proteins may, in their natural structure, bind undesirable flavors, which are released during freezing.

Many of the changes to proteins during cold storage are connected to ice formation:

- Ice crystals may physically destroy protein structures and cell membranes.
- The natural structure of proteins normally depends on whether they are bound to water. When the water freezes, the proteins are dehydrated and thus changed.

- The high concentration of soluble substances in the nonfreezable water leads to high salt concentrations in particular, which in turn cause the proteins to change.

The unique enzymatic degradation of trimethylaminoxide (TMAO) in frozen codfish moreover leads to protein alterations and, subsequently, to deterioration of quality. During TMAO degradation, small amounts of formaldehyde are formed, which, apparently bind the proteins together and thus make them less able to bind water.

Changes to proteins in fish meat during freezing and cold storage may be examined and monitored via proteome analysis, a relatively new technique for examination of the protein composition in cells and tissue, such as fish muscle.

Can Protein Changes Be Avoided?

It is relatively simple to hinder protein changes in frozen fish. Processing the raw material shortly after catching, quick freezing, and cold storage at a low and stable temperature will reduce protein denaturation significantly.

CONCLUSION

It is possible to produce high quality frozen fish if the physical and chemical changes described in this chapter are taken into account during handling immediately after catching, and in connection with freezing and cold storage of the fish.

Temperature and temperature fluctuations are the most significant factors. It is important to cool the fish as quickly as possible immediately after it has been caught in order to minimize the biochemical and microbiological reactions from the very beginning. Fishing methods and temperature are likewise significant in relation to rigor mortis. In addition, it is essential that fish that are frozen, which will subsequently be thawed and processed, are frozen before rigor sets in, as this will result in the best eating quality and greatest yield. The fish must be cleaned and bled before freezing, among other things because of the content of compounds in the blood that intensify the degradation processes and because of the very high enzyme activity in the guts. Thus, a first-class frozen product can be achieved only when using entirely fresh fish and freezing them and storing them at low and stable temperatures. If subject to poor handling in connection with freezing and cold storage, for example in terms of a freezing process spanning a whole day and cold storage temperatures that fluctuate over a longer time, the product will be ruined. Short-term storage of the raw material, gentle processing, quick freezing, short-term storage as a semiprocessed product, and short-term cold storage at temperatures lower than −30°C, will result in the best product.

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Handbook of Meat, Poultry and Seafood Quality
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Appendix
Standards for Meat, Poultry, and Seafood in the United States

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Appendix Standards for Meat, Poultry, and Seafood in the United States

Y.H. Hui

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INTRODUCTION

Apart from numerous factors that determine the quality of a processed food product, the most basic one is what the product actually contains. Some simple examples follow:

1. When we purchase a package of frozen beef sukiyaki, the first question that usually comes to mind is: how much beef? How many other ingredients (leeks, onions, peas, and so on). To most of us, good quality means lots of beef.
2. When we purchase packaged breaded chicken, we want to know chicken quantity versus breading quantity. Good quality means more chicken.
3. When we purchase a package of frozen prawns, we pay attention to the following: type, size, broken pieces, etc. Undesirable quality means deviations from labels: wrong type and size; too many broken pieces.

Some products do not match the description on the labels. The federal government obviously does not have enough resources to police every manufacturer. Instead, it has issued minimal standards for most processed muscle foods, as illustrated here:

1. A frozen beef sukiyaki must contain at least 30% beef.
2. Beaded chicken must contain 30% or less breading.
3. When it comes to shrimp, let us look at some forms specified by the federal government:
   a. Heads on (head, shell, and tail fins on).
   b. Headless (only head removed; shell, tail fins on).
   c. Peeled, not deveined, round, tail on (all shell removed except last shell segment and tail fins, with segments not slit).

So, if the label states headless, the shrimp in the packages must also be headless. Unfortunately, this is not always so. The same applies to 30% beef or 30% breading. This chapter describes the minimal standards for most processed meat, poultry, and seafood as issued by the federal government.

STANDARDS FOR MEAT AND POULTRY PRODUCTS

In the United States, the federal government has total control over the safety of all land muscle foods, i.e., beef, pork, chicken, and many others. This approach is similar to that of major Western countries. The federal agency is called Food Safety Inspection Service (FSIS). FSIS is one arm of the United States Department of Agriculture (USDA). FSIS has the major responsibility of assuring that nation's meat supply and other land muscle foods are safe for the

public to consume. However, apart from safety, the FSIS has another responsibility—assuring that economic fraud is kept to a minimum. Because meat and many land muscle foods are high-priced items, many dishonest food operations cannot resist the temptation to cheat the consumers when the opportunities arise. Fortunately, most operators are honest. Still the FSIS has spent an enormous amount of effort to prevent economic fraud.

All information in this chapter has been derived from publication documents issued by the USDA, with legal citation and languages removed. To obtain original documents, please consult the USDA with respect to standards and labeling of products under USDA jurisdiction.

DEFINITIONS

For ease of reference, some important terms are defined here.

Red Meat

Required percentages of meat required for red meat products are shown on the basis of fresh, uncooked weight unless otherwise indicated. Whenever the terms beef, pork, lamb, mutton, or veal are used they indicate the use of skeletal muscle tissue from the named species, in accordance with 9 CFR 301.2.

Poultry

Required percentages for poultry products are based on a cooked, deboned basis unless otherwise stated. When the standards indicate "poultry," the skin and fat are not to exceed natural proportions in accordance with 9 CFR 381.117(d).

ABBREVIATIONS

AMS Agriculture Marketing Service
BHA Butylated Hydroxyanisole (antioxidant)
BHT Butylated Hydroxytoluene (antioxidant)
CH China
CRDSM Calcium Reduced Dry Skim Milk
FDA Food and Drug Administration
FR French
FSIS Food Safety and Inspection Service
FTC Federal Trade Commission
GK Greece
GR Germany
GRAS Generally Recognized as Safe
HVP Hydrolyzed Vegetable Protein
IMPS Institutional Meat Purchase Specifications
IT Italian
LCPS Labeling and Consumer Protection Staff
MPR Moisture Protein Ratio
MSG Monosodium Glutamate
NAMP National Association of Meat Purveyors
NFDM Nonfat Dry Milk
OPPD Office of Policy Program Development
PDBFT Partially Defatted Beef Fatty Tissue
PDCB Partially Defatted Chopped Beef
PDCP Partially Defatted Chopped Poultry
PDPFT Partially Defatted Pork Fatty Tissue
PER Protein Efficiency Ratio
PFF Protein Fat Free
pH Measure of Acidity
ppm Parts Per Million
PR Puerto Rico
SP Spanish
TBHQ tert-butylhydroquinone
TK Turkish
TVP Textured Vegetable Protein
URMIS Uniform Retail Meat Identity Standards
USA United States of America
USDA United States Department of Agriculture
VPP Vegetable Protein Product

STANDARDS, LABELS, AND OTHER INFORMATION

Andouille (FR)

The product is made with pork and/or pork by-products stuffed into large intestines. Product can be sold cooked or uncooked. Andouille is a coined name and must be accompanied by a true product name, e.g., "sausage" or "pudding" depending on formulation. If beef is used, it must be shown in the product name, e.g., "Beef Andouille Sausage" or "Beef Andouille Pudding."

Arroz Con Pollo (SP)

The product must contain at least 15% cooked chicken meat. The label must show, the true product name, in English, i.e., "Rice with Chicken," except if the product is distributed solely in Puerto Rico.

Au Gratin Potatoes and Bacon

At least 8% fully cooked bacon (based on 40% yield).

Baby Food

High Meat Dinner—At least 26% meat.
High Meat Poultry Dinner—At least 18.75% cooked poultry meat, skin, fat, and giblets.
Meat and Broth—At least 61% meat.
Vegetable with Meat—At least 8% meat.
Poultry with Broth—At least 43% cooked poultry meat, skin, and giblets.
Poultry and Rice—At least 5% cooked deboned poultry meat.

Note: Wine, mechanically separated species, nitrites, and nitrates are not acceptable in baby and toddler foods.

Baby Food with Fresh Ham or Bacon

Ham or bacon without nitrates or nitrites must be shown in the ingredients statement as ham or bacon (water, salt, sugar, etc., without nitrates or nitrites).

Bacon

The term "bacon" is used to describe the cured belly of a swine carcass. If meat from other portions of the carcass is used, the product name must be qualified to identify the portions, e.g., "Pork Shoulder Bacon."

"Certified" refers to products that have been treated for trichinae.

Bacon and Pork Sausage

Product is formulated with a high percentage of bacon (usually bacon ends and pieces) with at least 20% pork.

Bacon Arkansas and Arkansas Style Bacon

Product that is identified as Arkansas Bacon or Arkansas Style Bacon is produced from the pork shoulder blade Boston roast. The pork shoulder blade Boston roast includes the porcine muscle, fat, and bone; cut interior of the second or third thoracic vertebrae, and posterior of the atlas joint (first cervical vertebrae); and dorsal of the center of the humerus bone. For Arkansas Bacon, the neck bones and rib bones are removed by cutting close to the underside of those bones. The blade bone (scapula) and the dorsal fat covering, including the skin (clear plate), are removed, leaving no more than one-quarter inch of the fat covering the roast. The meat is then dry cured with salt, sugar, nitrites, and spices, and smoked with natural smoke. The meat may not be injected or soaked in curing brine, nor may any artificial or liquid smoke be applied to the meat. Product that is prepared outside the state of Arkansas but in the manner prescribed may be identified as "Arkansas Style Bacon." The true product name must be shown as "Boneless Cured Pork Shoulder Butt."

Bacon (Canned, Pasteurized)

A shelf stable item, which must have at least 7% brine concentration.

Bacon (Canned, Prefried)

In "Canned Prefried Bacon," e.g., "Bacon Crumbles," the following criteria should be applied:

1. M/SP Index of 0.4 or more. M/SP = Moisture/(Salt × Protein)
2. A Brine Ratio of 9.0 or less. Brine Ratio = Moisture/Salt
3. A Brine concentration of 10% or more. Brine concentration = Salt/(Moisture + Salt)
4. Maximum 40% yield Bacon (Cooked)—Not to yield more than 40% bacon −60% shrink required. BHA and BHT may be used as antioxidants in precooked bacon at level of 0.01% individually or 0.02% collectively, based on fat content. TBHQ can be used in products as an antioxidant in combination with BHT and BHA, but it cannot be used alone except in cooked bacon.

Bacon Dressing for Stuffing

The product must contain at least 8% bacon.

Bacon-like Products

Bacon-like products, including poultry bacon, labeled with "bacon" in the name must follow the

same requirements as those applied to pork bacon. These requirements include, but are not limited to, limits on restricted ingredients and the requirement that the bacon must return to green weight.

Beef bacon is a cured and smoked beef product sliced to simulate regular bacon. It is prepared from various beef cuts and offered with a variety of coined names, including "Breakfast Beef," "Beef Bacon," etc. A common or usual name is required, e.g., "Cured and Smoked Beef Plate," and should be shown contiguous to the coined name.

Poultry bacon products are acceptable and may be designated as (Kind) Bacon. However, a true descriptive name must appear contiguous to (Kind) Bacon without intervening type or design, in letters at least one-half the size of the letters used in the (Kind) Bacon, and in the same style and color and on the same background. An example of an acceptable designation is "Turkey Bacon-Cured Turkey Breast Meat-Chopped and Formed."

The descriptive name can serve alone as the product name.

Bacon Products

The bacon products intended for further cooking before consumption, i.e., slab bacon for deli slicing, can be labeled "certified," "roasted," or "partially cooked" provided the product is cooked to 148°F and the labeling clearly indicates the product is intended to be further cooked before consumption.

Bangers

A sausage-like product prepared with meat and varying amounts of rusk or other cereals. The label must show percentage of rusk (or other cereal) adjacent to product name in prominent lettering. May be labeled British, Scottish, or Irish Style.

Barbecue (BBQ) Products

Barbecue (BBQ) products that are composed of uncured red meat products that are injected, massaged, tumbled, etc., and which are cooked back to or below the weight of the raw meat product (green weight), must use the term "seasoned" or "flavored," in conjunction with the meat product in the product name, e.g., "BBQ Seasoned Pork," or "Sliced Seasoned Beef with Barbecue Sauce."

The labeling for uncured red meat products containing some solutions that are used to make BBQ products (9 CFR 319.312 or 319.80), which are not cooked back to green weight or are not in compliance with the cooking yield must have a containing statement on the label. A containing statement is required in the product name when the cooking yield is not met, e.g., "BBQ Pork Containing up to 15% of a solution." Similarly, a containing statement is required in the product name when the product does not have sufficient quantities of meat minus the solution to meet the minimum meat requirement. However, in limited situations when the minimum meat requirement (minus the solution) is met and when cook yield is compensated for by adding additional meat, the containing statement can either be placed in the product name or attached to the meat component in the ingredients statement, e.g., "Ingredients: Beef Containing up to 25% . . .sugar, spices."

Red meat components that contain binders and extenders and do not meet one of the barbecue standards (9 CFR 319.80, 319.312) should be descriptively labeled to include the extender, nomenclature in the product name, e.g., "BBQ Seasoned Beef, Modified Food Starch and Gelatinized Wheat Starch," "Pork and Binder Product with Barbecue Sauce," or "BBQ Cooked Beef and Binder Product" followed by a parenthetical list of all of its ingredients. Bone-in red meat products do not have to comply with federal meat regulation 9 CFR 319.312 or 319.80 with regard to cooking yield and must indicate the presence of bones in product name, e.g., "Seasoned Cooked Pork Ribs with Barbecue Sauce" or "Barbecue Beef Ribs."

When bone-in red meat products are injected, massaged, tumbled, etc., and do not return to green weight after cooking, the containing statement should appear once on the label (1) in the ingredients statement as part of the red meat component (only if there is enough beef ribs without solution to meet the requirement for "Beef Ribs and BBQ Sauce"), or (2) in the product name, e.g., "Beef Ribs, containing 10% of a solution and BBQ Sauce."

Barbecue (Infrared Cooked)

The label must indicate heat source, e.g., "infrared cooked," with lettering no less than one-half the size of the largest letter in the word "barbecue."

Barbecue Meat or Poultry "Eastern North Carolina Style"

Acceptable identification for a product that is enhanced in a vinegar-based solution, apple or white. The solution is seasoned with pepper, i.e., black pepper, red pepper, or cayenne pepper. Other ingredients may include salt, sugar, and hot pepper sauce.

Barbecue Sauce with Chicken

The product must contain at least 15% cooked chicken meat. Changing the size of the term "chicken" does not change the 15% cooked chicken meat requirement.

Barbecue Sauce with Meat

The product must contain at least 35% cooked meat. When the name of the product shows meat in smaller letters, not more than one-half the size of the largest letter in the product name, 25% cooked meat is required.

Beef a La King

The product must contain at least 20% cooked beef.

Beef a La Mode

A product consisting of sliced beef (marinated in wine, cognac, vegetable stock) with carrots, onions, and other ingredients covered with wine sauce. The product must contain at least 50% beef.

Beef Almondine with Vegetables

The product must contain at least 18% cooked meat on the ready-to-serve basis. The product must contain almonds.

Beef and Dumplings with Gravy

The product must contain at least 25% meat and not more than 25% water-blanched dry dumplings.

Beef and Gravy

The product contains at least 50% cooked beef.

See: Gravy and Beef.

This is an acceptable ingredient for beef patties provided the product name is qualified, such as "Beef and Blood Patties" or "Beef Patties with Blood."

Beef Blood Glaze

A coating of beef blood is permitted on cured products (e.g., ham, hamette, etc.) if the product name is prominently qualified to reflect the coating. Nitrite is not permitted in the glaze.

Beef Brisket (Canned)

The minimum brine concentration required is 5.5%.

Beef Burgundy or Bourguignonne

The product must contain at least 50% beef. Product contains beef cubes, mushrooms, onions, and red wine or burgundy gravy. May include other vegetables, e.g., carrots, shallots, tomato paste, or potatoes. Other acceptable names include "Boeuf A La Bourguignonne," "Beef Burgundy Style," "Beef Burgundy," and "Burgundy Beef."

Beef Burgundy with Noodles

The product must contain at least 50% beef in the beef burgundy portion. Total product should not contain more than 50% cooked noodles.

Beef Cheek Meat and Beef Head Meat and Pork Cheek Meat and Pork Head Meat (Use and Labeling as an Ingredient in Meat Food Products)

Beef cheek meat and pork cheek meat refers to beef and pork cheeks from which the glandular material has been removed.

Beef head meat and pork head meat refer to muscle tissue remaining on the beef and hog skull after removal of the skin, cheeks, tongue, and lips. The meat normally attached to and considered as part of the tongue trimmings when detached from the tongue trimmings may also be included as beef head meat or pork head meat although it can be labeled as "beef" or "pork."

When beef cheek meat and/or beef head meat are included in boneless beef, its presence must be specifically declared. Examples include "Boneless

Beef—Contains Beef Cheek Meat and Beef Head Meat," "Boneless Beef Head Meat," "Boneless Beef—Ingredients: Beef, Beef Head Meat, Beef Cheek Meat," or "Boneless Beef—20% Beef Head Meat, 15% Beef Cheek Meat."

Beef cheek meat and/or beef head meat may be used in unlimited quantities and identified as "beef" in meat food products unless restricted by regulatory standards for specific products as indicated in 9 CFR 319.15(a) (chopped beef, ground beef), 319.15(b) (hamburger), 319.15(d) (fabricated steak), 319.81 (roast beef parboiled and steam roasted), 319.100 (corned beef), 319.300 (chili con carne), 319.301 (chili con carne with beans), and 319.303 (corned beef hash).

The presence of pork head meat is not required to be identified on the labeling of boneless pork. However, pork cheek meat and/or pork head meat may be used in unlimited quantities and identified as "pork" in meat food products, unless restricted by regulatory standards as indicated in 9 CFR 319.300 (chili con carne), and 319.301 (chili con carne with beans).

Beef Concentrate and Salt

Broth derived from cooking fresh beef containing 3% to 4% solids is centrifuged and evaporated to approximately 60% solids under vacuum. The water fraction is salted to a level of 25.5% of the water weight (100 pounds concentrated stock at 60% will have 10.2 pounds of salt added, making a total weight of 110.2 pounds). There is no need for refrigeration.

Beef Consommé

The standard requires beef as an ingredient and a minimum protein content of at least 3% in the finished product.

"Beef stock" or "beef broth" (or mixture of both) may be used to comprise the beef ingredient. Additional optional ingredients are gelatin, beef extract, tomato puree, hydrolyzed plant protein, and seasoning.

Beef (Dried or Air Dried)

Product name is "Air Dried Beef" or "Dried Beef" (Moisture Protein Ratio [MPR] 2.04:1). It is usually cured by rub and/or stitch pump followed by cover pickle for 4 to 8 weeks with several overhauls (turned over for the application of additional cure), then placed in smokehouse or drying chambers for 3 to 10 days.

Beef Fibrin

This is a component mixture of beef fibrinogen and beef thrombin plasma protein used to bind pieces of meat or poultry together. It is limited to 10%.

1. If used from 7 % to 10%, it must appear in the product name, e.g., "Bacon Wrapped Beef Tenderloin Steak Formed with Beef Fibrinogen and Thrombin." Therefore, the smallest letter in the product name must be at least one-third the size of the largest letter in the product name.
2. If used at less than 7%, it must be a product name qualifier, e.g., "Formed with Beef Fibrinogen and Thrombin." As a product name qualifier, there is no size requirement, however, it must be contiguous to the product name and be prominent and conspicuous. Additionally, the terms "Beef Fibrin" or "Fibrin" may be used in the product name as a qualifier and its components identified elsewhere on the principal display panel. In this situation, the terms "Beef Fibrin" or "Fibrin" and its components are linked to each other by means of asterisks. Acceptable terminologies for the components are "Beef Fibrinogen and Thrombin Plasma Protein" or "Beef Fibrinogen and Thrombin."

Beef Gravy Mix

The product must contain at least 15% dried beef.

Beef Marsala

The product must contain at least 50% beef. Product contains beef cubes, Marsala wine sauce, and usually mushrooms and onions. White wine may be used, but it may not replace Marsala wine.

Beef Oriental or Oriental Beef

The product must contain at least 12% meat and oriental-style vegetables and sauce. The label must show true product name, e.g., "Beef Oriental with Vegetables."

Beef Roulade

The product must contain at least 50% cooked meat. Usually a thin strip of flank meat wrapped around vegetables and cooked.

Beef Slices A-La Pizzailoa

The product must contain at least 50% cooked beef.

Beef Stroganoff

A dish with a creamy sauce prepared with beef cut into narrow strips or cubes and sautéed. Product labeled "Beef Stroganoff" should be prepared with a formula, which includes at least 45% beef, or 30% cooked beef.

1. The product must contain at least 10% sour cream, or
2. 7.5% sour cream, and 5% wine, or
3. 9.5% whole milk, 2% sour cream, and 2½% wine.

Beef Stroganoff with Noodles

Meat and sauce portion must meet the standard for Beef Stroganoff. Total product should contain no more than 50% cooked noodles.

Beef Sukiyaki

The product must contain at least 30% meat based on total product. Consists of thinly sliced beef and various vegetables cooked in a flavored beef stock. This is not a stew as the vegetables and components are mixed during the cooking process. Vegetables used with this food are celery, bean sprouts, leeks, onions, mushrooms, Chinese cabbage, carrots, spinach, water chestnuts, bamboo shoots, and bean curds.

Beef Tripe Stew

There are two versions of this product. One is of Mexican origin and merchandised in association with the term "Menudo."

Corn is a prominent ingredient in its formula. The standard for an item of this nature requires that it contain not less than 33% beef tripe computed on the basis of the uncooked tripe in relation to total ingredients.

The second product is popular in Puerto Rico. It is referred to as "Mondungo." The product is made with 25% raw beef tripe. The remainder consists principally of potatoes, a squash with pumpkin-like appearance and flavor, and a native vegetable called "Tanier." When the vegetables are not distinguishable, this product can be labeled as "Dominican Style Mondungo."

Beef Wellington

It is made with beef tenderloin that is roasted very rare. It is then spread with a liver paté, covered with pastry, and baked in a hot oven until pastry is brown. The product must contain at least 50% cooked meat and no more than 30% pastry.

Alternatively, mushroom duxelles is an acceptable substitute for liver paté, but a true descriptive product name is required, e.g., "beef tenderloin covered with mushroom duxelles and wrapped with pastry."

Beerwurst, Bierwurst

A cooked smoked sausage. Same requirements as beef salami, with the exception that pork may be used.

Berliner

A cooked smoked sausage usually made from coarsely cut cured pork in large casings. When beef is used, it should not exceed 50% of the meat block. Pork stomachs or beef tripe not permitted.

Berliner Blood Sausage

A cooked blood sausage containing diced bacon. After cooking, it is dried and smoked. Ham fat, snouts, and lips are not permitted.

See: Blood Sausage.

Bier Schinken (GR)

The literal translation is "Beer Ham." If product is made of all pork, it may be labeled "Bier Schinken."

Binders in Poultry, Boneless, Raw or Cooked

Binding agents may be added individually or collectively in amounts not to exceed 3% for cooked

poultry products and 2% for raw poultry products based on total finished product. When binders are added in excess of these levels, the common or usual name of the binder or the generic term "Binders Added" should be included in a product name qualifier, e.g., "Turkey Breast—Gelatin Added." In all cases, the presence of these ingredients must be shown in the ingredients statement.

This policy is intended to apply to binders that are used in chopped or chunked poultry products that are formed into rolls, loaves, etc., but not to binders added directly into whole muscle by injection, massaging, tumbling, etc., which then act as extenders.

Blockwurst

A semidry type sausage. The maximum MPR is 3.7:1.

Blood and Tongue Sausage

Same as blood sausage, except cured and cooked pork or beef tongues are used.

Blood Sausage

A cooked sausage formulated with blood and some meat. Usually contains pork skins and/or pork jowls. May also contain sweet pickled ham fat, snouts, and lips. If the product does not contain meat, it must be labeled as "Blood Pudding."

Boingghetti

This label must show a true product name, "Spaghetti with Chicken Sauce." The product must contain at least 6% cooked chicken meat.

Bone-In Meat with Sauce

Must have at least 50% meat (cooked basis). Product with barbecue sauce must comply with 9 CFR 319.312.

Boneless Breast Trimmings

Boneless breast trimmings (turkey or chicken) are defined as trimmings that are removed from the breast portion only. When a product is formulated with boneless breast trimmings, the amount of skin should be indicated in order to determine that the meat requirement is met for a standardized product and that the product is properly labeled. Trimmings from the ribs may be identified as white turkey or white chicken trimmings, or white turkey or white chicken rib meat (excluding skin).

Breakfast Links or Patties

The names "Breakfast Links" and "Breakfast Patties" can be considered fanciful names, which must be followed by a descriptive product name. Such products are acceptable without compliance with the fresh pork sausage or breakfast sausage standard. If the names "Breakfast Links" or "Breakfast Patties" are used without further qualification, the products must meet either the fresh pork sausage standard or the breakfast sausage standard.

Breakfasts (Containing Meat)

The product must contain at least 15% cooked meat or poultry or meat or poultry food product based on the total net weight of breakfast.

Broth, Beef, or Pork

No distinction has been made between "broth" and "stock." They may be used interchangeably as the resulting liquid from simmering meat and/or bones in water with seasonings. Both products have an MPR of 135.1 or a 67.1 MPR for concentrate.

Brotwurst

A cured and cooked sausage that may be smoked.

Brown and Serve Sausage

The standard is based on one of the four options as listed below:

1. MPR is no more than 3.7:1, fat limited to 35%, and 10% water at formulation.
2. No more than 10% added water at formulation and a yield of no greater than 80%.
3. No more than 8.8% added water at formulation and a yield no greater than 85%.
4. Product must meet fresh sausage standard before cooking. The label must show true product name, e.g., "Brown and Serve Pork Sausage."

Brown and Serve Sausage (Canned)

A cooked sausage, usually without cure, and not more than 8% water. The weight of the sausage at canning should not exceed the weight of fresh uncured meat ingredients plus weight of curing and seasoning ingredients.

Brunswick Stew

The product must contain at least 25% (fresh basis) of at least two kinds of meat, one of which may be poultry. Product must contain corn as one of the vegetables.

See: Poultry, Brunswick Stew.

Buffalo Style

Buffalo style is acceptable on any product (e.g., poultry parts other than wings, chicken patties, nuggets, etc.) that is cooked and coated with a mild or spicy sauce containing cayenne red pepper, vinegar, salt, and garlic. It is no longer exclusive to chicken wings only. It would also be acceptable on any product made in Buffalo, NY.

Burgundy Sauce with Beef and Noodles

The product must contain at least 25% cooked beef in the product, with up to 20% cooked noodles. Product must contain enough wine to characterize the sauce.

Burritos

A Mexican-style sandwich-like product consisting of a flour tortilla, various fillings, and at least 15% meat or 10% cooked poultry meat. The flour tortilla is rolled and may or may not have tucked ends. Fillings may contain, in addition to meat or poultry meat, such major ingredients as beans, potatoes, cheese, rice, tomatoes, and chilies.

Examples of product names are "Beef Burrito," "Turkey Burrito," "Chicken Fajita Burrito," and "Chili Verdi with Beans Burrito." If ingredients, e.g., rice or beans, are declared in the product name, they must appear in the proper order of predominance. Ingredients cannot be mentioned in the product name unless all other ingredients present in amounts equal to or above the declared ingredient are included in the name, e.g., "Beans, Beef, Tomato, Onion, and Rice Burrito."

The use of "Red Chili" or "Green Chili" or a similar designation of the chili content in a starburst, flag, or similar display, separated from the product name, is acceptable. If such designations are used as part of the descriptive name, the presence of the chilies must appear in the correct order of predominance, and all other ingredients present in amounts equal to or greater than the chilies must appear in the product name.

A claim or name that identifies the use of shredded meat or shredded poultry meat is permitted. However, if ground meat or ground poultry meat is also used, its presence must also be identified in the claim or name, e.g., "Shredded Beef and Ground Beef Burrito."

"Burrito" alone may be used to name the product without a descriptive name. However, the ingredients statement must appear directly beneath "burrito."

Burritos with Sauce or Gravy

Product must contain at least 50% burritos.

Butifarra Sausage

An uncured sausage. Labeling that features the term "Butifarra" would require an additional product name:

Pork Sausage—for those products that meet the fresh pork sausage standard.
Fresh Sausage—for those products that include by-product but do not meet the standard for pork sausage.
Sausage—for those products that are incubated or fermented.

The term Puerto Rican Style would be applicable if manufactured in Puerto Rico.

Caddies

Caddies or display cards used to display fully labeled product should not bear an inspection legend and, therefore, can be reused. The caddies or display cards may contain a picture of a product that has a legend on it.

Cajun

Refers to product made in Louisiana.

Cajun-Style/Cajun Recipe

Acceptable identification for products containing onion/onion powder/dehydrated onion, garlic/garlic powder/dehydrated garlic, white pepper, red pepper, and black pepper.

Calabrese (IT)

A salami originating in southern Italy. Usually made entirely of pork seasoned with hot peppers.

Calzone, Calzoni (IT)

Turnover-like product made with dough stuffed with meat or poultry, cheese, and seasonings and baked. It must contain 25% meat or 14% poultry meat. The label must show a true product name, e.g., "Sausage and Cheese Calzone."

Canadian and Canadian Style Bacon

"Canadian Bacon" and "Canadian Style Bacon" are synonymous and should not be considered geographical terms.

The term "Canadian Style Bacon," when featured on the label as a product name or part of a product name (i.e., as a description, etc.), may stand alone without an additional qualifier indicating the true geographical origin of the product.

"Chunked and Formed" and "Water Added" products are permitted, provided proper labeling is applied.

Uncooked and/or unsmoked "Canadian Style Bacon" is also permitted, provided labeling describes the product as uncooked and/or unsmoked.

Product that is identified as "Canadian Style Bacon" is made from a trimmed boneless pork loin. On the shoulder end, the cross section of the longissimus dorsi muscle should be equal to or larger than the combined cross sectional areas of the splenius and semispinalis capitis muscles. The ham end should be removed anterior to the ilium. The exposed faces should be approximately perpendicular with the skin surface. The dorsal and ventral side on each end of the "Canadian Style Bacon" should not be more than 1.0 inch different in length. The belly is removed adjacent to the longissimus dorsi muscle. All bones and cartilage should be removed. The tenderloin and the flesh overlying the blade bone are excluded. The surface fat (and false lean when necessary) should be trimmed to 0.3-inch thick at any point. The fat on the ventral and dorsal sides is neatly beveled to meet the lean.

Canadian Style Bacon Made with/from Pork Sirloin Hips

The sirloin is obtained by removing a 5- to 7-inch section of the pork loin immediately in front of the hip or pelvic bone. The sirloin hip is obtained by removing the half of the sirloin that comprises the posterior end of the pork loin. The tenderloin is not included and surface fat should be trimmed to 0.3-inch thickness.

The labeling for these Canadian Style Bacon products must bear a qualifying statement, adjacent to the product name, clarifying that pork sirloin hips are included or that the product is made entirely from pork sirloin hips, e.g., "Canadian Style Bacon—Includes Pork Sirloin Hips" or "Canadian Style Bacon—Made from Pork Sirloin Hips." The smallest letter in the qualifier should not be less than one-third the size of the largest letter in the product name. The qualifier must be of equal prominence to the product name.

Chunked (or chopped) and formed varieties and substances controlled by the protein fat free (PFF) regulation for cured pork products 9 CFR 319.104 should be labeled in accordance with applicable guidelines.

Use of this type of product in a secondary product, e.g., a pizza, requires complete identification only in the ingredients statement; the product name of the secondary product need only refer to Canadian Style Bacon, e.g., Canadian Style Bacon Pizza.

Canned Chopped Beef or Pork

Cured product with no more than 3% water in formula.

Canned Meat

"Canned meat with Natural Juices" is acceptable for product that has been pumped or contains up to 10% of a solution before canning and processing. Processed canned uncured meat products, when water or broth is added to the can, may not be called "with

natural juices," but the acceptable name would be "with juices."

Cannelloni (IT)

Product must contain at least 10% meat or 7% cooked poultry meat. Cannelloni is an Italian term referring to a product with the same characteristics as "Ravioli" except Cannelloni has a tubular form. The product name should show the type of species, e.g., "Beef Cannelloni."

Cantonese Style Species

Marinated in a solution of soy sauce, cooked and returned weight. In addition, product is mildly seasoned with sugar, salt, wine, and spices.

Capacolla, Cooked (Capicola, Capocolla, Capacola, Capicollo, Cappicola, Capacolo) (IT)

Boneless pork shoulder butts that are cured and then cooked. The curing process may be dry curing, immersion curing, or pump curing. The cured product is coated with spices and paprika before cooking. This product should always be labeled with "Cooked" as part of the product name. Water added is permitted.

Caramel Coloring

Caramel is considered a natural color. However, when caramel coloring is added to a product, the product name must be qualified to indicate the presence of artificial coloring, e.g., "Cooked Roast Beef—Caramel Coloring Added" or "Artificially Colored." This requirement does not apply to gravies, sauces, and similar products where the use of such coloring is customary. Seasoning mixes containing small quantities of caramel coloring may be used if the caramel coloring does not impart color to the finished product.

Caramel coloring may be used on the surface of raw products, e.g., beef patties, if the name is appropriately qualified. However, caramel coloring may not be added directly to the formulation of a raw product where the caramel coloring becomes an integral part of the total product.

Carbonade (FR)

Product must contain at least 50% meat. It may contain beef, pork, or mutton, and beer or wine. Product is slowly cooked, either by braising or stewing.

Label must show a true product name, e.g., "Beef Carbonade."

Carriers

Substances, as defined by the Food and Drug Administration, that carry flavoring compounds, e.g., essential oils, on their surface, and are not expected to provide a functional effect, e.g., binding and emulsifying, in the finished food product and are considered incidental. Some substances, e.g., maltodextrin and modified food starch, are not carriers but actually diluents or bulking agents, and must be declared in the ingredients statement.

Dextrose and/or sugar are commonly used as carriers for spice extracts and resins of spices. The carrier must be declared in the ingredients statement, except in those cases where a sweetening agent is used separately in formulating the meat or poultry product and the use of the spice mixture will not result in the quantity of the carrier being more than 0.75% of the seasoning mix. When a determination cannot be made from the information on the label application, declaration is required.

Salt, when used as a carrier, will always be declared regardless of amount used.

Casing, Artificial

Frankfurters packaged in retail containers with the artificial casing left on must bear a prominent statement, e.g., "Remove casing before eating," contiguous to the product name on the label.

Casserole

Product must contain at least 25% meat or 18% cooked meat.

Cassoulet (FR)

Product must contain at least 25% meat. A complex stew consisting of dried white beans and a combination of pork, lamb, game, and sausages. The ingredients are cooked, then put into a casserole, usually

covered with crumbs, and baked. Label must show true product name, e.g., "Beans and Bacon in Sauce."

Center Slice

When the term "Center Slice" is used on labels for slices of ham from smoked and cooked, smoked, or water cooked hams, product must be sliced from an area of the original ham positioned about 1 inch on each side of a center cut.

Certified

With the exception of the term "Certified Pork," the term "certified" implies that the United States Department of Agriculture (USDA) and the Agriculture Marketing Service (AMS) have officially evaluated a meat product for class, grade, or other quality characteristics. When used under other circumstances, the term should be closely associated with the name of the organization responsible for the "Certification" process (e.g., "XYZ Company's Certified Meat," or "Our Certified Meat").

Cervelat

A cured and cooked sausage, often a semidry or dry summer sausage. Hog stomachs, beef tripe, and extenders are permitted. There is no MPR requirement.

Cha Shu Bow (CH)

A steamed bun with a dry roasted pork filling requiring 15% cooked pork. Label must show true product name, e.g., "Steamed Bun with a Pork and Cabbage Filling."

Cheek Meat, Beef

Natural proportions are considered to be 2%.

See: 9 CFR 319.15.

The use of cheek meat is limited to 25% in ground beef, chopped beef, and similar type products. If cheek meat exceeds 2% (natural proportions), its presence must be declared.

Cheese

1. When cheese is declared in the ingredients statement of a fabricated product, cheddar cheese must be used in the product's formulation.
2. Swiss, Gruyere—The term "Gruyere" pertains to a cheese that closely resembles "Swiss cheese" both in its appearance and on analysis, although it has smaller holes than Swiss cheese. FDA advises that Gruyere Cheese is a suitable substitute for Swiss cheese and gives the same character to a finished food product, e.g., "Chicken Cordon Bleu."
3. The term "cheese" may appear in the product name, e.g., "Ham and Cheese Loaf," provided the common name is declared in the ingredients statement.
4. When a cheese product and meat or poultry food product are packaged together, the product name shown on the label must show the name of each component product. For example, if slices of ham and slices of a cheese product are packaged together, the product name should include "Ham" and the name of the cheese product (e.g., Ham and Pasteurized Processed American Cheese). Alternatively, the Pasteurized Processed American Cheese could be parenthetically qualified contiguous to the product name (e.g., "Ham and Cheese" ("Pasteurized Processed American Cheese"). The name "Ham and Cheese" alone would be acceptable if the cheese used was "Cheddar Cheese."
5. Use of substitute or imitation cheese in products where real cheese is expected (e.g., Cordon Bleu) requires the product name be changed or qualified to indicate the presence of the ersatz cheese. Substitute and imitation cheeses cannot be described as "cheese" in the product name. There is no limitation on the amount of ersatz cheese used.
6. Reduced fat cheeses may be identified on the label as "Reduced Fat Cheese." However, the name of a standardized cheese may not be associated with the phrase "Reduced Fat Cheese" (e.g., Reduced Fat Cheddar Cheese).
7. Cheese is a standardized product. See: 21 CFR 130.10 and 133 for a listing of standardized cheeses.

Cheese (Pasteurized Processed Cheese Food or Spread)

A cheese food product with a standard of identity, but is not considered a cheese. Therefore, it cannot be used in meat food products where cheese is an expected ingredient, e.g., "Cheesefurters" or "Veal Cordon Bleu." It is acceptable in nonspecific loaves, etc.

Cheese Products Containing Meat

Homogeneous cheese products, e.g., cheese balls, must contain more than 50% meat to be amenable. When cheese and meat are separate components, the products are amenable with 2% meat.

Cheese Standardized Products

Cheese standardized products that require real cheese, e.g., chicken cordon bleu, must use FDA standardized cheese or those FDA standardized cheeses specified. Use of a substitute, imitation cheese, or other non-FDA standard cheeses, if permitted, must be declared in the product name, or a suitable qualifier, e.g., chicken cordon bleu made with reduced fat cheese. The 90/10-cheese rule is only applicable to pizza.

Chicharrones (PR)

The Spanish name for fried pork skins. Product must have an English product name, "Fried Pork Skins" except in Puerto Rico.

Chicharrones de Pollo (PR)

An acceptable product name for "Marinated Cut-up Fried Chicken" sold in Puerto Rico. When product is destined for sale only in Puerto Rico, "Chicharrones de Pollo" can be the product name. When destined for sale in other places, "Chicharrones de Pollo" must be explained with true product name.

Chicken, Aloha

"Aloha Chicken" is acceptable as a coined name, which must be followed by a true product name, e.g., "Chicken and Sauce with Rice." The standard for the product is 22% cooked poultry meat.

Chicken and Noodles Au Gratin (FR)

Product must contain at least 18% cooked chicken meat.

Chicken Cordon Bleu (FR)

Product must contain not less than:

1. 60% chicken breast meat (sliced). If it is made from any other part of the chicken, then the product name must be qualified to indicate the part used.
2. 5% ham or Canadian Style Bacon.
3. Cheese (either Swiss, Gruyere, Mozzarella, or Pasteurized Processed Swiss).
4. Not more than 30% batter and breading (if used).

Chicken Enchilada Suiza

The product consists of chicken enchiladas with a cream sauce. The sauce used must be made with sour cream, heavy cream, or whipped cream in an amount sufficient to characterize the sauce. The label must show a true product name, e.g., "Chicken Enchilada with Cream Sauce."

Chicken Ova

These cannot be used for human consumption without first going to an egg products plant for pasteurization (because of problem with potential Salmonella contamination). Chicken Ova cannot use the poultry inspection legend.

Chicken Paprika

Product must contain at least 35% chicken. A Hungarian dish. Sauce must contain either sour or sweet cream and enough paprika to give a pink color.

Chicken Tocino

Acceptable with a true product name such as sliced, marinated, cured chicken thigh meat.

Chicken Wellington

It is made with roasted chicken that is spread with liver paté, covered with pastry, and baked in a hot oven until pastry is brown. The product must contain at least 59% cooked meat and no more than 30% pastry.

Chili

1. "Brick Chili" or "Condensed Chili" requires 80% meat. Cereal is limited to 16%.
2. Chili with reconstitution directions should meet the chili standard when reconstituted.

3. When beef heart meat, cheek meat, or head meat is used in excess of 25% of the meat block, it must be reflected in the product name, e.g., "Chili with Beef and Beef Heart Meat."
4. When beef appears in the product name, **Beef May Be The Only Meat Source Used**. Beef Chili may not contain beef fat or other beef by-products.
5. "Chili Gravy with Meat" requires at least 40% fresh meat and no more than 8% cereals.
6. Cured meat is not an expected ingredient in chili; when used, it must be shown as part of the product name.
7. The terms "Chili" or "Chili con Carne" may be used interchangeably.
8. Since "con carne" means "with meat," products labeled as chili con carne should include only red meat and not poultry. Products that meet the chili standard and include poultry may be labeled "beef and chicken chili," "beef chili, chicken added," etc., as appropriate. The binder and extender limitation of 8% is based on total formulation.

See: 9 CFR 319.300.

Chili Colorado

Product must meet 9 CFR 319.300 requirements. Chili peppers must be exclusively of the red variety. If a prepared chili powder is used, it must be prepared exclusively from red chili peppers.

The term "Colorado" is used for red more than "Rojo" in Mexico. The term "Rojo" is used more in Spain, Puerto Rico, and Cuba.

Chili Mac

Product must contain at least 16% meat. The label requires a true product name, e.g., "Bean, Macaroni and Beef in Sauce."

Chili Pie

Chili component of the total product must have at least 40% fresh meat.

Chili Pups

An emulsion stuffed in casing and smoked. Label requires a true product name, e.g., "Chili con Carne and Ground Beans Product." Product must contain at least 60% fresh meat in total formulation.

Chili Relleno

Product must contain at least 12% fresh meat and be coated with a batter and then fried. Sometimes product is called "Chili Pepper Relleno." Relleno means stuffed.

Chili Spaghetti

Product must contain at least 16% meat.

Chili Verde (SP)

Product must meet 9 CFR 319.300 requirements. Chili peppers must be exclusively of the green chili or Verde chili pepper varieties. If a prepared chili powder is used, it must have been prepared exclusively from green chili or Verde chili peppers. Products, e.g., "Chili Verde with Beans" should comply with 9 CFR 319.301 and the above requirements for "Chili Verde."

Chili with Beans

1. "Brick Chili with Beans" or "Condensed Chili with Beans" requires 50% meat and cereal is limited to 16%.
2. Chili with Beans with reconstitution directions should meet the Chili with Beans standard when reconstituted.
3. When beef heart meat, cheek meat, or head meat is used in excess of 25% of the meat block, it must be reflected in the product name, e.g., "Chili with Beef and Beef Heart Meat with Beans."
4. When beef appears in the product name, beef may be the only meat source used. Beef Chili with Beans may not contain beef fat or other beef by-products.
5. Cured meat is not an expected ingredient in Chili with Beans; when used, they must be shown as part of product name.
6. "Chili with Beans" formulae usually contain up to 25% of beans in a product. About one-fourth of these beans may be incorporated in the product as ground beans and should be listed in the ingredients statement as ground beans.

7. The terms "Chili with Beans" or "Chili con Carne with Beans" may be used interchangeably.
8. The binder and extender limitation of 8% is based on total formulation. See: 9 CFR 319.301.

Chimichanga

Product must contain at least 15% meat or 10% poultry meat. A Mexican specialty from the state of Sonora. Like burritos, product is made by wrapping a flour tortilla around a filling; but unlike the burrito, chimichanga is fried until brown and crisp. "Fried Burritos" is acceptable.

Chinese Brand Links

Raw nonspecific sausage-like products. These products are permitted to contain artificial red coloring; however, if pork is used it must be certified. Unlike the term "links," "Chinese Brand Links" is considered a coined or fanciful name, and [as a] nonspecific product, it must be accompanied by an ingredients statement. Furthermore, "made in USA" must be contiguous to the word "brand" but cannot intervene between "links" and the ingredients statement.

Chinese Pepper Steak

A Chinese main dish, usually served with rice, must contain at least 30% cooked beef. Beef steak is cut into thin strips, browned in fat or oil, and added to a soy flavored sauce. Vegetables are also added to the sauce. Green pepper strips are always used and other vegetables may be included.

Chinese Style Barbecue Meat

Acceptable identification for a product that is enhanced in a solution with soy sauce, grain alcohol or dry sherry wine, and a sweetener, i.e., sugar or honey. Other ingredients may include garlic or scallions, ginger or ginger juice, and sesame or peanut oil. The product may be artificially colored. If artificially colored, a qualifier is needed.

Chinese Style Beef

Product must contain grain alcohol and soy sauce.

Chinese Style Sausage

Product must contain grain alcohol and soy sauce.

Chipped Beef

Beef that is dried, chipped, or sliced, and may be cured or smoked. An MPR 2.04:1 is required. It may be chunked, ground, chopped, and formed. If so, the product name must be qualified, e.g., "Chipped Beef, Chunked and Formed."

Acceptable fill:

1. 2 oz. in a 4 fluid oz. glass, or
2. 2 1/2 oz. in a 5 fluid oz. glass, or
3. 5 oz. in a 9-5/8 fluid oz. glass.

Chitterlings

Approved label must identify the species of food animal from which the product is derived. Hog bungs may be labeled "Pork Chitterlings." The purge under normal conditions should not exceed 20% of the net weight of frozen chitterlings.

See: 9 CFR 317.8(b)(30).

Choice Grade, Fancy Grade Poultry

"Choice" or "Fancy" may not be used in conjunction with "Grade" on poultry labels. These terms and others like "Prime" and "Top Quality" on poultry labels indicate only that product is equal to U.S. Grade A.

Chopped Chicken Livers

Total product must contain at least 50% cooked chicken livers. Wheat flour and similar ingredients are acceptable.

Chopped Chicken Livers Combined with Other Characterizing Components

Product must contain at least 30% cooked livers, e.g., "Chopped Chicken Livers with Eggs and Onions."

Chopped Ham

A total of 15% shank meat is permitted. This is 3% above the normal proportion of 12% shank meat found in a whole ham.

See: 9 CFR 319.105.

Chop Suey, American

Product must contain at least 25% fresh meat in total formulation. A stew-like dish prepared with beef, pork, or veal. Vegetables include onion and celery. Macaroni, noodles, or rice are usually incorporated in the product, although recipes suggest serving chop suey over one of these.

Chop Suey (Vegetables with Meat)

Product must contain at least 12% fresh meat.

Chorizo (SP)

The product name "Chorizo" can be used for any type of chorizo sausage that is cooked, dry, semi-dry, cured, and fresh without further product name qualification. Other requirements for various types of chorizo apply, including the sausage standard. It is seasoned with Spanish pimento and red pepper.

Partially defatted pork fatty tissue is acceptable in chorizo. Wine is considered a flavoring and need only appear in the ingredients statement. However, the liquid is credited as added water.

Chorizo, Fresh

These products may contain vinegar. The vinegar used must have a strength of no less than 4 grams of acetic acid per 100 cubic centimeters (20°C).

See: 9 CFR 318.7(c)(1).

Chorizo in Lard

Product must contain at least 55% chorizo.

Chorizo in Lard (Canned)

Canned chorizos that are packed hot, usually in lard, and are not thermally processed must have an MPR of 1.8:1 and a pH of not more than 5.5. An alternative standard is a water activity (Aw) of 0.92.

Chow Mein with Meat

Product must contain at least 12% fresh meat.

Chulent (Cholent)

Product must contain at least 25% fresh meat. A meal-in-one dish of Jewish cuisine made in various ways. The product name can stand without qualification.

Coarse Ground Meat Trimmings

Coarse ground trimmings may be shipped from an establishment without meeting the 30% fat limitation if a specific fat content is declared, e.g., "Coarse Ground Beef Trimmings—40% fat beef." If the labeling terminology is "Coarse Ground Beef" or "Ground Beef," the 30% fat limitation should apply.

Colored Casing

Colored casings on meat and poultry products that do not transfer color to the product, but that change and give a false impression of the true color of the products, must be labeled to indicate the presence of the casings. Acceptable terminology includes "Casing Colored" or "Artificially Colored." These phrases must appear contiguous to the product name.

Casings that are the same color as the product and not misleading or deceptive, e.g., a white opaque casing on a summer sausage, do not have to be so labeled. Also, products consisting of whole muscle bundles, e.g., hams, pork butts, etc., packaged in colored wrappings where a cut surface is not visible through the casing are exempt. The color agent must be specifically identified on the label either in the product name qualifier or ingredients statement.

See: 9 CFR 319.15(d).

Composite Ingredients Statement

Processors who use a multi-ingredient product, e.g., pepperoni from various sources, as an ingredient, may identify all the ingredients that may be present from all the various formulations (i.e., a composite ingredients statement). However, the ingredients identified as those that may be present can only be those ingredients that are minor in nature and cannot include ingredients, e.g., the meat component that have a bearing on the overall characteristics or value of the product. The minor ingredients must be identified using one of the following examples of acceptable formats:

1. Pepperoni (pork, beef, water, salt, spices, sodium nitrite, and may also contain lactic acid starter culture, sugar, and sodium ascorbate)
2. Bacon bits (cured with water, salt, dextrose, and/or sugar, sodium nitrite)

3. Pepperoni (pork, beef, water, sweeteners [contains one or more of the following: sugar, dextrose, fructose, corn syrup], salt, spices, sodium nitrite)

Labeling records must identify all of the ingredients of each type of component that is used so the accuracy of the composite ingredients statement can be determined. All labeling for meat and poultry products must either comply with this type of format or, alternatively, accurately list all ingredients used in the product.

Cooked Beef, Equivalency

In lieu of fresh beef, a 70% yield figure is used if no yield information is provided.

Cooked Breakfast Sausage

Antioxidants are permitted when product is formulated on a raw basis (no more than 3% water).

Cooked Red Meat Products Containing Added Substances

Cooked corned beef products and cooked cured pork products not addressed by the cured pork products regulation (9 CFR 319.104), that weigh more than the weight of the fresh uncured article, may be prepared if they are descriptively labeled to indicate the presence and amount of the additional substances. Acceptable product names include "Cooked Corned Beef and X% Water" or "Cooked Cured Pork and Water Product, X% of Weight is Added Ingredients," and "Cooked Pastrami and Up to 20% of a Solution." The ingredients of the solution may accompany the product name or appear in locations prescribed for ingredients statements. Product name prominence guidelines are found in Policy Memo 087A and Policy Memo 109. If product name qualifiers, such as "X% of Weight is Added Ingredients," are used, the labeling prominence guidelines used for cured pork products as found in 9 CFR 319.104(b) apply.

Uncured red meat products that weigh more than the weight of the fresh article after cooking should be labeled with a qualifying statement indicating the amount of solution remaining after cooking, e.g., "After cooking, contains X% of a seasoning solution of . . ." The ingredients of the solution may accompany the qualifying statement or appear in locations prescribed for ingredients statements. The qualifying statement must be one-fourth the size of the largest letter in the product name. If the ingredients of the solution accompany the qualifier, they must appear in print one-eighth the size of the most prominent letter in the product name. Other labeling prominence guidelines are found in Policy Memo 087A.

If cooked, uncured red meat products that contain added solutions/substances prior to cooking are cooked back to or below the weight of the fresh (green weight) article, words, such as "seasoned" and "flavored," are to be used to reflect the addition of the added substances, e.g., "Seasoned Cooked Beef."

For cooked products, the percent added substances for the label statement is determined by subtracting the fresh (green) weight of the article from the weight of the finished cooked product (i.e., after injecting, marinating, etc., and cooking), dividing by the weight of the finished product, and multiplying by 100.

This policy is intended to apply to solutions that impart favorable flavor and other sensory characteristics, but not to solutions containing ingredients used to extend a product, such as isolated soy protein and carrageenan.

Uncooked red meat products containing added substances are addressed in Policy Memo 066C.

Corn Dog or Korn Dog

A coined name that must be accompanied by a true product name, e.g., "Batter Wrapped Franks on a Stick." Product is limited to 65% batter and a minimum of 35% frankfurter.

Corn Dog or Korn Dog (Poultry)

"Corn Dogs" made from poultry cooked sausage, e.g., poultry franks or poultry frankfurters, must show the "kind" of poultry used in conjunction with the coined name "Corn Dogs," e.g., "Chicken (or Turkey) Corn Dogs." The "kind" name should be shown in type size at least one-third the size of the largest letter of the coined name. A descriptive name, e.g., "Batter Wrapped Chicken Frank on a Stick," must accompany the coined name. If the descriptive name is at least one-third the size of the coined name, the "kind" name need not precede the coined name.

Corn Meal Mush with Bacon

Product must contain at least 15% cooked bacon.

Corned Beef and Cabbage

Product must contain at least 25% cooked corned beef.

Corned Beef (Canned, Cooked with Natural Juices)

Canned product labeled "Cooked Corned Beef with Natural Juices," is limited to 10% added solution before cooking. If the added solution is greater than 10%, the label must indicate the total added solution, e.g., "Cooked Corned Beef and Water product—X% of weight is added ingredients."

See: Cooked Red Meat Products containing Added Substances.

Corned Beef, Gray

Gray corned beef is not a cured product but one that contains water, salt, sugar, flavorings, etc. It should be labeled as "Gray Corned Beef," "Gray Corned Beef Rounds," etc. The label must show an ingredients statement rather than a curing statement as shown on other corned beef labels.

Corned Beef with Juices

Uncooked corned beef with juices (or without juices and spices) is unacceptable terminology for corned beef products meeting standards in 9 CFR 319.101 and 319.102 for corned beef brisket and corned beef round (and other cuts). The presence of free flowing juices in a package does not change this policy. The net weight includes free flowing juices.

Cornish Style Pasty

Product must contain at least 25% beef. Product consists of a round or square of piecrust with a filling of chopped beef, potatoes, and onions.

Cotechino (IT)

Pork skin sausage. Meat and meat by-products other than pork skin can be used in this product. It could also be given the name of pork skin sausage in parentheses as a common name. Italian sausage. A variety of cooked sausage.

See: 9 CFR 319.140.

Country

A geographical term that refers to an unincorporated area. To use country, the product must be made in the country.

Country Fried

Refers to a fried product that is usually breaded. It is not considered a geographical term.

Country of Origin

Statement, "Product of . . ." need only appear beneath the product name on the Principal Display Panel on imported product.

Country Style Chicken

Cut up chicken in which the wishbone is left whole.

Country Style (Farm Style) Sausage

When sausage products are labeled "farm style" or "country style," they must be prepared with natural spices with the exclusion of oleoresins, essential oils, or other spice extractives. Sugar is the sweetening agent for "farm style" or "country style." HVP, MSG, and antioxidants are permitted ingredients. Products so labeled are not necessarily prepared in the country (on the farm) but are expected to have these characteristics.

See: 9 CFR 317.8(b)(2).

Creamed Beef (Chipped or Dried)

Product must contain at least 18% dried beef. It may be produced using a cured beef, or beef product that has been chopped, pressed, or cooked.

Creamed Cheese with Chipped Beef

Product consists of cream cheese, chipped beef, cream, and chopped onions. The meat component must be at least 12% of the total formulation.

Creamed Sauce with Meat or Creamed Meat Products (Chipped Beef, Cooked Beef, Sausage, Ham, Franks, Meatballs, Etc.)

Product must contain at least 18% meat or meat products (on a cooked basis). The kind of meat product used should be reflected in the product name (e.g., "Creamed Cured Beef, Chopped, Pressed, Cooked").

Creole Style

Term applies to many dishes made with tomatoes, spices, and green peppers. Spices include onion, garlic, bell pepper, white pepper, red pepper, black pepper, parsley, and other Louisiana seasonings, e.g., bay leaf, paprika, or pepper sauce.

Crepe Filling

Must contain at least 40% cooked meat or 20% cooked meat if filling has one other characterizing ingredient, e.g., cheese, and at least 14% cooked meat when the filling has two other characterizing ingredients, e.g., cheese and mushrooms. This is based on the total weight of the filling.

Crepes

Product must contain:

1. At least 20% cooked meat when the filling contains no other major characterizing component.
2. At least 10% cooked meat when the filling contains one other major characterizing component (e.g., cheese).
3. At least 7% cooked meat when the filling contains two or more other major characterizing components (e.g., cheese and mushrooms).

These percentages are based on the total weight of the product.

Croissant

A crescent-shaped roll requiring 18% cooked meat. Label must show a true product name, e.g., "Croissant with a ham and cheese sauce filling."

Croquette

Product must contain at least 35% cooked meat, based on total formulation. Beef, ham, etc., must appear as part of the product name.

Curdlan

A substance identified by the common or usual name "curdlan" has been approved for use in foods (see 12/16/1996 *Federal Register*), and for nonstandardized meat products, poultry products, and in Policy Memo 123 and 121B products as a binder/stabilizer/thickener/texturizer.

Cured Meat Products—Labeling of Mechanically Reduced

The traditional names of cured meat products, e.g., bacon, may be used even though mechanical reduction-like chopping or chunking has taken place before the product has acquired the characteristics expected of the product, provided the finished product acquires the characteristics expected. Furthermore, the mechanical reduction must be noted in the product name or in a qualifier to the product name (e.g., chopped bacon or bacon, chopped and formed).

Cured Meat Products—Packed in Brine

Cured meat products, e.g., pork tails, pork snouts, and cured boneless beef brisket, that contain 120–200 ppm nitrite and are packed and sold in brine solution, do not require a handling statement, e.g., "Keep Refrigerated," provided the finished product has at least 10% brine concentration, and the packing medium contains a sufficient quantity of salt to maintain the 10% brine concentration in the product.

Cured Pork Bellies

Such products are assumed to be further processed into bacon. Therefore, cured pork bellies must meet the restricted ingredient requirement for bacon.

Cured Pork

Cured pork products, that contain modified food starch, X% solution ISP, carrageenan, or sodium caseinate, that fall into the PFF value of "Ham, Water

Added" and the "Ham and Water product X% solution" category, must be labeled with the appropriate PFF Nomenclature, Descriptive Labeling, e.g., "ham, water and binder product," will be used if:

1. Binders are at levels above those permitted by the regulations
2. Binders other than those permitted are used
3. Two or more binders are used in combination or
4. If the PFF value of the finished product falls in the "ham" or "ham with natural juice" category that does not permit binders

Cured Turkey Thigh Meat

A product labeled "cured turkey thigh meat" (without turkey ham in the name) must follow the turkey ham standard. The product "cured turkey thighs" (which includes skin and bone), is not required to meet the standards for turkey ham and cannot be labeled "turkey ham."

Curried Sauce with Meat (Poultry) and Rice Casserole

Product must contain at least 35% cooked meat or poultry meat based on the sauce and meat portion only.

Curry Product

1. Meat Curry—Must contain at least 50% meat (lamb, beef, etc.)
2. Poultry Curry—Must contain at least 35% cooked poultry meat.

Cutlet, Beef

Beef cutlet may be chopped and formed.

Cutlet, Pork

"Pork Cutlet" may consist of pork temple meat, inside masseter muscles, and small pieces of lean from the tip of pork jaws. These are flattened and knitted together in "cutlet" size products by means of "cubing" or "Frenching" machines, or by hand pounding with "cubing hammers." The term "cutlet" relates to thin slices of meat. They can be identified as sliced pork meat product when the designation clearly states the specific part of the carcass from which the meat in the product is derived (e.g., "Pork Loin Cutlets"). All of the terms should be conspicuously displayed on labels.

Cutlet, Poultry

Poultry cutlets may be fabricated as opposed to using whole pieces of poultry meat. However, the term "cutlet" must be properly and distinctly qualified to describe the product, e.g., "Turkey Cutlet from a Turkey Loaf," "Chicken Cutlet from Chicken Roll," "Turkey Cutlet, Chopped and Formed."

Cooked poultry cutlets, which are solid pieces and contain added water, should not be labeled as patties. A solution statement is not needed.

Cutlet, Veal

Must be a solid piece of meat from the round; slice thickness may vary. However, combining several thin slices to represent a single cutlet is not permitted.

Dehydrated Meat Calculation Factor

The fresh meat equivalent based on a given amount of dehydrated meat can be found by multiplying the weight of the dehydrated beef by the factor 2.8. This factor was derived as follows: Assuming canners and cutters grade beef was used, the composition of meat would be approximately 12% fat, 18% protein, 69% water, and 1% ash. Then 100 pounds of beef, when dehydrated to 5% moisture, would be 100 less 64 or 36 pounds dehydrated meat. Thus, 100 divided by 36 equals 2.8.

Assuming that the amount of dehydrated beef equivalent of 100 pounds of fresh beef is that quantity containing 18 pounds of protein, then 18 divided by the percentage of protein found by analysis of dehydrated beef would be the amount of dehydrated beef equivalent to 100 pounds of fresh meat.

Dehydrated Poultry Calculation Factor

The moist deboned cooked poultry or poultry meat equivalent based on a given amount of dehydrated poultry or poultry meat that can be found by multiplying the weight of the dehydrated poultry or poultry meat by the factor of 4.0.

Dehydrated Products When Water is Added

Three methods are acceptable for listing dehydrated products. Listing of the ingredients (1) As "water, dehydrated potatoes" or "dehydrated potatoes, water," whichever is the proper order, (2) As "reconstituted potatoes," or (3) As "rehydrated potatoes." If the reference was to meat instead of potatoes, the word beef, pork, or whatever was appropriate would be substituted for the word "potatoes."

Deviled Poultry

A semiplastic cured poultry food product made from finely comminuted poultry in natural proportions and containing condiments. Deviled poultry may contain poultry fat, provided that the total fat content should not exceed 35% of the finished product and the moisture content should not exceed that of the fresh unprocessed poultry. When skin is in excess of natural proportions, skin must be included in the product name (e.g., "Deviled (Kind) with (Kind) Skin Added").

Dinner Dog

A coined name, must show true product name, e.g., "A Meat and Soy Protein Concentrate Product."

Dinners and Suppers, Frozen

Frozen products labeled as "dinner" or "supper" must weigh at least 10 ounces and should contain at least three components consisting of the following: meat, poultry, cheese, eggs, vegetables, fruit, potatoes, rice, or other cereal-based products (other than bread or rolls). This is not intended to include products like casseroles and stews that have all of the components combined. Sauces and gravies are not considered one of the components. They may also contain other servings of food, e.g., soup, bread or rolls, appetizer, beverage, and dessert, and these components may be included in the minimum 10-ounce net weight requirement. If meat is featured in the product name, e.g., Beef Dinner, the requirement is 25% or 2.5 ounces cooked meat. If a meat food product is featured in the product name, e.g., Beef Burgundy Dinner, then 25% or 2.5 ounces of meat food product is needed. If poultry is featured in the name, e.g., Chicken Dinner, the standard is 18% or 2 ounces cooked deboned poultry meat, whichever is greater. However, if a poultry food product is featured in the product name, e.g., Chicken a La King Dinner, the 25% or 2.5 ounces of poultry food product, whichever is greater, is needed. The meat requirement for products with net weights greater than 10 ounces may be established exclusive of the appetizer, bread, and dessert, provided the remaining components weigh not less than 10 ounces.

The name for dinner and supper products should consist of or include a listing of each of the dish components in descending order of predominance by weight, for example, Fried Chicken Dinner—Fried Chicken, Mashed Potatoes, Peas, and Carrots. Dinner or supper identification may appear on side panels without the complete product name shown, for example, "Fried Chicken Dinner" or "Beef Dinner."

When a dessert is one of the components of a frozen dinner or supper, i.e., a multicomponent item, it may appear out of the order of predominance in the product name and appear as the last component in the product name.

Dipped Steaks

Steaks made from a solid piece of meat may be dipped in a solution of water and flavoring. The result in gain should not be more than 3% above the weight of the untreated product. A prominent statement, such as "Dipped in a Solution of contiguous to the product name," should appear.

Dixie Bacon

True product name, e.g., "Pork Jowl Dixie Bacon, Cured and Smoked" should appear on the label.

Dixie Square

Same as for Dixie Bacon.

Dog Food

See: 9 CFR 355.29.

Dough Conditioner

A generic or class name that cannot stand alone in the ingredients statement. The term "Dough Conditioner"

must be followed immediately by the common or usual name of all ingredients present.

Dried Egg White Added

See: Wheat Gluten.

Dried Soup Mixes (Meat)

Dried meat soups are not amenable.

Poultry—See: 9 CFR 381.15.

Dry Aged

Fresh meat is held (without vacuum packing) for various periods of time (usually 10 days to 6 weeks) under controlled temperatures (34°F to 38°F), humidity, and airflow to avoid spoilage and ensure flavor enhancement, tenderness, and palatability.

There is a difference of opinion regarding the best cooler humidity. Some prefer low humidity of from 70 to 75% so that exposed surfaces of meat remain dry. Others use humidity levels up to 85 to 90% in order to purposely develop a mold growth on the outside of the meat and reduce evaporation losses. Ultraviolet light may be used to reduce microbial load in the aging room. The number of days aged does not have to appear on the label when the product is identified as "Dry Aged" (e.g., "Dry Aged Beef.")

Dry Cured

Product labeled as "dry cured" should not be injected with a curing solution or processed by immersion in a curing solution.

Dry Milk Products

Approved dry milk items include whole dry milk, nonfat dry milk, calcium-reduced dried skim milk, dried whey, and lactose-reduced dried whey. If nonfat dry milk is reconstituted prior to addition to product, it would be declared on the label as "Reconstituted Skim Milk."

Dry Salt Cured

Dry salt cured product may contain a curing solution injected directly into the tissue but not through the circulatory system before it is covered with a dry curing mixture. It may be momentarily moistened to facilitate initial salt penetration, but should not be immersed in a curing solution.

Dual Weight Requirement for Stuffed Poultry Labels

Poultry products that consist solely of bone-in poultry and stuffing, e.g., a "Stuffed Turkey," should bear weight statements on the label indicating the total net weight of the product and a statement indicating the minimum weight of the poultry in the product.

When a stuffed poultry product is a component of a dinner or an entree, only the total net weight needs to be shown on the label.

Duck, Salted

This product should reach an internal temperature of 155°F.

Dumplings with Beef

The product must contain at least 18% meat in total formulation.

Dutch Brand Loaf

A nonspecific loaf that must be qualified as "Made in USA."

Easter Nola

Salami that is made with pork that is coarsely chopped and mildly seasoned with black pepper and garlic.

Egg Foo Young with Meat

The product must contain at least 12% meat.

Egg Foo Young with Poultry

The product must contain at least 3% poultry meat.

Egg Roll, Vietnamese Style

The product must contain soybean noodles or cellophane noodles, and fish sauce or anchovy extract. They are usually rolled in a thin spring roll skin or a dry rice paper skin.

Eggs Benedict

The product must contain at least 18% cured smoked ham. A poached egg on a toasted English muffin, topped with a slice of ham, and covered with hollandaise sauce.

Eggs, Fresh

For breakfast-type foods, the egg portions may be referred to in the product name and the ingredients statement as "Fresh U.S. Grade A Large." The eggs must be received in shells or broken and blended and not in dry or frozen form.

Empanadillas (SP)

A turnover containing 25% fresh meat or poultry (raw basis). The species or kind is part of the product name, e.g., "Beef Empanadillas." The product may vary in size from large to hors d'oeuvre size.

Empanadillas, Chorizo

An empanadilla that contains at least 25% fresh chorizo or 17% dry chorizo.

Encapsulation

An encapsulated additive, e.g., salt is an acceptable name. It does not require a sublisting if encapsulated in vegetable oil. If encapsulated in an animal fat, the specific animal fat must be identified in the ingredients statement.

Encapsulated lactic acid starter culture does not need to be sublisted.

Enchilada (SP)

The product must contain at least 15% meat or 10.5% poultry meat. A Mexican-type food consisting of a "tortilla" that has been filled with a variety of fillings and then rolled.

The species must appear in the product name, e.g., "Beef Enchilada."

Enchilada with Beef Chili Gravy or Enchilada Prepared with Meat and Sauce

The product must contain at least 50% enchilada.

Enchilada, Sonora Style

The product consists of two or more tortillas stacked "pancake style" with filling spread between each tortilla. Cheese may be mixed into the tortilla dough prior to frying.

Entree (Principal Dish or Main Course)

Product labeled entree should fall into one of the following categories:

1. All meat or meat food product—100% meat or meat food product
2. Meat or meat food product and one vegetable; or meat or meat food product and gravy—50% cooked meat or meat food product
3. Meat and Vegetable with Gravy—30% cooked meat portion; meat and gravy portion at least 50% (e.g., Salisbury Steak with Potatoes and Gravy)
4. Meat or Entree portion of a meal type product —25% cooked meat or meat food product (e.g., Meat Loaf Dinner would require 25% meat loaf)

Enzyme-treated Product

Product from carcasses of animals injected with papain; liver, heart, tongue, cheek and head meat, trimmings, boneless beef, tenderloin, tails, tripe, and cuts of meat not showing an imprint of the roller brand reading, "tenderized with papain," should be properly identified and kept separate from other product. Kidneys must be segregated and properly labeled.

When such product leaves an official establishment, the immediate container should bear a label showing, in addition to the other required labeling, a statement like "tenderized with papain" prominently displayed contiguous to the product name.

The establishment will furnish retail dealers handling such product with labels bearing the statement, "tenderized with papain" prominently displayed contiguous to the product name for use by such dealers on consumer packages or on product prepared from carcasses of animals injected with papain. Inspection personnel visiting retail markets should observe the effectiveness of this requirement. When retail outlets do not follow this identification, these facts should be immediately reported to the Food Labeling Division.

Enzyme Trimmings from Antemortem Injected Beef

Beef trimming from this operation may be used in fresh meat products without label declaration.

Enzymes, Proteolytic

A 3% limit permitted pickup on dipped items, e.g., steak and solid pieces of meat. The label must declare the presence of the enzyme, e.g., "Tenderized with Papain." Trimmings from this method may be used in fresh meat products up to 25% of the formula, provided the finished product is immediately frozen and that distribution is limited to institutional use only. The labeling record should state the conditions and means of inspection control. Meat from this method may be used in cooked ground beef products up to 25% of the formula without showing the ingredients of the solution.

See: 9 CFR 317.8(b)(25), 9 CFR 318.7(a)(1), 9 CFR 381.120.

Exotic/Nonamenable Products—Use of Cure Agents

Only amenable meat/poultry products can contain curing agents (i.e., nitrites, etc.), with the exception of ratites (ostrich, rhea, emu) and squab. The prior function of nitrite and nitrate, according to FDA regulations, applies only to those species that were considered "meat" or "poultry" prior to September 1958. Therefore, amenable species that can contain cure agents are identified as the following:

1. Poultry—domesticated birds, such as chicken, turkey, duck, geese, and guineas.
2. Meat—cattle, sheep, swine, and goat. Nonamenable products, such as buffalo, reindeer, and pheasant, cannot contain curing agents; such products are considered to be regulated under FDA regulations. However, if nonamenable products are included in an amenable product, curing agents would be permitted. The curing agents would be calculated based on both the amenable meat/poultry product and nonamenable meat/poultry product. For example, the formula includes 3 pounds cooked chicken and 97 pounds buffalo. The calculation for the curing agents would be based on 100 pounds of meat. In addition, in those situations where the meat block consists of an amenable product and a nonamenable product (refer to the example), the appropriate inspection legend should represent the amenable product. Therefore, using the example above, the label would have a poultry legend.

Product derived from exotic/nonamenable species that contain over 3% raw meat (cattle, sheep, swine, goat, horses, or other equine) are subject to inspection. The game meat used in these products must be derived from carcasses slaughtered under the Food Safety and Inspection Service. Products made with meat from exotic and nonamenable exotic species with 3% or less of meat or edible portion from cattle, sheep, swine, goat, horses, or other equine, or up to 30% fat from these species are nonamenable provided the only reference to meat or meat by-products on the labeling is in the statement of ingredients and the product name includes the term "flavored with (amenable species)."

Custom prepared products composed of meat from exotic/nonamenable species and up to 30% animal fat are not amenable. Labeling such products with the term applies.

See: 9 CFR 303.1(a)(2).

Products made with meat from game animals with 3% or less of meat or edible portion from cattle, sheep, swine, goat, or up to 30% meat fats provided the only reference to meat or meat by-products on the labeling is in the statement of ingredients or referred to as "flavored with."

Custom prepared products composed of meat from game animals and up to 30% animal fat. Labeling "Not For Sale" applies.

See: 9 CFR 303.1(a)(2).

Buffalo and venison must be federally or state inspected; however, venison may also be produced under the supervision of inspection officials of a country approved to export meat products into the United States. All other meat from exotic/nonamenable species that is used in formulating amenable products must be derived from carcasses slaughtered under the Food Safety and Inspection Service.

Extra and More Than

The terms "extra" or "more (component) than" may be used provided the following guidelines are followed:

1. There is at least a 10% increase in the particular component of interest over the amount that is found in the usual or "regular" formulation.

2. Information must be provided with the label application that compares the product formulation containing the "extra" amount of the component to the regular formulation of the same product to establish that at least a 10% increase in the component has occurred. Therefore, the usual or "regular" component claims at the time of label review must be presented so that the necessary comparison of formulations can be made.
3. In the situation where production of the "regular" product formulation ceases, the "extra" or "more (component) than" product labels would be given a 6-month temporary approval.
4. A comparison to a similar product on the market may be made to support the "extra" or "more than" type claim, provided suitable market basket data are submitted with the label application that establish the similarity of formulations and show the increased amount of the component over the "usual" amount.

Fabricated Steak

1. Steaks that include large sections or pieces of meat that are molded or shaped to form one large piece and then sliced. A qualifier such as "formed" must be included in the product.
2. Fabricated steaks may contain added solutions if labeled in accordance with Policy Memo 066B.
3. Antioxidants are permitted.
4. When made from simulated fat covering and/or marbling, the name must reflect this fact, e.g., Artificially marbled-simulated fat covered.

Fajitas

The Spanish translation is "little belts" or strips of meat. Fajitas are strips of seasoned or marinated red meat or poultry meat, which have been cooked. Red Meat Fajitas require labeling in accordance with the current policy memo on added solutions. Fajitas may also be a sandwich-like product, requiring 15% strips of cooked meat or poultry meat (excluding the marinade), topped with onions, peppers, and sauce, and rolled in a flour tortilla. Fajita, including the name of the meat or poultry, may stand alone, e.g., "Beef Fajita," "Chicken Fajita."

Farm Style Sausage

See: Country Style (Farm Style) Sausage.

Farmer Sausage Cervelat

Is usually a semidry sausage, but may be made in dry form. Usually made of equal parts of pork and beef delicately seasoned without garlic.

Farmer Summer Sausage

This is a special type of sausage made of beef and pork, salt, spices, nitrite or nitrate, and heavily smoked. It is classed as "Cervelat," and no extenders are permitted. It is dry with an MPR of 1.9:1. The word "Farmer" is considered a generic term, and labels can be approved without any qualifying words like "Style" or "Brand." Such labels are not required to bear a statement identifying the place of manufacture. The product must be *trichinia*-treated.

Fiber Products

Fiber products such as bran are acceptable only in nonspecific products.

Fiber-type foods are permitted in meat and poultry products and must be identified by their common or usual name, such as oat bran. However, fiber is not permitted in meat or poultry products, e.g., soy fiber, oat fiber, and wheat fiber. Currently, there is no recognized definition for fiber.

Fillet Style

"Fillet style" must be qualified, e.g., "chunked and formed," if the meat or poultry product is not made from a solid piece of meat or poultry. The term "fillet" is defined as a solid piece of meat or poultry.

Flanken in the Pot

The product must contain at least 25% beef. Product is made from beef plates and may contain such components as Matzo Balls, Noodles, and Vegetables. True product name, e.g., "Flanken in the Pot with Matzo Balls, Noodles, and Vegetables" must be used.

Flavored with —

Any product with a standard in Section 9 CFR 319 and 9 CFR 381 of the regulations must meet that standard and may not be designated "Flavored with." If a product does not meet the standard as it appears in the Policy Book it can be labeled "Flavored with."

"Flavored with" can be anything from over 3% fresh meat or 2% cooked meat to below the standard for the product.

Flavoring

Ingredients, e.g., thiamine hydrochloride, monosodium glutamate, disodium inosinate, disodium guanylate, hydrogenated vegetable oil, and other commonly used materials must be listed separately.

Such ingredients as diacetyl, hexanal, ethyl alcohol, dimethyl sulfide, diallyl sulfide, and furfuryl mercaptan may be declared as artificial flavors or artificial flavorings without naming each.

When spices and/or flavorings are presented on labels coming from foreign countries, the identity of the spices and/or flavorings must be made known.

Foie Gras Products, Duck Liver, and/or Goose Liver

Goose liver and duck liver foie gras (fat liver) are obtained exclusively from specially fed and fattened geese and ducks. Products in which foie gras is used are classified into the following three groups based on the minimum duck liver or goose liver foie gras content:

(A) FRENCH PRODUCT NAME
Foie Gras d'Oie Entier
Foie Gras de Canard Entier

ACCEPTABLE ENGLISH PRODUCT NAME
Whole Goose Foie Gras
Whole Duck Foie Gras

These are products in which goose liver or duck liver foie gras are the only animal tissues present. They may contain added substances, e.g., seasonings and cures, and when truffles are featured in the product name, they are required at a minimum 3% level.

(B) FRENCH PRODUCT NAME
Foie Gras D'Oie
Foie Gras de Canard
Bloc de Foie Gras D'Oie
Bloc de Foie Gras de Canard
Parfait de Foie Gras D'Oie
Parfait de Foie Gras de Canard

ACCEPTABLE ENGLISH PRODUCT NAME
Goose Foie Gras
Duck Foie Gras
Block of Goose Foie Gras
Block of Duck Foie Gras
Parfait of Goose Foie Gras
Parfait of Duck Foie Gras

These products are composed of a minimum 85% goose liver or duck liver foie gras, although "parfaits" may contain mixtures of goose liver and/or duck liver foie gras. These products may also contain a wrapping or stuffing consisting of the lean or fat of pork, veal, or poultry, pork liver, and/or aspic jelly. When these ingredients are used, their presence must be indicated in a product name qualifier. Truffles, when featured in the product name, are required at a minimum 3% level.

(C) FRENCH PRODUCT NAME
Paté de Foie D'Oie
Paté de Foie de Canard
Galantine de Foie D'Oie
Galantine de Foie de Canard
Purée de Foie D'Oie
Purée de Foie de Canard

ACCEPTABLE ENGLISH PRODUCT NAME
Paté of Goose Liver
Paté of Duck Liver
Galantine of Goose Liver
Galantine of Duck Liver
Purée of Goose Liver
Purée of Duck Liver

These products must contain a minimum of 50% duck liver and/or goose liver foie gras and may also contain a wrapping or stuffing of the lean or fat of pork, veal, or poultry, pork liver, aspic jelly, extenders, and/or binders. When these ingredients are used, their presence must be indicated in a product name qualifier. Truffles, when featured in the product name, are required at a minimum 1% level.

In all groups, an English translation of the term "foie gras" is not required, although all other product name terms must be translated into English. The kinds of poultry liver(s) used must be indicated in the product name. Also, other species and/or binders used must be indicated in a product name qualifier immediately following the product name, while the ingredients statement must follow the product name or qualifier as the case may be.

For Further Processing

Products that require further processing at another federally inspected plant may leave a federally

inspected plant under one of the following three conditions:

1. With the name of the finished product qualified by a "For Further Processing" statement (e.g., Turkey Ham For Further Processing)
2. With a fully descriptive name (e.g., uncooked ham contains up to 30% of a solution)
3. Not acceptable on a label when a product is formulated or processed in a manner contrary to the regulations

Fresh, "Not Frozen" and Similar Terms when Labeling Poultry Products

The word "fresh" may not be used to describe the following:

1. Any cured product, e.g., corned beef, smoked cured turkey, or prosciutto
2. Any canned, hermetically sealed shelf stable, dried, or chemically preserved product
3. Any raw poultry, poultry part, or any edible portion thereof whose internal temperature has ever been below 26°F
4. Any injected, basted, marinated poultry, poultry part, or any edible portion thereof whose internal temperature has ever been below 26°F
5. Any other finished processed poultry product (including cooked poultry products) where its temperature has ever been below 26°F, e.g., turkey sausage, chicken meatballs, cooked breaded chicken nuggets, etc.
6. Any uncured red meat product permitted to be treated with a substance that delays discoloration, such as, ascorbic acid, erythorbic acid, or citric acid
7. Any product treated with an antimicrobial substance or irradiated
8. The phrase "never frozen" or similar verbiage is not permitted on an unprocessed or processed poultry product where the internal temperature of the product has ever been below 0°F or on any red meat product that has ever been frozen. Further, the phrase "never frozen" or similar verbiage is not permitted on refrigerated secondary products where the meat or poultry component has ever been frozen, e.g., multicomponent meals, dinners, etc.

Generally, trademarks, company names, fanciful names, etc., containing the word "fresh" are acceptable, even on products produced in a manner described in one through seven above, provided the term is used in such a manner that it remains clear to the purchaser that the product is not fresh.

Secondary products, e.g., pizza, multicomponent meals, dinners, etc., sold in the refrigerated state, i.e., not frozen or previously frozen, may be labeled as "fresh" when the term is used to describe the product as a whole even when made from components processed in a manner described in one through seven above.

This entry cancels Policy Memo 022C dated January 11, 1989, since 022C is out of date.

Fresh Thuringer

Not an acceptable name.

Fried Noodles with Pork

The product must contain at least 12% fresh pork in total formulation.

Fried Products

1. Frying medium need not be shown on the label.
2. Breading is not limited to 30% unless breaded is in the product name.
3. Fried chicken labels do not need to state "fully cooked" or refer to breading because fried denotes fully cooked and breading is expected. Fried poultry products in dinners are limited to 30% breading.

Fried Rice with Meat

The product must contain at least 10% meat and may contain eggs and vegetables.

Fries

1. Beef testicles may be labeled as "Beef Fries." They are not permitted to be used as an ingredient in meat food products.
2. "Fries" is not a required part of the product name, "species mountain oyster."

Fritters

The product must contain at least 35% raw red meat/poultry or red meat/poultry food product in

the total formulation depending on the name, i.e., "Beef Fritter" must contain 35% beef and a "Chicken Patty Fritter" must contain 35% chicken patty. Fritters can contain up to 65% batter/breading (coating). If "breaded" is included in the product name, the batter/breading is limited to 30%.

Frizzes

An acceptable name. Similar to pepperoni but not smoked. MPR of 1.6:1.

Galician Sausage

Cured beef and pork is seasoned and stuffed into beef rounds. It is then smoked at a high temperature. Cooling is done in a blast of air that produces a wrinkled appearance that is characteristic of Galician sausage.

Gelatin

Gelatin is a binder/extender and is only permitted in a few meat and poultry products. Examples where gelatin is permitted include:

1. Nonspecific products
2. Jellied products, e.g., souse, jellied beef loaf, and head cheese
3. As a covering for products such as paté, to bind two pieces of meat together and in products where "gelatin" would be part of the product name

Gelatin is permitted as a thickening agent in menudo (i.e., beef tripe stew). If it is used in red meat paté products, its presence must be indicated by product name qualification.

It is not permitted in products like sausage, luncheon meat, and meat loaves. Gelatin is an acceptable ingredient in souse, jellied beef loaf, headcheese, canned whole hams require qualifier if gelatin is added.

Gelatin in Poultry Rolls

If gelatin or some other binder comprises more than 3% of the formula, the name of the product must be qualified by wording, e.g., "Gelatin Added."

General Offices

The company's grant of inspection permits the general office address to be used in the signature line for any firm "doing business as."

Genoa or Genoa Salami

Is a dry sausage product with an MPR not in excess of 2.3:1. It is prepared with all pork or with a mixture of pork and a small amount of beef. The meat is given a coarse grind and enclosed in a natural casing. No smoke is used in its preparation.

Geographic and Related Terms (Requirements for the Use on Product Labels)

Any label representation that expresses or implies a particular geographical origin of the product, or any ingredient of the product, should not be used except when such representation is:

1. A truthful representation of geographical origin, e.g., "Virginia Ham" for a ham produced in the state of Virginia; or
2. A trademark or trade name which:
   a. has been so long and exclusively used by a manufacturer or distributor that it is generally understood by consumers to mean the product of the particular manufacturer or distributor, e.g., "Swiss Chalet;" or
   b. is so arbitrary or fanciful that it is generally understood by the consumer not to suggest geographical origin, e.g., "Moon Sausage;" or
3. A part of the name required or allowed by an applicable federal law, regulation, or standard, e.g., "Frankfurter," "Vienna;" or
4. A name whose market significance is generally understood by consumers to connote a particular class, kind, type, or style of product, or preparation rather than to indicate geographical origin of the product, e.g., "Mexican Style Dinner," "Italian Style Pizza." Such terms must be qualified with the word "style" or "type," unless specifically approved by the Administrator as a generic term, e.g., "Lebanon Bologna," "Genoa Salami," Milan Salami."

Any geographical representation that does not meet the aforementioned guidelines should be

qualified by the word "brand," provided that the word "brand" is not used in such a way as to be false or misleading. A qualifying statement identifying the place where the product was actually made is required in proximity to the brand name, e.g., "Milwaukee Brand Bacon, Made in Chicago, Illinois." The word "Brand" must be in the same size and style of type as the geographical term. If the product has a foreign brand name, it may be identified as having been made in this country, e.g., "Scandinavian Brand Bacon, Made in U.S.A.".

Geographic Terms

1. Country, Ranch, and Farm in Trade, Branch, and Fanciful Names—trade names, brand names, or fanciful names that include the words country, ranch, or farm, e.g., "Country Kitchen," "Ranch House," "Hickory Farms," or "Carabeef Ranch Brand" do not invoke section 9 CFR 317.8 of the regulations regarding the use of the term "Country" or "Farm." However, if the terms are used alone in conjunction with the product name, e.g., "Country Stew," then such products must be prepared in the country or on the ranch or farm and meet any other requirements prescribed.
2. Southern—The term "Southern" is restricted to use only in areas south of the Mason-Dixon Line and east of the Mississippi River as well as Arkansas, Louisiana, and Missouri, which are also considered southern states.

German Potato Salad with Bacon

The product must contain at least 14% cooked bacon in total formulation.

See: Salad—German Style Potato Salad with Bacon.

German Sausages with Milk

Whole milk is a permitted ingredient in the following meat food products when the ingredients statement is shown immediately under the name of the product or the milk is shown in a qualifying statement contiguous to the product name: Speckblutwurst, Kalbsbratwurst, Langblutwurst, Blutwurst, Gelbwurst, Zengenwurst, and Brand Tongue and Blood Pudding Kalbslebenwurst. (Swiss Liver Sausage, Kalbslebenwurst should be considered on the same basis as Bockwurst [e.g., no limit on water or milk]).

Milk is a characterizing ingredient in German sausages and not an extender. Products that contain milk should be called by their proper names.

Giblet Gravy (Kind)

Requires 7.25% giblets. The product must contain an equal number of livers, hearts, and gizzards.

Giblets and/or Necks Sold with Carcasses

Poultry giblets consist of approximately equal numbers of hearts, gizzards, and livers, as determined on a count basis.

Although often packaged with them, the neck is not a giblet. Rabbit giblets consist of the liver and heart. Giblet packs are expected within the cavities of eviscerated whole birds or eviscerated whole rabbits or when packaged with cut-up whole birds or cut-up whole rabbits, therefore, qualifying the presence of giblets is not required on labeling. However, when giblets are not expected, therefore, a product name qualifier is required, e.g., "Packed with Giblets."

In situations where parts of giblets are missing, a product name qualifier is required, e.g., "Parts of Giblets Missing" or "Parts of Giblets May Be Missing." In situations where the giblets are missing entirely from an eviscerated carcass or a cut-up whole carcass, a product name qualifier is required, e.g., "Packed Without Giblets." In addition, an excess of one of the giblet components can be added to make up for another missing giblet component. In this case, a proper qualifying statement is required, e.g., "Packed with 2 Gizzards, 1 Liver."

A neck, when not attached to the carcass of a whole bird, is also expected to be present within the carcass or packed with the cut-up whole carcass. Accordingly, a product name qualifier is not required to flag the presence of the neck. Rather, when the neck is missing, a product name qualifier is required, e.g., "Without Neck."

See: 9 CFR 381.170(b)(21) and 9 CFR 354 (m).

Glazes

If a glaze component contributes to the flavor profile of the product, we will allow the ice glaze coating to

be counted toward the total net weight of the finished product. However, the coating and coating percentage must be included as part of the descriptive product name in accordance with Policy Memo 044A, e.g., "chicken breast filets with rib meat, coated with a butter garlic flavoring and containing up to 22.5% of a solution of . . ." or "chicken breast filets with rib meat, coated with a 6.5% butter garlic flavoring and containing up to 16% of a solution of. . . ."

Glycerin

May not be added to any product as such; may be used in proprietary mixes.

Goetta

An oatmeal product similar to scrapple. Goetta is prepared with a formula containing not less than 50% meat and meat by-products. The cereal component should consist of oats or oat products and just enough water to prevent product from sticking and burning during the preparation process. The term "Old Fashioned" when noted on a label for "Goetta" refers to the round shape.

Goettinger Cervelat

A dry cervelat with no by-products or binders.

Goose Liver or Goose Liver Sausage

At least 30% cooked goose liver. When pistachio nuts are added, product name must be qualified, i.e., "pistachio nuts added."

Gorditas, Meat/Poultry

The product must contain at least 15% cooked meat/poultry based on the weight of the total product. The "species" or "kind" gorditas name (Beef Gordita, Chicken Gordita) may stand-alone. If other characterizing ingredients such as potatoes, rice, beans, etc., are included in the name, they must be reflected in their order of predominance, e.g., Beef and Potato Gorditas, Rice and Chicken Gorditas, as determined by the formula.

Goteborg

A Swedish dry sausage made of coarsely chopped beef and sometimes pork. Mildly seasoned with thyme. It has a somewhat salty flavor and is heavily smoked, usually in long casings and air dried.

Gothaer Cervelat

Originated in Gotha, Germany. Usually made of very lean pork finely chopped and cured.

Goulash

A stew-like product with at least 25% meat or 12% poultry meat. Unless designated "Hungarian," generally means stew, whether veal, pork, beef, turkey, etc., are used. Product may be just meat and gravy or meat and gravy with vegetables served with or without rice, potatoes, or noodles.

Goulash, Hungarian Style

The product must contain paprika and at least 25% meat or 12% poultry meat. May not contain noodles, potatoes, or dumplings.

Grade Marks

There are no acceptable grade marks for products imported from foreign countries. Foreign countries do not have a grading service exactly like AMS.

The grading term "good" on poultry is considered puffery and is acceptable.

Red meat grading terms, "prime," "choice," "select," may not be used immediately preceding "kind" poultry, unless the poultry is equivalent to USDA Grade A. For example, "Choice Turkey" or "Select Chicken" must come from Grade A birds.

Gravies

The product must contain at least 25% meat stock or broth, or 6% meat. Mono- and diglycerides are allowed in amounts of 1% in gravies.

Gravy and Beef

The product must contain at least 35% cooked beef (beef same size lettering as gravy). For 25% cooked beef (beef lettering no larger than one-half size gravy).

Gravy and Dressing with Pork or Gravy and Pork with Dressing

The product must contain at least 14% cooked pork.

Gravy and Poultry Salisbury Steak

Not more than 65% gravy and at least 35% poultry salisbury steak.

Gravy and Swiss Steak

The product must contain at least 35% cooked meat.

Gravy and Yankee Pot Roast

The product must contain at least 35% cooked beef. Beef is cooked with or without vegetables.

Greek Sausage

The product must contain orange peel.

Ground Beef

May not contain added fat. Maximum total fat 30%. Cheek meat is permitted up to 25% and must be declared in the ingredients statement. For more than 25%, show as "Ground Beef and Cheek Meat," all the same size.

Beef of skeletal origin, or from the diaphragm or esophagus (weasand) may be used in the preparation of chopped beef, ground beef, or hamburger. Heart meat and tongue meat as organ meats are not acceptable ingredients in chopped beef, ground beef, or hamburger.

Ground Beef Chuck and Round

Product to be labeled "Ground Beef Chuck" or "Ground Beef Round" must comply with the following guidelines:

1. "Ground Beef Chuck" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Chuck," except as provided for in No. 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
2. "Ground Beef Round" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Round," except as provided for in No. 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
3. Generally, shank meat may be added but may not exceed the natural proportion of the beef carcass, which is considered to average 6%. Higher quantities of shank meat may be used if the shank meat remains attached during the cutting and boning of the boneless chuck or round, or if the processor can demonstrate that a higher percentage is applicable.

Ground Beef—Hamburger and Soy Products

Combinations of ground beef or hamburger and soy products may be descriptively labeled, e.g., "Hamburger and Textured Vegetable Protein Product" or "Ground Beef and Isolated Soy Protein Product" if the combination product is not nutritionally inferior to hamburger or ground beef. If the combination products are nutritionally inferior, they are to be labeled as Imitation Ground Beef (or Imitation Hamburger) or Beef Patty or Beef Patty Mix in accordance with Section 9 CFR 317.2(j)(1) and Section 9 CFR 319.15(c), respectively.

Gum Arabic

May be used up to 2% in breading and batter mixes.

Gum Tragacanth

A carrier and stabilizer in liquid spice extractives not to exceed 0.1% in finished product. Not permitted in sausage products.

Gum—Vegetable

Spice extractive products that employ vegetable gums as emulsifiers have been approved. The addition of vegetable gum is limited to no more than 15% in the seasoning blend emulsion.

Gumbo

A Creole word for okra. It is now recognized as meaning a dish or a soup thickened with okra. To qualify, the dish must have okra as an ingredient. Either the soup or the stew standard would apply, depending on product name ("Chicken Gumbo"). Product identified as "Creole Style _____ Gumbo" does not contain okra, however, it must contain a roux (flour, milk, or water, etc.) or gumbo file (dried powder young leaves and leaf buds of sassafras).

Gyros

Products identified with this term must contain at least 65% meat and no more than 12% extenders and binders. Examples include gyro loaf, gyro cone, gyro portions, and gyro slices.

Halal and Zabiah Halal

Products prepared by federally inspected meat packing plants identified with labels bearing references to "Halal" or "Zabiah Halal" must be handled according to Islamic law. The federal meat and poultry inspection program does not certify to the "Halal" or "Zabiah Halal" preparation of products, but rather accepts the statements and markings in this regard offered and applied under the supervision of any Islamic organization. The words "Halal" or "Zabiah Halal" may be used only on the labeling of meat and poultry products prepared under such Islamic authority. The identity of the Islamic organization should be available upon request by agency official. Qualification of the words "Halal" or "Zabiah Halal" by such terms as "style" or "brand" does not negate these requirements.

Ham a la King

Must contain at least 20% ham (cooked basis).

Ham and Bacon Loaf

There is a limit of 3% water in this product.

Ham and Cheese Loaf

Nonspecific loaf. Cheese is chopped into small cubes and combined with finely ground ham.

Ham and Cheese Salad

Product must contain at least 25% ham (cooked basis).

See: Salads, Ham and Cheese Salad.

Ham and Dumplings and Sauce or Gravy

Product must contain at least 18% cooked ham.

Ham, Boiled

A fully cooked, boneless product that must be cooked in water and may be processed in a casing or can. The product may be of various shapes and may be partially cooked in boiling water.

Hamcola

Not an acceptable product name; should be accompanied by true product name, i.e., "Boneless Cooked Ham Coated with Spices."

Ham Capacolla, Cooked

Ham that has been cured and then cooked.

Ham Chowder, Condensed

Product must contain at least 10% cooked ham.

Ham—Cooked, Sectioned, and Formed

The qualifying phrase "sectioned and formed" is no longer required on boneless ham products, e.g., "ham" and "ham-water added." The addition of small amounts of ground ham added as a binder to such products may be used without declaration. The amount of ground ham that may be used can represent no more than 15% of the weight of the ham ingredients at the time of formulation. Products containing more than 15% ground ham trimmings must be labeled to indicate the presence of the ground ham, e.g., "a portion of ground ham added." Policies regarding the required use of terminology such as "chunked and formed" and "ground and formed" will continue.

Whole hams require a cooking temperature to differentiate the ready to eat products from *trichinae*-treated products. The reason that the temperature is required is to determine the label requirements (e.g., safe handling) and proper serving size.

Ham Croquettes

Product must contain at least 35% cooked ham. If chopped ham is used, the product name must be "Chopped Ham Croquettes."

Ham, Fresh (or Uncured)

Ham that does not contain a cure must be labeled either "Fresh" or, if the ham meets the requirements of 9 CFR 319.2, "Uncured." This also applies to cooked product, and must be labeled cooked product "Cooked Uncured Ham."

Ham Half

"Half Ham" is permitted on labels for semiboneless ham products, which during their processing have had the shank muscles removed. The two halves of the finished product have approximately an equal amount of bone. The term "No Slices Removed" has also been deemed suitable for use with a ham item referred to as "Half Ham."

Ham Omelet

Product must contain at least 18% cooked ham.

Ham/Parma Ham/Prosciutto di Parma

Ham, when labeled "Parma Ham" and/or "Prosciutto di Parma," would have to be produced in the region of Parma, Italy, in accordance with Italian law, which defines the denomination of origin, the territorial limits of production, characteristics of the product, and the method of manufacture.

Ham, Quarter, Semiboneless (No Slices Removed)

The product consists of a ham prepared as a "Regular Semi-Boneless, Half Ham" which is sectioned again to result in four pieces just about equal not only in weight but also in content of bone.

Ham Roll Sausage

Ham trimmings and ham shank meat are permitted.

Ham Salad

Product must contain at least 35% cooked ham. Chopped ham may be used without it appearing in the product name.

See: Salads.

Ham, Scotch Style

A cured, uncooked, boned, and rolled whole ham either tied or in a casing.

Ham, Shankless

When the term "shankless" is used in reference to a ham, it indicates that the shank has been removed by a cut through the joint at a right angle to the femur bone. The distal tip of the semitendinous muscle may be severed above its tendinous attachment, leaving an extension approximately 2 inches long. The extension is considered an integral part of the ham's body and is usually folded over the femur's end.

Ham Shortcake

Product must contain at least 25% cooked ham.

Ham, Smithfield

This is an aged, dry cured ham made exclusively in Smithfield, Virginia. The use of the words "brand" or "style," e.g., "Smithfield Brand Ham," "Smithfield Style Ham," does not eliminate this requirement.

Ham Trimmings

Ham trimmings, to be labeled as ham, cannot contain excess shank meat. The fat content will not exceed 35%. It will consist of at least 65% lean meat as determined by chemical analysis.

Ham, Westphalian or Westphalian Style Ham

Ham is cut with bone in, the hip bone cut out, cured in a combination of dry and pickle cure but not a pickle alone. It is smoked in a medium warm (no greater than 100°F) smokehouse until a shining red brown or chestnut color is acquired. Beech wood may be used and will impart the characteristic Westphalian flavor. Other hard woods are also acceptable. Juniper berries are permitted.

Handling Statements

Acceptable handling statements, in addition to those required in sections 9 CFR 317.2(k) and 9 CFR 381.125, include "Keep Refrigerated—May be Frozen" or "Keep Refrigerated—Can be Frozen."

Handling Statements on Retorted Products

Handling statements may appear on labels for shelf stable product, even though such product does not have to be refrigerated or frozen, and provided the statement will accurately reflect conditions of

distribution and sale. These products are to be handled in the plant as shelf stable items including incubation and condition-of-container examinations. Once the product is refrigerated or frozen for shipment, distribution, and display for sale, it is to be handled as a refrigerated or frozen item.

The statement "previously handled frozen for your protection, refreeze or keep refrigerated" is now acceptable on poultry products under the usual restriction of use for such statements.

Head Meat

After removal of the cheeks, lips, snout, skin, and tongue from the head there remains small pockets and areas on the skull to which muscle tissue is attached. This muscle may be removed and used in product and declared on labeling as beef or pork as the case may be. However, there are a few standardized products in which the regulations limit the amount of this meat that may be used and require that it be specifically declared on the label (e.g., chili, chili with beans, and corned beef hash).

See: Beef Cheek Meat, Beef Head Meat, Pork Cheek Meat, and Pork Head Meat (use and labeling as an ingredient in meat Food Products).

Headcheese

A jellied product consisting predominantly of pork by-products and seasoning ingredients. It must contain some product from the head. Extenders like cereal, soy derivatives, nonfat dry milk, etc., are not permitted ingredients of headcheese.

Hearts/Heart Meat

Hearts/heart meat may not be labeled as "beef," "pork," etc., in the ingredients statement. When used in a product, they must be identified by species, e.g., "Beef Hearts." Hearts/Heart Meat, including the heart cap, may be considered meat for calculating the meat to textured vegetable protein ratios.

Heat and Eat Sausage

Not the same as Brown and Serve Sausage. When the "heat and eat" term is used, product must comply with cooked sausage regulations, e.g., limitation of 10% added water and not more than 3½% binder.

Hickory Smoked

"Hickory flavored" and "hickory taste" are acceptable terms on products that have been smoked with some hickory in the sawdust. They do not need to be smoked with 100% hickory smoke.

High Fructose Corn Syrup (HFCS)

HFCS may be used to flavor meat or poultry products in amounts sufficient for its intended purpose, provided the following conditions are met:

1. HFCS must contain not less than 40% fructose on a solids basis.
2. HFCS must have a dextrose equivalence (DE) of not less than 93.
3. HFCS must have a sweetening power greater than or equal to sugar (sucrose).
4. HFCS must be identified on the label as High Fructose Corn Syrup in the ingredients statement, curing statement, etc.

Holstein or Holsteiner

Product is the same as Farm Style Sausage, except that it is stuffed into wide casings and heavily smoked, usually in long casings, and air dried. No extenders are permitted.

Honey Claim in Product

A honey claim may be made or implied on a product label if:

1. The product contains at least 3% honey.
2. Honey contains at least 80% solids, U.S. Grade C or above.
3. When other sweeteners (sugar, dextrose, maltose, invert sugar, corn syrup solids, and similar ingredients) are used, the quantity may not exceed one-half that of the honey. If 3% honey is used, then no more than 1½% of all other sweeteners may be used.
4. Product to be identified as "Honey Glaze" must contain honey to other sweeteners at a ratio no less than 2:1. If dried honey is used, the ratio is to be no less than 1.6:1.
5. When honey is included in a breading, a honey claim may be made regardless of the quantity of honey used.

Honey Cured or Sugar Cured

"Honey Cured" may be shown on the labeling of a cured product if:

1. The honey used contains at least 80% solids or is U.S. Grade C or above.
2. Honey is the only sweetening ingredient or when other sweetening ingredients are used in combination with honey, they do not exceed one-half the amount of honey used.
3. Honey is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

Traditionally, cured products that are labeled to indicate the presence of honey, e.g., Honey ham, must meet the parameters prescribed herein.

"Sugar Cured" may be used on the labeling of a cured product if:

1. The sugar used is cane sugar or beet sugar.
2. Sugar is the only sweetening ingredient or when other sweetening ingredients are used in combination with sugar, they do not exceed one-half the amount of sugar used.
3. Sugar is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

"Honey and Sugar Cured" or "Sugar and Honey Cured" may also be used on labeling if:

1. The honey and sugar are of the nature described above.
2. The honey and sugar are the only sweetening agents or when other sweetening ingredients are used in combination with the honey and sugar, they do not individually exceed either the amount of honey or sugar used and collectively do not exceed one-half the total amount of honey and sugar.
3. The honey and sugar are used in amounts sufficient to flavor and/or affect the appearance of the finished product.

Hors d'Oeuvre (Snack)

Product must contain at least 15% cooked meat or 10% bacon (cooked basis). True product name must be shown, e.g., "Puffed Pastry Wrapped Frank."

Hot Dog Chili Sauce with Meat

Product must contain at least 6% meat.

Hot Dog Chili with Meat

Product must contain at least 40% meat. Sausages and bologna rework not permitted.

Hunan Style Seasoned Pork

Acceptable for pork shoulder sliced into 1-inch pieces and marinated in a solution of soy sauce, garlic, and ginger, cooked, and returned to green weight. The product may be flavored with other seasoning ingredients, e.g., star anise and coriander.

Hydrolyzed Beef Stock

A beef stock that has been treated with acid, alkali, or enzymes to digest the protein. The protein molecules are broken down into amino acids, peptides, polypeptides, and peptones. As the digestion is carried out for longer periods of time, more and more of the larger molecules are broken down into amino acids, with free alpha-amino groups. By analyzing these alpha-amino nitrogens, one can determine the degree of hydrolysis. 100% hydrolysis would mean that all the nitrogen (protein) is in the form of amino acids. 10% of hydrolysis would mean that only 10% of the nitrogen is in the form of free amino acids, while the rest is still present in polymeric form.

The label should indicate the degree of hydrolysis. This is determined from the ratio of amino nitrogen to total nitrogen.

amino nitrogen = % hydrolysis total nitrogen

A product labeled 50% Hydrolyzed Beef Stock must, therefore, have 50% of the total nitrogen present as amino nitrogen. Adding % solids is optional. The % solids would not necessarily be the same % as hydrolysis depending on the thickness (consistency) of product.

Hydrolyzed Gelatin

Hydrolyzed gelatin is permitted in frankfurters and similar products (9 CFR 319.180) at levels typically used for flavorings (less than 2%). Hydrolyzed gelatin may currently be used as 2% in 9 CFR

319.180 and like products as a flavoring. If gelatin is used in a product, make sure it is permitted for use in the product. Hydrolyzed gelatin is acceptable in poultry franks according to 9 CFR 319.180 and like products at levels not to exceed 2% of total formula until otherwise notified.

Hydrolyzed gelatin is recognized as a binder rather than flavoring and may be used at levels of 2% or less in 9 CFR 319.180 type products.

Hydrolyzed Oat Flour

Hydrolyzed oat flour is safe and may be used in non-standardized meat/poultry products as a binder at below typical binder use levels, i.e., 3%. It may be used in low fat hamburger, water, and hydrolyzed oat flour product in accordance with Policy Memo 121.

Hydrolyzed Protein

"Hydrolyzed Protein (milk, egg, soy)" is an acceptable common or usual name provided all components are hydrolyzed. "Hydrolyzed Protein (potato, gelatin)" is an unacceptable ingredient declaration and must be declared as "hydrolyzed potato protein and hydrolyzed gelatin."

Salt is present in hydrolyzed protein and must appear in the sublisting of the hydrolyzed protein if it does not appear elsewhere in the ingredients statement.

Hydroxypropyl Methylcellulose (HPMC)

Emulsifying agent, binder, thickener, and a stabilizer. This is accepted for its emulsifying qualities when prepared as a solution and applied as a dip.

1. Not more than 2% in solution.
2. Not more than 4% weight gained in product.
3. Not more than .08% hydroxypropyl methylcellulose in finished product.
4. Must be identified in the ingredients statement for purpose.
5. Approved on individual basis only.

Ice-Glazed Breaded Chicken Nuggets

If an ice glaze is applied for the purpose of setting the breading, the term "ice glazed" needs to appear close to the product name. The water cannot be included as part of the net weight statement and the transmittal form should indicate this.

Imitation Flavors

Imitation beef flavor, imitation mushroom flavor, flavor base for gravies, and similar substances which enhance, fortify, or help to simulate a flavor are usually composed of food additives and, as such, are not "artificial flavors" for labeling purposes. This class of imitation flavors can be composed of such ingredients as flour, fats, oils, salt, hydrolyzed vegetable protein, vegetable gums, thiamine hydrochloride, beta alanine, disodium inosinate, glutamic acid, and a host of other ingredients. These flavorings must be identified on labels by showing each individual ingredient by its common name. Class names, e.g., amino acids are not acceptable. Each specific amino acid must be listed.

Incidental Additives

As defined in the Food and Drug Administration regulations 21 CFR 101.100(a)(3), incidental additives are substances present in foods at insignificant levels and that do not serve a technical or functional effect in that food. In determining whether a substance is an incidental additive, the following criteria may be applied:

1. Substances that are present in a food as a result of having been present in an ingredient added to the food and have a technical or functional effect on the ingredient but not on the finished food, or
2. Substances that are processing aids, defined as:
   a. substances added during processing but removed before the food is packaged in its finished form, or
   b. substances added during processing but that are converted to constituents normally present in the food, and do not significantly increase the amount of those constituents naturally found in the food, or
   c. substances that are added to a food for their technical or functional effect in the processing, but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food.

Ingredient Labeling

1. All ingredients in FDA standardized products, e.g., Cheddar Cheese (water, salt, cheddar, etc.), and on standardized products, e.g., soy sauce, Worcestershire sauce, require complete disclosure of all ingredients on the labels of meat and poultry products.
2. Protein hydrolysates must identify the common and usual names and identify the source from which the protein is derived, e.g., "hydrolyzed vegetable protein" would be declared as "hydrolyzed corn protein."
3. FDA-certified color additives require the listing of the common or usual names, e.g., FD&C Blue No. 1, Blue 1, or Blue 1 Lake. Color additives not subject to certification may be declared as "artificial color," "artificial color added," or "color added." Alternatively, color additives not subject to certification may be declared as "colored with _____," or "_____ color," with blank space filled in with the name of the color additive listed in 21 CFR 73, e.g., "colored with annatto" or "caramel color."
4. Cured meat products used as ingredients, regardless of their level of use, require complete disclosure of all ingredients in the formulation of meat and poultry products.

Insert Labels for Use at Retail Stores

No inspection legend is permitted on insert labels.

Inspection Legends (Dual)

Products consisting of mixed meat and poultry ingredients should bear either the official meat inspection legend or poultry legend, depending on which ingredients are present in the greater amounts. If meat or poultry ingredients exist in equal proportions, either official legend may be used. If meat and poultry ingredients exist in exact proportions and both appear in the product name, the official legend must reflect the ingredient appearing first in the product name.

Containers of products intended for sale to household consumers can bear only the official mark of inspection of the product enclosed. Containers of products intended for distribution to other than the retail trade may bear both the official meat inspection legend and the official poultry products inspection legend.

Inspection Mark on Wing Tag

When the inspection mark is shown on a wing tag, either the plant number or the firm's name and address must also appear.

Intestines

Intestines can be prepared as edible product and bear the mark of inspection.

Irish Stew

Product does not require a geographical qualifying statement nor the words, "Style," "Type," or "Brand." Usually it contains lamb or mutton but beef may be used. It must meet the stew standard. Vegetables include onions, carrots, potatoes, and turnips. Dumplings are often used. Beans are not acceptable in "Irish Stew."

Isolated Soy Protein

This food ingredient is similar to soy protein concentrate except that additional extraction has removed more of the nonprotein fraction, thereby increasing its protein content. It is prepared by alkaline solubilization of the soy protein and then precipitation of same in an acid bath. It may be powdered, extruded, or spun into fibrils and has a protein content of 90 to 95%. Products of spun fibrils may be referred to as "Textured Soy Protein Isolate," "Isolated Soy Protein Fibers," or "Spun Isolated Soy Protein." The PER of isolated soy protein is about 1.9 and indicates a poorer quality protein than that of soy flour or soy protein concentrate (PER 2.2). When hydrated textured (structured) protein isolate is added to meat food products, the ingredients statement should read "Hydrated Textured (Structured) Isolated Soy Protein."

Italian Sausage

Beef and pork Italian sausage is acceptable. Tomato products and other unexpected ingredients can be added if the product name indicates their presence. Red pepper is permitted under 9 CFR 319.145(b)(1).

See: 9 CFR 319.145.

Italian Style

Acceptable term for products containing anise or fennel or Italian type cheese (e.g., Mozzarella, Parmesan, Provolone, Ricotta, Romano) or at least three of the following: basil, garlic, marjoram, olive oil, or oregano. Sausage products must meet the Italian Sausage requirements in accordance with Regulation 9 CFR 319.145.

Italian Style Smoked Sausage

This is a smoked sausage (10% added water) and is not a 9 CFR 319.145 (Italian sausage) product. However, the product must contain Italian style ingredients found in the policy book under Italian style.

Jagwurst

The product is the same as yachtwurst (the Americanized name for the item). It is a cooked sausage made from a fine emulsion with cubes of lean meat rather than fat (as in mortadella).

Jamaican Style

This term may be used to identify meat and poultry food products made with allspice, garlic, onion, red pepper, and thyme. The name of the product must be further qualified with a statement, like "with Jamaican Style Seasoning," e.g., "Jamaican Style Chicken Wings—with Jamaican Style Seasonings." If the product formula contains textured soy product, then the ration rules apply.

Jamaican Style Patties

Product has at least 25% meat enclosed in a crust. The label must show true product name, e.g., "Beef Turnover." If the formula contains textured soy product, then the ratio rules apply.

Jambalaya

Product must contain at least 25% cooked ham and one other meat or seafood must be included. A New Orleans dish involving rice and ham and usually tomatoes (shrimp or other shellfish, other meat, or poultry), together with seasonings. Must show true product name, e.g., "Ham and Shrimp Jambalaya."

Jamon

Spanish word for "ham." In the usage of Spanish-speaking people outside Spain, it has come to mean cured pork. "Jamon di Cocinar" is cured pork for cooking as opposed to slicing. When the term "Jamon" appears before the name of a limb, it means the product is cured. With the exception of products available for sale in Puerto Rico, all Spanish product names must be followed with the English translation.

Examples of acceptable product names are:

Jamon de Paleta—Cured Pork Shoulder
Jamon de Pierna—Cured Pork Leg

Jardinière (FR)

Must contain at least 50% cooked meat based on total product. It means "in the manner of the gardener." The term applies to dishes made with diced garden vegetables that have been cooked with meat. Jardinière should be followed by a true product name, e.g., "Beef with Vegetables."

Jerk or Jerk Style

The terms "Jerk" or "Jerk Style" can be used to describe red meat or poultry whole muscle, fabricated products, and other meat poultry food products, that are mixed or placed in a "spicy seasoning." The seasoning usually contains scallion, onion, thyme, allspice (pimento), hot peppers, and usually contains at least one or more of the following: nutmeg, cinnamon, sugar, brown sugar, garlic, and rice or wine vinegar. The seasoning may be in the form of paste, marinade, sauce, or dry seasoning mixture. The product is mixed or placed in the spicy hot seasoning raw or the product may be grilled, cooked, or smoked. Examples of acceptable product names are, e.g., "Oven Roasted Jerk Chicken," "Jerk Pork Sausage," or "Jerk Style Smoked Beef Sausage."

Jerky

All Jerky products must have a MPR of 0.75:1 or less; "species" or "kind" must be in the name. Products may be cured or uncured, dried, and may be smoked or unsmoked, air or oven dried. A reference to the particular type of drying method is not a labeling requirement.

1. "Beef Jerky"—Produced from a single piece of beef. May also be classified as "Natural Style Beef Jerky" provided this product name is accompanied by the explanatory statement "made from solid pieces of beef" or comparable terminology. When a "Natural" claim (not natural style) is made, the policies as outlined in Policy Memo 055 are to be applied.
2. "Beef Jerky Chunked and Formed"—Produced from chunks that are molded and formed and cut into strips.
3. "Beef Jerky Ground and Formed or Chopped and Formed"—Produced as described, molded and formed, and cut into strips.
4. Jerky products that contain over 3½% binders (2% ISP) must reflect the binder in the product name, i.e., "Beef Soy Protein Concentrate" jerky, ground and formed. Jerky products that contain binders at levels below 3½% should express the binder in a qualifying statement, e.g., beef jerky, soy protein added.
5. "Species (or Kind) Jerky Sausage": The word "Jerky" can appear on labels for product in which the "species" or "kind" has been processed by chopping or grinding and stuffed into casings under the following conditions only:
   a. The word "Sausage" must appear immediately contiguous to "Jerky" whenever it is shown. "Sausage" must be in type at least one-third as high as "Jerky" in the same color ink and on the same background. The words "stick," "piece," etc., cannot be used as substitutes for "sausage" in the product name. "Sausage" means that the product has been chopped.
   b. The product may be dried at any stage of the process.

Junior Meat Snacks

Product must conform to the sausage standards going into the jar before processing. Limited to 3½% extenders.

Juniper Berries

Juniper berries and twigs are normally thrown on the fire from which dry cured hams are smoked. Juniper berries have been approved in the curing ingredients of Westphalian ham.

Kabobs

Product consists of chunks of red meat or poultry and vegetables placed on a metal or wooden skewer. "Kabob" may be included in the descriptive name, e.g., "Beef, Mushrooms, and Onion Kabob." A kabob may be cooked or uncooked, but the label must clearly indicate this. This product may contain but does not require vegetables.

Kalbelwurst

Product is similar to Bockwurst with no limit on water or milk.

Katrifitas

A coined word used to describe a type of empanadilla. The product consists of dough containing yucca made to resemble a meat turnover and has a special meat filling. The product must contain at least 25% raw meat (beef) in total formulation. Label must include a true product name in conjunction with "Katrifitas," e.g., "Katrifitas, Beef Turnover made with Yucca Shell," or similar wording.

Kelco-Gel

A thickening agent used in sauces. It contains sodium alginate, calcium carbonate, and disodium phosphate. The amount of disodium phosphate in the finished product is approximately 0.099%. Its use should be judged on an individual basis.

Kidneys from Enzyme Injected Beef

Product may be exported to other countries. They must be labeled "Beef Kidneys, Tendered with Papain—For Export Only."

Kielbasa

A sausage that is cured, cooked, and usually smoked. Kolbassy is the Czechoslovakian spelling; other variations include Kielbassy, Kolbasa, and Kolbase. Kielbasa is made from coarsely ground pork or coarsely ground pork with added beef or mutton. "Hungarian Style Kolbase" is finely ground product, seasoned, and stuffed into casings. The 70/30 rule can be used, however, pork must always be the predominant meat

ingredient. "Beef Kielbasa" is prepared with only beef as the meat ingredient. By-products are not permitted ingredients in these sausages.

An uncured (fresh), uncooked variety, with no more than 3% water exists. "Fresh" should be used in the name when the product is uncured. When fresh Kielbasa is cooked or smoked, then cooked or smoked is required in the product name. The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

Kippered Beef

A cured dry product similar to beef jerky but not as dry. MPR of 2.03:1 is applied to product.

Kiska, Kisba, Kishka, or Stuffed Derma

Ingredients statement is part of the product name. A meat food product prepared two ways:

1. Prepared with meat by-products, including beef blood, pork snouts, pork livers, pork cheeks, etc. Packaged in fully labeled retail size packages or individually banded. When beef blood is used, it must be shown as part of product name.
2. Prepared with no more than 30% animal fat, mixed with farinaceous (consisting of or made of flour or meal) materials containing no other meat by-products and ordinarily stuffed into beef casings and cooked. Product containing 30% or less fat is not considered amenable to the Federal Meat Inspection Act.

Knishes

Product must contain at least 15% cooked meat or poultry or 10% bacon (cooked basis). Same as snack standard. The type of meat or poultry should be identified in a true product name, e.g., "Chicken Knishes."

Konjac Flour

Food ingredient that provides the effects of thickening, gelling, texturizing, and water binding, e.g., "binder," similar to that of starch vegetable flours, such as potato flour. Konjac flour can be used in meat and poultry products in which starch vegetable flours are permitted, e.g., 3.5% in cooked sausage products such as frankfurters and bologna.

Kosher and Kosher Style

Products prepared by federally inspected meat packing plants identified with labels bearing references to "Kosher" or Rabbinical markings must be handled under Rabbinical supervision. The federal meat and poultry inspection program does not certify to kosher preparation of products, but rather accepts the statements and markings in this regard offered and applied under the supervision of the Rabbinical authority. The word "Kosher" may be used only on the labeling of meat and poultry products prepared under Rabbinical supervision. The identity of the Rabbinical authority must be made available upon request from agency official. Qualification of the word by such terms as "Style" or "Brand" does not negate the requirement.

Kosher (Product Containers)

Containers must be labeled "Kosher tags attached" when used for hearts, livers, and other products or tissues with attached metal tags indicating kosher inspection.

Krakow

Acceptable name for a cooked sausage similar to "Berliner."

Kreplach

Product must contain at least 20% meat. The type of kreplach should be identified in a true product name, e.g., "Beef Kreplach."

Kubbee

Other acceptable names are Kubbe, Kibbe, Kabeda, Kilin, Kibbes, Kibby, Kabbo, or Kabe. A product popular in Syria and Lebanon. It must contain at least 25% meat based on total formulation; it must contain soaked cracked wheat and show the true product name, e.g., "Fried Cracked Wheat and Beef Balls," "Baked Stuffed Wheat and Beef Patty." Products may be shaped like a hamburger and fried or shaped into balls and fried.

Kuemmelwurst

An acceptable name. The product is the same as Carawaywurst and is a cooked sausage of the ring variety, with whole caraway seeds. Usual ingredients are beef, pork, salt, caraway, flavorings, and cure.

Kurma

Product must contain at least 50% meat or at least 35% poultry meat.

Labeling, Check-off Blocks

The use of check-off blocks on immediate containers for identifying products that look alike but are different in composition is not permitted.

Examples of product that may look alike but are different in composition are as follows:

- Ground Beef and Beef Patty Mix
- Partially Defatted Chopped Beef and Partially Defatted Beef Fatty Tissue
- Frankfurters and Frankfurters with Variety Meats
- Finely Ground Chicken and Finely Ground Chicken Meat
- Comminuted Chicken and Comminuted Chicken with Kidney and Sex Glands Removed

However, exceptions to this policy may be granted. Exceptions would require that the establishment operators develop a procedure that the assigned inspector can readily monitor to ensure correct labeling. Such procedures, accompanied by written comments from the assigned inspector and where possible, the circuit supervisor, must be forwarded to the area supervisor for review and approval.

Approved procedures must be attached to the label records accompanying new or modified labels submitted for approval.

Labeling for Substitute Products

If a product fails to comply with a standard only because the meat or poultry content is lower than required and the product has generic identity as a nonmeat product (e.g., pizza, stew, pies), then the product may be designated by the nonmeat terminology in the standardized name (e.g., "Pizza," "Stew," "Pie"), provided the meat/poultry content of the product is conspicuously disclosed contiguous to the product name along with a statement of the amount of meat/poultry in the standardized product. (For example, Pizza contains 5% sausage; Sausage Pizza contains 12% sausage.) Such product may not be nutritionally inferior to the standardized product it resembles. For this purpose, nutritional inferiority is defined, consistent with the requirement of 21 CFR 101.3(e)(4), as any reduction in the content of an essential nutrient that is present at 2% or more of the U.S. Recommended Daily Allowance (USRDA) per serving of protein or any of the vitamins or minerals for which USRDAs are established. A quality control procedure must be approved for such products by the Processed Products Inspection Division before the label can be used.

If a product is nutritionally inferior to the standardized product it resembles, it must be labeled "imitation" in accordance with 9 CFR 317.2(j) and 9 CFR 381.1(b).

Labeling of Modified Breakfast Sausage, Cooked Sausage, and Fermented Sausage Products Identified by a Nutrient Content Claim

Modified breakfast sausage, cooked sausage, and fermented sausage products are substitute versions of the standardized or traditional products that have been formulated and processed to reduce the fat contents to qualify for use of nutrient content claims, but do not comply with the standard of identity or composition as described in the meat and poultry regulations or the Standards and Labeling Policy Book (Policy Book) because of the use of ingredients used for fat replacement, which are precluded or restricted by these standards. The deviation from the standard or the traditional, i.e., "regular product," is conveyed by associating an expressed nutrient content claim for the appropriate reduction in fat content and the standardized or traditional product name, e.g., "Reduced Fat Frankfurter" or "Low Fat Pepperoni." The nutrient content claims that may be used are those related to a reduction in fat contents that are identified in the regulations for meat products in 9 CFR Part 317 and for poultry products in 9 CFR Part 381.

Maintaining Product Integrity—The following guidelines must be applied to assure that the modi-

fied versions of the subject meat and poultry sausage products do not violate the integrity of the standardized or traditional product for which they purport to be substitutes: (1) the product must be similar in shape, flavor, consistency, and general appearance to the product as prepared according to the regulatory or traditional standard; (2) the meat or poultry used to formulate the modified product must come from the same anatomical location when the standardized term is related to an anatomical region on an animal, e.g., "ham" is expected to be from the hind leg of the hog and cured; thus, "lean smoked ham sausage" would be comprised of meat from the hind leg of a hog that has been smoked and cured; (3) the modified sausage product must result from the same processing procedures as those specified for the subject sausage products described by regulatory or Policy Book standards; (4) there must not be deviations from product safety criteria (e.g., salt content, curing agents, pH, water activity and/or moisture/protein ratio) that are provided in the regulatory or Policy Book standards for sausages; and (5) the modified product must achieve the appropriate reduction in fat content to be eligible to use a nutrient content claim in conjunction with the standardized or traditional product name.

Performance Characteristics—In producing modified, substitute versions of sausages, the deviations from ingredient provisions of the regulatory and Policy Book standards should be the minimum necessary to qualify for the nutrient content claim while maintaining the performance characteristics similar to the standardized or traditional product, i.e., similar preparation, cooking, and handling characteristics. If a modified version of the standardized or traditional sausage does not perform in substantially the same way as the standardized or traditional item, the label must include a prominent statement informing the consumer of such differences. For example, a "low fat frankfurter" that essentially has all of the characteristics of a frankfurter, but cannot be grilled, would indicate "not recommended for grilling." A "reduced fat pepperoni" that displays essentially all the characteristics of pepperoni, but cannot be cooked, would, for example, indicate "not recommended for cooking" or "do not cook."

Safe and Suitable Ingredients—A modified, substitute sausage product must be formulated with approved safe and suitable ingredients, e.g., those identified in 9 CFR 318.7(c)(4) and 9 CFR 381.147(f)(4), and those determined to be safe and suitable by the Food Standards and Ingredients Branch, Product Assessment Division. Such ingredients are to be used at the lowest level necessary to achieve the intended effect of reducing fat as compared to the standardized or traditional product. Safe and suitable ingredients are those used to replace fat, improve texture, and prevent syneresis.

An ingredient or component of an ingredient that is specifically required by the regulatory or Policy Book standard for characterizing purposes, e.g., cheese in a cheesefurter, fresh livers in liver sausage, cured ham in a ham sausage, and fennel or anise in an Italian sausage, should be present in the required amount, if applicable, or otherwise in a significant amount to provide a characterizing identity to the product. Moreover, an ingredient or component of an ingredient that is not permitted by regulations for use in any meat or poultry sausage product, e.g., sodium benzoate, should not be added to a modified, substitute product.

Product Identity—The name of the modified version of the standardized or traditional product that complies with all parts of the policy prescribed herein is the appropriate expressed nutrient content claim for the meat and/or poultry product with a reduction in fat content and the applicable standardized or traditional term, e.g., "Lean Sausage," "97% Fat-Free (or 'Low Fat') Kielbasa," "Low-Fat Frankfurter Made with Beef, Pork and Turkey," "Reduced Fat Pepperoni," "Extra Lean Turkey Italian Sausage," and "Lite Genoa Salami." The size and style of type must conform to the nutrition labeling regulations.

Ingredients Statement—To assist the consumer in differentiating between the standardized or traditional sausage product and the modified, substitute version, ingredients that are not provided for by regulatory or Policy Book standards, or used in excess of the allowable levels specified, must be appropriately identified with an asterisk in the ingredients statement. The statement(s) defining the asterisk(s), e.g., "*Ingredient(s) not in regular (fill in name of the standardized or traditional product)," or "*Ingredients(s) in excess of amount permitted in regular (fill in name of the standardized or traditional product)," or both as appropriate, must be legible and conspicuous, and should immediately follow the

ingredients statement in the same size and style of type.

Labeling of Meat and Poultry Stick Items

Stick items such as beef jerky, pepperoni sticks, and beef sticks must be labeled (i.e., contain the required label features as outlined in 9 CFR 317 and 9 CFR 381, Subpart N) according to the following guidelines:

1. If sold in fully labeled bulk containers, i.e., canisters, caddies, or similar containers, stick items do not have to be fully labeled unless they are individually wrapped. This type of container cannot be reused.
2. If sold in bulk containers, i.e., canisters, caddies, or similar containers that are not fully labeled, stick items must be fully labeled. Bulk containers such as these may only be refilled with fully labeled product.
3. If sold in small, fully labeled cartons, boxes, or similar containers (e.g., 3 oz., net weight) that are only intended for retail sale intact, stick items may be individually wrapped and unlabeled.

Labeling of Modified Substitute Versions of Fresh (Species) Sausage, Hamburger, or Ground Beef Products

This policy allows modified versions of fresh (species) sausages, ground beef, or hamburger to contain nonmeat or poultry, "fat-replacing ingredients" (e.g., binders such as carrageenan, modified food starch) and to be identified by certain nutrient content claims in accordance with nutrition labeling regulations effective on August 8, 1994, in conjunction with descriptive labeling, e.g., "Lean Pork Sausage with a X% Solution of . . . ," or "Low Fat Ground Beef, Water, and Carrageenan Product."

This policy allows for the use of terms defined in regulations, e.g., "Lean," "Reduced Fat," "Low Fat," etc., to be used to describe fresh (species) sausage, ground beef, or hamburger products with a reduction in fat content resulting from the use of added ingredients (i.e., "fat replacers" such as carrageenan and isolated soy protein). These products must meet the criteria for use of the nutrient content claim associated with the fat reduction. The nutrient content claim may be used in conjunction with the standardized name provided the consumer is informed of the actual components of the product through labeling, i.e., descriptive product name, ingredients statement, and Nutrition Facts.

Meat products, including those that meet the criteria established for claims, such as "Lean," "Low Fat," "Lower Fat," "Reduced Fat," etc., that combine fresh (species) sausage, ground beef, or hamburger, and other safe and suitable ingredients, for the principal purpose of replacing fat, may be descriptively labeled. Examples of such products are "Lean Ground Beef, Water, and Carrageenan Product," "Low Fat Ground Beef With a X% Solution of . . . ," "Lean Beef Sausage, Water, and Carrageenan Product," or "Reduced Fat Pork Sausage, Water, and Binders Product," provided conditions prescribed in the regulations, viz., 9 CFR 317, for use of the nutrient content claim are satisfied. In contrast, modified versions of fresh (species) sausage, ground beef, or hamburger product containing added ingredients that do not qualify for use of a nutrient content claim prescribed in the nutrition labeling regulations must be labeled as Imitation Pork Sausage, Imitation Beef Sausage, Imitation Ground Beef, Imitation Hamburger, Beef Patty, or Beef Patty Mix in accordance with 9 CFR Section 317.2(j)(1) and Sections 9 CFR 319.141 (fresh pork sausage), 319.142 (fresh beef sausage), and 319.15 (miscellaneous beef products), respectively.

Descriptively labeled, modified, substitute versions of fresh (species) sausage, ground beef, or hamburger product with a reduction in fat content must comply with the following guidelines:

1. The descriptive name of a modified, substitute product with a reduction in fat content is the applicable nutrient content claim used in conjunction with the appropriate standardized name and fat-replacing ingredients, e.g., "Low Fat Ground Beef, Water and Carrageenan Product," or "Lean Pork Sausage With a X% Solution of Water, Modified Food Starch, Spices, and Salt." Words in the descriptive name may be of a different size, style, color, or type, but, in all cases, the words must be prominent, conspicuous, and legible. Moreover, no word in the descriptive name should be printed in letters that are less than one-third the size of the largest letter used in any

other word in the descriptive name. The solution statement, when used, is considered to be part of the descriptive product name and must comply with descriptive name sizing requirements.
2. Fat-replacing ingredients (e.g., binders and water) and fat in the finished product may not exceed 30% of the product as formulated for the modified, substitute ground beef, hamburger, or fresh beef sausage product, and no more than 40% of the product formulation for the substitute fresh pork sausage. The fat content must be in accordance with requirements for use of the applicable nutrient content claim.
3. The product includes mandatory nutrition labeling prescribed in the meat inspection regulations, viz., 9 CFR 317.
4. The product is formulated with approved safe and suitable ingredients, e.g., those identified in 9 CFR 318.7(c)(4), and which are determined to be safe and suitable by the Labeling and Consumer Protection Staff, that are used at the lowest level necessary to achieve the intended effect as a fat-replacing ingredient (i.e., binder).

Labeling of Product Names, Fanciful Names, Word Size

Words in product names or fanciful names may be a different size, style, color, or type, but in all cases, the words must be prominent, conspicuous, and legible. Moreover, no word in a product name, i.e., a common or usual name, a standardized name, or a descriptive name should be printed in letters that are less than one-third the size of the largest letter used in any other words of the product name. The same guidelines apply to letters of words in fanciful names that may accompany the product name.

For example, for a product labeled Chili Mac—Beans, Macaroni, and Beef in Sauce, "Chili Mac" is the fanciful name and "Beans, Macaroni, and Beef in Sauce" is the product name. No letter in "Chili Mac" may be smaller than one-third the size of the largest letter in "Chili Mac." Similarly, no letter in the descriptive name may be smaller than one-third the size of the largest letter in the descriptive name. This policy is not intended to address the relative size of words in fanciful names versus product names. The size of words in qualifying statements, e.g., "Water Added," "Contains up to . . . ," "Smoke Flavoring Added," etc., are not affected by this policy memo.

Labeling of Products Containing Meat with Added Solutions or Other Nonmeat Ingredients in Secondary Products

In those situations where meat containing an added solution or other nonmeat ingredients, e.g., Ham-Water Added, Corned Beef and Water Products, Beef-Containing up to 10% of a solution, are used in secondary products in sufficient quantities to meet the minimum meat requirement without including the added solution, or nonmeat ingredients, the product name need not include any reference to the added solution or nonmeat ingredients; e.g., Corned Beef and Cabbage would be an acceptable name for a product if the corned beef portion of the corned beef and water product was present in a sufficient quantity to satisfy the 25% cooked corned beef requirement. The ingredients statement, however, must include nomenclature as required by the regulations or policy. In this example, the ingredients statement would list "Corned Beef and Water Product-X % of added ingredients are . . . ."

For products in which the added solution ingredient as a whole is used to meet the minimum meat requirement, the product name must include nomenclature required for the component, e.g., Beef (containing up to 10% of a flavoring solution) Burgundy. The ingredients statement must also include the same nomenclature for the meat ingredient.

Labeling of Products That Are Artificially Colored

Labels of products that are artificially colored either by artificial colors or natural colors must bear a statement to indicate the presence of the coloring, e.g., "artificially colored" or "colored with annatto." Products whose true color is disguised by packing media, e.g., colored pickling solutions, must also have labels that include a statement that indicates the presence of the color. The statement must appear in a prominent and conspicuous manner contiguous to the product name. When a component within a product is artificially colored, e.g., breading, sauce, and sausage, a qualifying statement is necessary. However, in all cases, the presence of the coloring must appear in the ingredients statement. Whenever FD&C Yellow No. 5 is used, it must be declared in the ingredients statement as FD&C Yellow No. 5 or Yellow 5. Some products, e.g., chorizos and some of

the sausages of the longaniza variety, are expected to be characterized by coloring. In these situations, the presence of the coloring need only be indicated in the ingredients statement.

Labeling of Products That Include Packets of Other Components

Wording indicating that the product contains, in addition to the meat or poultry product, another component, e.g., a gravy, sauce, or seasoning packet must appear in conjunction with the name of the product in such a manner that it is obvious to the purchaser that he or she is also purchasing that packet along with the meat and/or poultry product. The wording must be shown in print no smaller than one-third the size of the largest letter in the rest of the product name, of such color that will ensure it is not overlooked at point of purchase, and positioned contiguous to the rest of the product name, so as not to appear in whole or part on any panel except the main display panel. The net weight individual components may be shown but are not required.

Labeling of Safe Thawing Instructions on Consumer Packages

Thawing instructions that appear on the label of a frozen meat or poultry product must be given in accordance with FSIS' recommendations for safe thawing procedures. These procedures are as follows:

1. Thawing product in the refrigerator.
2. Thawing product in cold water, changing water every 30 minutes until product is thawed.
3. Thawing product in a microwave oven for less than 2 hours. Cook immediately.

Upon request, alternative thawing procedures may be considered. However, scientific evidence that thoroughly establishes the safety of an alternative thawing procedure must be presented with the procedure when it is submitted for review.

Labeling Prominence Guidelines for Cured, Cooked Products with Added Substances That Do Not Return to Green Weight

The cured, cooked products covered by sections 9 CFR 319.100 ("corned beef"), 319.101 ("corned beef brisket"), 319.102 ("corned beef round and other corned beef cuts"), and 319.104(a) ("cured pork products" under PFF) of the federal meat inspection regulations; and by Policy Memos 057A ("Labeling Turkey Ham Products Containing Added Water") and 084A ("Cooked Corned Beef Products and Cured Pork Products with Added Substances"), whose weights after cooking exceed the weight of the fresh uncured article, should bear the product name and qualifying statements on the principal display panel using the following guidelines:

1. The product name and the qualifying statements must be prominent and conspicuous.
2. The label will bear the product name on the principal display panel in lettering not less than one-third the size of the largest letter in terms commonly associated with the product name, e.g., cooked, boneless, chopped, pressed, smoked, or words that could be a part of the product name, e.g., steak, butt portion, shank portion.
3. The product name will be judged prominent if the lettering is of the same style and color, and on the same color background as that which is used for the terms commonly associated with the product name or words that could be a part of the product name (see guidelines No. 2.). If other styles, colors, and/or backgrounds are used, the prominence must be judged equal to those terms and words that could be associated with or part of the product name.
4. The product name must be distinct and separate from other label information. Thus, the product name should not be part of or embedded in qualifying phrases or descriptions that include a list of added solution ingredients. Examples of acceptable terminology are "Corned Beef and Water Product" and "Cured Pork and X% of a Solution."
5. The label for the products covered by this policy memo must also bear qualifying statements that conform to established policies on the size of the lettering in these statements in relation to product name (as outlined in Policy Memo 087A, FSIS Directive 7110.2, and Policy Memo 057A).

Labeling Requirements for Pump-cured Bacon Products Treated with D- or D1-α-Tocopherol in Surface Applications

Pump-cured bacon treated on the surface with d- or d1-α-tocopherol must be labeled with a product

name qualifier that identifies the substances involved and the method of application. The qualifier must identify both the carrier and active substance in their order of predominance. The specific names, d- or d1-α-tocopherol, or the term, vitamin E, may be used in the name qualifier. Examples of acceptable name qualifiers are "Sprayed with a solution of vegetable oil and vitamin E" or "Dipped in a solution of corn oil and d-α-tocopherol." The name qualifier must be contiguous to the product name and printed in a style as prominent as the product name. The type used for the statement must be at least one-fourth the size of the most prominent letter in the product name, except that the ingredients of the mixture may be in print not less than one-eighth the size of the most prominent letter in the product name. The specific name of the ingredients, d-α-tocopherol or dl-α-tocopherol, and of the carrier must be listed as such in the ingredients statement or curing statement, as required by 9 CFR 317.2(f)(1).

Lamb Curry

Product must contain at least 50% fresh meat.

Landjaeger Cervelat

A semidry sausage that originated in Switzerland. It is about the size of a large frankfurter but pressed flat, smoked, and dried giving it a black appearance.

Lard Continuous Process

This nomenclature identifies the commodity produced from clean and sound edible tissues of swine by a low-temperature separation process in which the oil is separated from the fatty tissue by means of a combination of heat and centrifugal force. Labeling records containing the above designation should identify in detail the process and equipment used in producing the commodity.

Lard—Cured Pork Tissue Use

Cured pork trimmings may be rendered to produce lard manufactured in compliance with the lard and leaf lard standard. Rendered bacon is not acceptable in lard.

Lard Refined

This term is applied to open-kettle rendered, prime steam, or dry-rendered lard put through a filter press, with or without bleaching agent.

Lasagna

Sauce is an expected ingredient of lasagna products and its declaration in the product name is optional.

Cheese Lasagna with Meat—12% meat
Lasagna with Meat and Sauce—12% meat
Lasagna with Meat Sauce—6% meat in total product
Lasagna with Poultry—8% poultry meat
Lasagna with Tomato Sauce, Cheese, and Pepperoni—8% pepperoni
Meat Lasagna—12% meat
Poultry Lasagna—8% poultry meat

Lau—Lau

Product must contain at least 25% meat. A Hawaiian dish made with pork and fish, wrapped in taro leaves. Label must have a true product name, e.g., "Pork and Fish Stuffed Taro Leaves."

Lebanon Bologna

A coarse ground, fermented, semidry sausage. If the product has an MPR of 3.1:1 or less and a pH of 5.0 or less, no refrigeration is required. It is made with beef. No extenders or hearts are permitted in the product. This is not a 9 CFR 319.180 product.

Legends

Products consisting of mixed meat and poultry ingredients should bear either the official meat inspection legend or poultry legend, depending on which ingredients are present in the greater amounts. If meat or poultry ingredients exist in equal proportions, either official legend may be used. If meat and poultry ingredients exist in exact proportions and both appear in the product name, the official legend must reflect the ingredient appearing first in the product name.

Lentil Soup with Bacon—German Style

Acceptable name for a lentil soup containing only bacon. The bacon requirement is 4.0% for condensed and 2.0% for ready to eat.

Leona

An acceptable name. A coarse ground cooked sausage.

Lima Beans with Ham or Bacon in Sauce

Product must contain at least 12% ham or bacon. See: 9 CFR 319.310.

Linguica

A Portuguese-type sausage containing pork and excluding other meat and meat by-products. Usually contains nonfat dry milk and condiments, e.g., vinegar, cinnamon, cumin seed, garlic, red pepper, salt, and sugar. Paprika and cures are acceptable in this product.

Links

This designation falls into four categories:

1. "Links" without further qualification refers to an all pork fresh sausage in links.
2. "Links Sausage" can be used to designate any sausage type formulation usually cured and smoked in links, except for those formulations containing poultry.
3. "Links cereal and nonfat dry milk added" usually formulated with meat and meat by-products cured and smoked, and approved with the understanding each link is banded with an approved band label.
4. "Links, A pork and textured vegetable protein product" followed immediately by the ingredients statement is acceptable. "Links," "Top's Links," "Joe's Links" are coined names and must be followed immediately by true product name.

Little Smokies

A smoked small variety sausage link made with beef and pork.

Liver and Onions

Product must contain at least 45% liver.

Liver, Chopped

Product must contain at least 50% liver.

Liver, Onions, and Eggs

Product must contain at least 40% liver.

Liver Products

The product name does not have to include the species for multi-ingredient liver products, such as chopped liver, liver paté, and pureed liver. However, the species must be identified in the ingredients statement. For single ingredient liver products, such as sliced beef liver, the species must be identified in the product name. "Kind" liver must always be identified.

Products with liver in the name (except for products listed) must contain a minimum of 30% liver.

Liver Spread (Streich Leberwurst)

The product name "Liver Spread (Stretch Leberwurst)" is acceptable. Product name must contain at least 30% liver in total formulation.

Liverwurst or "Paté de Foie-Style Liverwurst"

Product must meet liver sausage requirements. (See Regulation 9 CFR 319.182.)

Loaf

A "Loaf" (other than meat loaf) consists of meat in combination with any of a wide range of nonmeat ingredients. These products are not identified with the term "Meat Loaf," "Beef Loaf," or the like but with designations, e.g., "Olive Loaf," "Pickle and Pimiento Loaf," "Honey Loaf," "Luxury Loaf," and others that are descriptive.

Loaf, Canned, Perishable

Canned perishable products in the loaf category must:

1. Meet the perishable requirements. See 9 CFR 317.2(k).
2. Show a brine concentration of not less than 3.5% in finished product. Show a brine concentration of not less than 6.0% when the products contain cereal, starch, or other extenders.
3. Be cooked to a minimum internal temperature of at least 150°F.
4. When extenders are added, the product name must be qualified, e.g., "(Name of extender) added."

Lola and Lolita (IT)

Dry sausage products of Italian origin. Consists of mildly seasoned pork and contains garlic. Lolita comes in 14-ounce links, while Lola comes in 2½-pound links.

London Broil

Name can only be applied to a cooked product. Products including the expression "London Broil" on labels must be prepared with beef flank steak. Uncooked product must be labeled to indicate this, e.g., "Beef Flank Steak for London Broil." If prepared from another cut, the identity of that cut must accompany the term "London Broil," e.g., "Sirloin Tip London Broil."

Long Island Style or Type

Not acceptable for poultry products.

Longaniza

Longaniza is a fresh sausage product. If it is prepared otherwise, the product name must indicate its nature, e.g., "Cured Longaniza." Paprika is an acceptable ingredient because it is expected.

Longaniza and Puerto Rican Style Longaniza

Longaniza is an acceptable name for Puerto Rican sausage made from pork that may contain beef, but does not contain annatto. Added fat is not permitted.

Puerto Rican Style Longaniza is acceptable labeling for sausage made from pork that may contain beef and does contain annatto. Added fat is not permitted, although up to 3% lard may be used as a carrier for annatto. When annatto is used, it should be included in the ingredients statement as "annatto" in accordance with Section 9 CFR 317.2(j)(5) of the meat inspection regulations.

Loukanika

An acceptable name for cooked fresh Greek sausage. It is usually made with lamb and pork, oranges, allspice, whole pepper, and salt.

Lumpia or Loompya

A Philippine-style or Filipino-style egg roll. There are no special ingredient requirements. It refers to a shape of the egg roll. Lumpia or Loompya are generally longer and thinner than traditional egg rolls.

Luncheon Meat

1. "Luncheon Meat" cannot contain livers, kidneys, blood, detached skin, partially defatted pork or beef tissue, or stomachs.
2. On the label the meat components of "Luncheon Meat" are identified in the ingredients statement as "beef," "pork," "beef tongue meat," "pork tongue meat," "beef heart meat," and "pork heart meat."
3. In the ingredients statement, "Beef" and "Pork" means lean meat with overlying fat and the portions of sinew, nerve, and the blood vessels that normally accompany muscle tissue and that are not separated in the process of dressing but not including bone and skin. Up to 10% of the meat portion of the formula can consist of cured and smoked meat trimmings, which does not require special declaration in the ingredients statement except included under "pork" and "beef."
4. Heart or heart muscle, tongues, or tongue meat and cheek meat can be included in "Luncheon Meat" under the following restrictions:

    a. Hearts or heart meat or tongues or tongue meat must be declared individually by species in the ingredients statement on the label.

b. No restriction on the percentage limits of hearts, heart meats, tongues, and tongue meats in the formulation.
c. The terms "heart meat" and "tongue meat" refer to the muscle tissue remaining after heart caps, glands, nodes, connective tissue, etc., are trimmed away.

5. Water added to "Luncheon Meat" during manufacture cannot exceed 3% by weight of the total ingredients. This is controlled by weighing ingredients and not by analysis. Care must be used to see that water is not added indirectly through the use of undrained hearts and tongues.
6. The only ingredients permitted in "Luncheon Meat" are curing ingredients, sweetening agents, spices, and flavoring. All of these substances must be declared in the ingredients statement by name, except the various "flavorings" and "spices," which need not be named individually. "Spices" refer to natural spices and not to extracts.

Lyoner Wurst

A cooked, smoked, and finely ground sausage originating in Germany. It is usually made with beef and/or pork (but no chicken) flavoring, cure, and contains green peppercorns.

Lyons Sausage (FR)

A dry sausage made exclusively of pork (four parts finely chopped lean and one or two parts small diced fat) with spices and garlic, which is stuffed into large casings, cured, and air-dried.

Macaroni and Beef in Sauce

Product must contain at least 12% beef.

Macaroni and Cheese with Ham

Product must contain at least 12% cooked ham.

Macaroni Salad with (Meat or Poultry)

Product must contain at least 12% cooked meat or poultry meat.

Made with. . . Qualifiers

Need only mention the species or kind in the statement even when only a by-product of the specific species or kind is used, e.g., pork, chicken, and beef hearts in a sausage would carry a qualifier "made with pork, chicken, and beef."

Made with 100% Real Cheese

This statement is acceptable on products as long as the cheese components are all 100% real cheese. It is not acceptable if a cheese food product or imitation cheese is included in the formula.

Malic Acid

Malic acid has been used extensively for many years as part of flavoring/seasoning mixtures that are added to components of meat or poultry products. It may be approved as a flavoring agent, and is acceptable as a component in a seasoning mix, e.g., in marinades and sauces, but may not be added alone to a product.

Manicotti (IT)

Product must contain at least 10% fresh meat. An Italian main dish consisting of rectangular-shaped pasta spread with a filling of meat (e.g., sausage, ground beef, or chopped prosciutto) and/or cheeses (e.g., ricotta and mozzarella). The pasta is rolled, edges pressed to seal, and covered with grated Parmesan cheese and tomato sauce. A true product name must be shown, e.g., "Beef Manicotti in Sauce."

Margarine Substitutes

Meat food products that are substitutes for margarine because they contain less than 80% fat and/or oil need not be labeled "imitation" if the product has a fully descriptive name and the finished product contains 15,000 international units of vitamin A per pound.

The descriptive name of the product may include the term "Spread" (or "Spred"), which has been widely adopted as a generic fanciful name for this class of product.

The following guidelines should be used in selecting the appropriate descriptive product name:

1. "Animal Fat Spread (or Spred)" is an acceptable product name for a product prepared from animal fat as the sole source of fat.
2. "Animal Fat and Vegetable Oil Spread (or Spred)" is an acceptable product name for a product prepared with a combination of animal fat(s) and vegetable oil(s) in which the vegetable oil(s) content is greater than 20% of the total of the fat(s) and oil(s) used but less than 50% of the total.
3. "Animal Fat Spread (or Spred)—Vegetable Oil Added" is an acceptable product name for a product prepared with a combination of animal fat(s) and vegetable oil(s) in which the vegetable oil(s) content is 20% or less of the total of the fat(s) and oil(s) used, but greater than 2% of the total.
4. The fanciful name "Spread" (or "Spred") accompanied by a list of all ingredients individually identified by their common or usual name in order of decreasing predominance is acceptable regardless of the nature and amount of fat(s) and/or oil(s) used.

In 1., 2., and 3. above, the descriptive product name may include the percentage of each fat and/or oil and may include the common or usual name of each fat and/or oil used.

Marengo

Product must contain at least 35% cooked meat or poultry meat. It has chicken or veal in a sauce containing tomatoes, mushrooms, onions, and wine, and the label must show true product name, e.g., "Chicken Marengo."

Marinated

To be labeled "marinated," a product must use a marinade that is a mixture in which food is either soaked, massaged, tumbled, or injected in order to improve taste, tenderness, or other sensory attributes, e.g., color or juiciness. Time allotted in a marinade depends on many factors, such as thickness and size of the meat and strength of the marinade. Marinade should be that amount necessary to affect the finished product, and limited to 10% pickup in red meat, 8% pickup in boneless poultry, and 3% in bone-in poultry.

Marine Oil

Herring oil and other marine species oils found by FDA to be satisfactory may be combined with animal and mixture of animal and vegetable oils processed as meat food products. Labels will bear statements identifying the presence of such substances, e.g., a shortening consisting of 50% herring oil and the remainder equal amounts of animal and vegetable oils would be "Shortening, Prepared with Herring Oil, Animal, and Vegetable Oils."

Marking

Labeling may consist of a combination of printing, stenciling, box dyes, etc., for large true containers and for shipping containers. Crayons are unacceptable for applying required labeling features except for figures indicating content quantity. Approval of official marks appearing in newspaper advertisements, billboards, etc., is not necessary; however, such marks may be reviewed locally before publication. Such markings should conform to the illustrations in the regulations and not be misleading.

"May Contain" Statements

The use of "may contain" or "and/or" labeling may be used in the ingredients statement's sublisting of sliced and/or diced products from various sources.

See Composite Ingredients Statement.

Meat and Dressing

Product must contain at least 50% cooked meat.

Meat and Dressing with Gravy

Product must contain at least 30% cooked meat.

Meat Base

A granular, paste-like product that is shelf-stable primarily because of its high salt content (30–40%).

1. Beef Base—15% beef or 10.5% cooked beef
2. Pork Base—15% pork or 10.5% cooked pork
3. Ham Base—18% ham Meat Broth or Meat Stock: MPR 135:1, Condensed 67:1.

Meat By-Products

By-products must be individually declared by species and specific name in the ingredients statement, e.g., Pork Liver, Beef Tripe, and Beef Fat.

Meat Casseroles

Product must contain at least 25% meat or 18% cooked meat.

Meat Curry

Product must contain at least 50% meat.

Meat Flavoring

When characteristic meat flavorings such as bacon are added in amounts less than 2% in addition to the required meat component of a product, such meat flavorings need not appear in the product label.

Meat Foldover Mit Dressing

Product must contain at least 50% meat (chopped and formed).

Meat Food Products Containing Poultry Ingredients—Labeling

Meat food products containing poultry ingredients in amounts that exceed 20% of the total livestock and poultry product portion of the meat food product must have product names that indicate the presence of the poultry ingredients, e.g., "Beef and Chicken Chili" or "Chili made with Beef and Chicken."

Meat food products containing poultry ingredients in amounts at 20% or less of the total livestock and poultry product portion of the meat food product must have product names that are qualified to indicate the presence of the poultry ingredients, e.g., "Beef Stew—Turkey Added."

However, meat food products that do not meet specified minimum livestock ingredient requirements because poultry ingredients are replacing any part of the required livestock ingredients must have product names that indicate the presence of the poultry ingredients, e.g., "Beef and Turkey Stew" or "Stew Made with Beef and Turkey."

This policy does not apply to the following: (1) red meat products that are expected to contain poultry ingredients, e.g., "Brunswick Stew and Potted Meat Food Product" (Section 9 CFR 319.761); (2) cooked sausages identified in Section 9 CFR 319.180 of the meat regulations (Policy Memo 005A); or (3) nonspecific loaves, rolls, logs, etc., e.g., Pickle and Pimento Loaf.

Meat Loaf

Uncooked or cooked pork, beef, veal or lamb, and other ingredients in loaf form, but not canned.

1. Ingredients, e.g., cracker meal, oatmeal, bread crumbs, nonfat dry milk, soy ingredients (untextured), milk, and whole eggs are not required in the product name.
2. Product may contain:
   a. Head meat, cheek meat, heart meat, and tongue meat under label declaration in the ingredients statement only.
   b. Not more than 12% extenders and binders.
   c. Partially defatted chopped beef or pork up to 25% and declared as meat in the ingredients statement.
3. Product must contain at least 65% meat.
4. Onion, tomato juice, water, and other liquid extenders are not directly controlled.

Meat Loaf, Canned (Perishable)

Canned perishable products in the loaf category must:

1. Meet the perishable labeling requirements. See 9 CFR 317.2(k).
2. Be cured with at least 1 ounce nitrate per 100 pounds of product and ½% dextrose or 1% sugar.
3. Have a brine concentration of at least 3.5% in the finished product. Products that contain cereal, starch, or other extenders must have a brine concentration of at least 6.1%.

Meat Loaf, Canned (Sterile Packed)

No head, cheek, heart, or tongue meat permitted. Other requirements are the same as uncanned cured meat loaf. Binders and extenders must be shown in the product name, e.g., "Meat Loaf, cereal added."

Meat Pasty or Pasties

Product must contain at least 25% meat. The label must show the true product name, e.g., "Beef Pasty."

Meat Pie Filling

Product must contain at least 37% meat.

Meat Pies (or Vegetable Meat Pies)

Product must contain 25% meat; meat in gravy may be counted toward meat content.

Meat/Poultry Extended Products

These should always be listed in the ingredients statement of the secondary product by their correct name, e.g., "Beef, water and binder product," unless it is included in the name of the product, e.g., "Chili made with beef and binder product."

Meat Ravioli

Product must contain at least 10% meat in ravioli.

Meat Ravioli in Meat Sauce

Product must contain at least 10% meat in ravioli and at least 50% ravioli in total product, and at least 6% meat in sauce.

Meat Ravioli in Sauce

Product must contain at least 10% meat in the ravioli and at least 50% ravioli in the total product.

Meat Sauce

Product must contain at least 6% ground meat.

Meat Spreads

Product must contain at least 50% meat or 35% cooked meat. When another major component is considered a significant source of protein, such as cheese, is added, the requirement is reduced to 25% cooked meat. Product must show a true product name, e.g., "Sausage and Cheese Spread."

Meat Stick and Cheese Combination Products

The following criteria are used for dry meat stick and cheese combination products that need not bear a "keep refrigerated" handling statement:

1. The dry meat stick portion must have a water activity of less than 0.90, the cheese portion must have a water activity of less than 0.94, and the equilibrium of the water activity of the two components must be no greater than 0.92.
2. The dry meat portion, if fermented, must be fermented by an active fermentation culture (typically to a pH of 5.0 or below).
3. For products where the meat portion and the cheese portion are packaged together, there must be a heat seal between the dry meat stick and cheese components, which separates the meat stick from the cheese stick by at least 4 mm.
4. Dry meat stick and cheese combination products not meeting these criteria must be labeled with a "keep refrigerated" statement in lieu of compelling data that establish safety.

Products not meeting the criteria stated above can be labeled without a "keep refrigerated" statement if a control program ensuring safety and shelf stability is established by the establishment.

Meatballs

Uncooked or cooked pork, beef, veal, and lamb, and other ingredients in a ball form.

1. Product must contain at least 65% meat.
2. Binders and extenders are limited to 12% of the total product. 6.8% of isolated soy protein is considered the equivalent to 12% of the other binders or extenders. The permitted binders and extenders include, but are not limited to, cereal, breadcrumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
3. Cheeks, hearts, and tongues are not allowed, but product may contain head meat, cheek meat,

heart meat, and tongue meat when declared in the ingredients statement.
4. Partially defatted chopped (PDC) (species) may be used up to 25% of the meat block. PDC (species) can be identified as (species) in the ingredients statements. (See entry for Partially Defatted Chopped (Beef or Pork) (PDCB, PDCP)).

Meatballs in Sauce

Requires a 50% minimum of meatballs, by weight in finished product.

Meatball Stew

Meatball stew contains at least 25% meatballs and usually contains vegetables such as potatoes, peas, carrots, etc., and gravy or thick broth resulting from cooking all ingredients together. The meatballs must meet the Meatball Standard.

Meatballs, Swedish Style

Product must contain at least 65% fresh meat. "Swedish Meatballs" or "Swedish Style Meatballs" are small in size and usually contain two or three different varieties of meat, nutmeg and/or allspice, potatoes, and milk. "Swedish Brand Meatballs Made in USA" means any meatball.

Meatballs, Turkey

Product must contain at least 65% raw turkey meat. Skin is permitted in natural proportions of meat used, if skin is in excess of natural proportions, it should be reflected in the product name.

Mediterranean Style

Acceptable identification for product containing onion or garlic, olive oil, and four of any of the following groups:

1. Vegetable or fruit—dried apricot, artichoke, dried date, dried fig, eggplant, tomato, pepper (green or red), squash, lemon or lemon juice, raisin and olives.
2. Legume or nut—fava bean, chick pea, white cannelloni bean, green bean, lentil, almond, pine nut, pistachio.
3. Seasoning—dill, coriander, cinnamon, cumin, fennel, basil, oregano, thyme, saffron, rosemary, parsley, mint, sumac, turmeric.
4. A regional dish as component, e.g., pita bread, yogurt, Italian or Greek type cheese, pasta, couscous, or bulgur.

Merguez, Mergues, or Merghez Sausage

A hot and spicy fresh sausage originating in North Africa and common in France, which contains hot pepper and/or paprika. The meat component must contain beef and may contain lamb or mutton when labeled as "Merguez Sausage." When pork is used as part of the meat component, the product is labeled as "Merguez Sausage with Pork." When pork is the only meat ingredient, the product is labeled "Pork Merguez Sausage."

Methyl Cellulose

May be used as an ingredient in formulas for meat and vegetable patties and various poultry products (mainly patties) at a level of 0.15% of the total weight of the product, which includes batter and breading of these products.

The internal technical effect is to extend and to stabilize products as well as to act as a carrier.

See: 421.24(4) and 9 CFR 381.147(f)(4).

Mettwurst

An uncooked cured smoked sausage in which by-products and extenders are not permitted. Beef heart meat is acceptable. Water is limited to 3% and the fat content should not exceed 50%.

Mettwurst, Cooked

Mettwurst that is cooked must be labeled "Cooked Mettwurst," and may contain up to 10% water based on the finished product.

Metz Sausage

Cured lean beef and pork and bacon are finely chopped, seasoned, and stuffed into beef middles. It is air-dried for 5 days, then given a cool smoke. It is classed as a semidry sausage.

Mexican Style

Acceptable for products that contain at least four of the following: jalapeno peppers, chili peppers, green chilies, cumin, cayenne peppers, red or green peppers, chili powder, jalapeno powder, Monterey Jack cheese, or cheddar cheese. This policy applies to a single food and does not supersede Policy Memo 068.

Mexican Style Dinners

Products like tamales, enchiladas, and tacos must make up 25% of the dinner or entree to qualify as "Mexican Style." The individual product standard must also be met.

Mexican Style Sauces

A garnish (decoration) of cheese in or on the sauce of Mexican style foods does not require the presence of the cheese to be declared in the product name or qualifying statement.

Milan or Milano Salami

A dry sausage with a maximum MPR of 1.9:1. It is an Italian-type salami, except the meat is finely cut. It is made with beef, pork fat, spiced with garlic, and has a distinctive cording.

Mince Meat

Product must contain at least 12% fresh meat or 9% cooked meat. Heart meat may be substituted. In addition to "Mince Meat," the product name should include kinds of meat, e.g., "Mince Meat with Beef" or "Mince Meat with (species) Heart Meat." When 2% or more cooked meat, but less than 9% cooked meat is present in the formula, the product is amenable and the name must state that the product is "Mince Meat Flavored with ____."

A product marketed as "Mince Meat" that contains less than 2% cooked meat or contains only beef suet as the ingredient of animal origin, is not considered as a meat food product and is not amenable.

Mixtures

Mixtures of nonfat dry milk (NFDM), calcium-reduced dry skim milk (CRDSM), or dried whey, reduced lactose whey, reduced minerals whey, and whey protein concentrate with other substances are not allowed, except in batter and gravy mixes and breaders. Mixtures of cereal, soy preparations, and/or sodium caseinate with other substances are permitted to come into the plant for use in batter and gravy mixes, but they must be labeled to show their intended use, e.g., "Patty Mix" or "Gravy Mix." The labels of the mixtures must show the ingredients in order of their predominance.

Mock Drumsticks

An imitation product; nonspecific.

Mock Turtle Soup

Product must contain at least 10% beef and may be made with beef and beef by-products.

Mofongo

Pork skins and plantain type product with at least 20% pork skins in the total formulation. It must show true product name, e.g., "Pork Skin Filling Wrapped in Plantain."

Moisture Protein Ratio (MPR)

Frizzes 1.6:1, Ukrainian Sausage 2.0:1, Jerky 0.75:1, Kippered Beef 2.03:1, Pepperoni 1.6:1, Dry Salami 1.9:1, Dry Sausage 1.9:1, Genoa Salami 2.3:1, Tropic Cure Pork 3.25:1, Sicilian Salami 2.3:1, Thuringer 3.7:1, Italian Salami 1.9:1, Dried Meat 2.04:1, Roast Beef, Canned 2.25:1, Chipped Beef 2.04:1, Farmer Summer Sausage 1.9:1.

Moisture Protein Ratio (MPR), pH

Nonrefrigerated or shelf-stable sausages must have an MPR of 3.1:1 or less and a pH of 5.0 or less, unless commercially sterilized. This does not apply to products containing more than 3.5% binders or 2% isolated soy protein.

Mondongo

A mixture of one or more of the following: (a) beef tripe, (b) cattle feet with or without hide on, (c) chitterlings, and (d) beef intestines.

See: Beef Tripe Stew.

Morcella Blood Pudding

Nonspecific. The product is made from pork fat, beef blood and/or pork blood, and may contain meat.

Mortadella

Normally a cooked sausage but can be dry or semidry. It is similar to salami and cervelat except that it has large chunks of pork fat. Red sweet peppers up to 4% and pistachio nuts up to 1% are acceptable as long as they are shown in the true product name.

Mortadella (Canned)

Canned items designated "Mortadella" must be labeled with the phrase "Perishable, Keep Under Refrigeration" and must have an MPR of 3.85:1 or less.

Mortadella—Poultry

Poultry Mortadella is a dry, semidry, or cooked sausage formulated with poultry. The sausage must contain large chunks of pork fat and may contain extenders and/or binders. Red sweet peppers are permitted up to 4% and pistachio nuts up to 1% and shown as added in the true product name.

If product is canned, the MPR must not exceed 3.85:1, the internal temperature must have reached 160°F, and the product labeled "Perishable, Keep Under Refrigeration" or similar wording.

Mortadella without Fat Cubes or Chunks

Product must meet the standard for Mortadella and the label be qualified to indicate the absence of Fat Cubes or Chunks, e.g., "Mortadella without Fat Cubes" or "Mortadella without Fat Chunks."

Mousaka, Moussaka, Musaka (GK)

Must contain at least 25% meat. Mousaka is a casserole containing layers of meat and eggplant made in various ways throughout the Middle East. A true product name is required, e.g., "Eggplant and Meat Casserole."

Mullicatawny Soup

Product must contain at least 2% cooked poultry meat and enough curry powder and pepper to characterize the product. The label must show a true product name, e.g., "Chicken Mullicatawny Soup."

Mulligan Stew

Product must contain at least 25% fresh meat or meat and poultry. Mulligan stew is a mixture of vegetables and meat combined in a gravy or sauce. The label must have a true product name, e.g., "Chicken and Meat Mulligan Stew."

Mustard Bran

This is not considered a spice and must be declared as "Mustard Bran." It is not acceptable in sausage.

Mustard Flour

It is a spice that is commonly used in sausage products.

Myvacet

(Distilled Acetylated Monoglycerides). Acceptable for use as a coating on sausage casings. Sausages coated with Myvacet should show, adjacent to the product name, a qualifying statement disclosing the presence of the compound, e.g., "Summer Sausage Coated with a Solution of Distilled Acetylated Monoglycerides."

Nacho Style, Nacho Flavor, and Similar Terms

Acceptable terminology for products possessing the commonly expected flavor characteristics associated with "Nachos," a Mexican hors d'oeuvre. The characterizing flavor components generally include, but are not limited to, cheese (Cheddar or Monterey Jack), tomato (tomato solids, tomato powder), spices, or other natural seasonings and flavorings (usually garlic and onion), and chili peppers (mild or hot). Romano and Parmesan cheese are also often present. However, these cheeses may not be used to satisfy the above cheese requirement.

Natural Claims

The term "natural" may be used on labeling for meat products and poultry products, provided the applicant for such labeling demonstrates that:

1. The product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and
2. The product and its ingredients are not more than minimally processed. Minimal processing may include (a) those traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or (b) those physical processes that do not fundamentally alter the raw product and/or which only separate a whole, intact food into component parts, e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices.

   Relatively severe processes, e.g., solvent extraction, acid hydrolysis, and chemical bleaching would clearly be considered more than minimal processing. Thus, the use of a natural flavor or flavoring in compliance with 21 CFR 101.22, which has undergone more than minimal processing, would place a product in which it is used outside the scope of these guidelines. However, the presence of an ingredient that has been more than minimally processed would not necessarily preclude the product from being promoted as natural. Exceptions of this type may be granted on a case-by-case basis if it can be demonstrated that the use of such an ingredient would not significantly change the character of the product to the point that it could no longer be considered a natural product. In such cases, the natural claim must be qualified to clearly and conspicuously identify the ingredient, e.g., contains refined sugar.

All products claiming to be natural or a natural food should be accompanied by a brief statement that explains what is meant by the term natural, i.e., that the product is a natural food because it contains no artificial ingredients and is only minimally processed. This statement should appear directly beneath or beside all natural claims or, if elsewhere on the principal display panel, an asterisk should be used to tie the explanation to the claim.

The decision to approve or deny the use of a natural claim may be affected by the specific context in which the claim is made. For example, claims indicating that a product is natural food, e.g., "Natural chili" or "chili—a natural product" would be unacceptable for a product containing beet powder that artificially colors the finished product. However, "all natural ingredients" might be an acceptable claim for such a product.

Natural Smoked Color

Approval can be properly granted to labels with this statement when the products involved are "Smoked" and not artificially colored. The results of the use of artificial smoke materials can, by means of a number of processing operations, result in a color characteristic being acquired by the frankfurters, bologna, and the like. The term "Natural Smoked Color" can be used to properly identify this point.

Navarin

Navarin is a stew containing lamb or mutton and vegetables and considered a national dish of France. It must meet the meat stew standard of 25% meat. Show true product name, e.g., "Navarin-Lamb Stew."

Negative Labeling

1. Negative labeling is allowed if it is unclear from the product name that the ingredient is not present. For example, the use of the term "no beef" on the label of "turkey pastrami" would further clarify that the product does not contain beef.
2. Negative labeling is allowed if the statement is beneficial for health, religious preference, or other similar reasons. For example, highlighting the absence of salt in a product would be helpful to those persons on sodium-restricted diets.
3. Negative labeling is allowed if the claims are directly linked to the product packaging, as opposed to the product itself. For example, flexible retortable pouches could bear the statement "no preservatives, refrigeration, or freezing needed with this new packaging method."
4. Negative labeling is allowed if such claims call attention to the absence of ingredients because they are prohibited in a product by regulation or policy. The statement must clearly and promi-

nently indicate this fact, so as not to mislead or create false impressions. For example, "USDA regulations prohibit the use of preservatives in this product" would be an acceptable statement for ground beef.

5. Negative labeling is allowed to indicate that absence of an ingredient when that ingredient is expected or permitted by regulation or policy. This could also apply to ingredients that are not expected or permitted by regulation or policy if the ingredients could find their way into the product through a component. For example, the use of "no preservatives" on the label of "spaghetti with meat and sauce" (where regulations do not permit the direct addition of preservatives) would be acceptable if the product contained an ingredient, such as cooking oil, which could contain antioxidants but do not.

   These guidelines do not preempt the requirements of the nutrition labeling regulation. Therefore, negative claims such as "unsalted" would have to comply with the provisions stated in the nutrition labeling regulations.

Net Quantity of Contents on Combination Packages

The guidelines for stating the net quantity of contents on combination packages containing both liquid and solid products are as follows:

1. The declaration of net quantity of contents for a combination package should be expressed in terms of fluid measure for individual products that are liquid and in terms of avoirdupois weight for individual products that are solid, semisolid, or viscous, provided the quantity statements for identical packages or units are combined. For example, the fruit drink would be expressed in fluid measure and the meat, cheese, crackers, and cookies would be expressed in the combined avoirdupois weight.
2. The declaration of quantity should be preceded by one of the following terms, as appropriate—"Net Weight," "Net Wt.," or "Net Contents."

The net quantity of contents declaration may appear in more than one line. Therefore, both stacked and side-by-side declarations would be considered appropriate.

- Descriptive terms may be used to identify the liquid and solid components of the package, e.g., entree, meal, or drink; however, such terms should not include brand names.
- Connecting words such as "and" or "plus" are permitted to be used as part of the declaration of contents.

Examples of acceptable net content declarations are as follows:

1. Entree Net Wt. 8 oz., Drink 4 fl. oz. (120 ml)
2. Net Contents—lunch 8 oz. plus fruit drink 4 fl. oz.
3. Net Wt. 8 oz. Drink 4 fl. oz. (120 ml)
4. Net Weight 8 oz. and 4 fl. oz.

Federally inspected meat and poultry products are exempt from the requirements of the Fair Packaging and Labeling Act (FPLA), including the mandatory metric labeling provisions that went into effect February 14, 1994. However, if metric labeling is included voluntarily, such labeling should comply with the FPLA.

The guidelines contained in this policy memo will be subject to the provisions prescribed in 9 CFR 317.2(h) and 9 CFR 381.121 of the federal regulations.

Net Weight Statement

Divider Pak—On a product where two cans are taped together, one of which contains the meat or poultry item and the other a vegetable, e.g., "Chicken Chow Mein," the meat or poultry label may include the net weight on the 20% panel. The vegetable can bears the true name of the product with the total net weight of the other can and the drained weight of the vegetable can.

Double Packing—When a poultry product and a nonpoultry product are separately wrapped and placed in a single immediate container bearing the name of both products, the net weight shown on the immediate container may be the total net weight of the two products or the net weight of the poultry product and the nonpoultry product separately.

Additional Net Weight Information—Nonregulatory information of a net weight nature, e.g., four 3-oz. packages, accompanying a net weight statement is acceptable and need not adhere to the size and spacing restrictions.

Open Net Weights—Open net weights may be presented in pounds and ounces, decimals, decimal fractions, or fractions, e.g., 1½ pounds, 1.6 pounds.

Net Weight Requirements—The statement of net quantity of contents is required on all products intended for sale at retail intact. In addition, shipping containers must bear a net quantity of contents statement if the product inside is not uniform in weight (i.e., random weight). Piece counts may not be used in lieu of a required net quantity of contents statement on a shipping container but may be used as additional information.

Multi-Unit Retail Packages—Fully labeled packages of more than one of the same meat or poultry product packages in an open (i.e., clear) overwrap do not have to include a net weight statement.

See: 9 CFR 317.2(h), and 9 CFR 381.121(b).

Net Weight Statements on Packages with Header Labels

The guidelines for determining the size and location of net weight statements on meat food product packages with header labels are as follows:

1. The entire front of the package is considered the principal display panel of the package, and its area is used to determine the size of the net weight statement. Print size specifications for the net weight statement specified by the regulations must be followed.
2. The net weight statement should be placed within the lower 30% area of the header label if no other mandatory labeling features are printed on the rest of the principal display panel of the package. If mandatory features do appear below the header label, the net weight statement must be placed within the lower 30% of the total area containing any mandatory information.

A "Header Label" is a small label applied across the top of a package usually bearing all of the mandatory labeling information. The rest of the package is most often a clear film containing a meat or poultry product, e.g., luncheon meat. This type of packaging is designed to be used on pegboard-type displays.

"New" and Similar Terms

Terms like "new," "now," "improved," and similar terms may be used within the following guidelines:

1. The terms may only be used for a period of 6 months from the date of the initial approval, except as noted in 2., 3., and 4. below.
2. Extensions to the 6-month period may be granted if:
   a. Processors can demonstrate that production or distribution delays precluded the use of the approved labeling as scheduled. In such situations, the lost time can be restored.
   b. Processors can demonstrate that labeling inventory needs for the 6-month period were overestimated due to poor sales. The processors must maintain records that indicate the amount and the date the labeling was originally purchased. In this situation, up to an additional 6 months can be granted. No further extension will be considered.
3. In those situations where it is customary to distribute "new" products to various geographical regions, each geographic area may receive a sketch approval for 6 months if the processor can assure adequate controls over the segregation and distribution of the products.
4. In situations where it is customary to test market product in no more than approximately 15% of the intended total marketing area before total distribution begins, labeling for the test market area can receive a sketch approval and also be included in the 6-month sketch approval given to the labeling of the product distributed to the total marketing area. Processors must be able to assure that only 15% of the total market is involved in test marketing.

New England Boiled Dinner

Product must contain at least 25% cooked "Corned Beef."

New Orleans Style

Acceptable for products that contain any five of the following ingredients: Roux base, rice, onion, green onions, garlic, celery, bell peppers, cayenne pepper, white pepper, parsley, or tomato.

The product may contain various protein sources including seafood and game.

Nitrite

Calculations should be based on the total meat block including the muscle tissue, fat, and blood (e.g., "Blood Pudding"). If the product is cured, the blood would be included and considered part of the meat.

Nonamenable Product/Voluntary Inspection

Examples of nonamenable products are sandwiches containing meat or poultry, clam chowder that has less than 1% bacon for export to Japan, and natural casings for export. Any nonamenable product can be produced under voluntary inspection when requested. See 9 CFR 318.13 and Subchapter B, Part 350.3(c). However, most FSIS requirements have to be met concerning labeling, i.e., mandatory labeling features, an accurate ingredients statement, handling statement, etc. Safe Handling Instructions are not required even for raw nonamenable products. FDA nutrition labeling rules apply to such products.

Nondairy White Sauce or Nondairy Sauce

A sauce made with a nondairy creamer. If this type of a sauce is proposed for use with "Chipped Beef," a suitable name would be "Non-Dairy White Sauce with Chipped Beef" or "Non-Dairy Sauce with Chipped Beef." The reference to "Cream" or any of its derivations should not appear in the product name.

Nonspecific Meat Food Products

Red meat items of this type do not have specific requirements, i.e., they do not possess a standard of identity or composition. Consequently, these products should be identified by one of two ways: (1) A descriptive name that identifies characterizing components and/or ingredients, or (2) a fanciful or coined name that is accompanied by an ingredients statement. The latter approach should be used when the use of a descriptive name is not practical, e.g., when the descriptive name would read like an ingredients statement.

When a fanciful name or coined name is used, the ingredients statement should appear contiguous to the product name on the principal display panel of an immediate container.

Nonstandardized Cooked Sausage Products Containing Both Livestock and Poultry Ingredients

The labeling of nonstandardized cooked sausage products must comply with 9 CFR 319.180.

Meat food products are those in which more than 50% of the livestock and poultry product portion consists of livestock ingredients. Such cooked sausage products that contain poultry ingredients at more than 15% of the total ingredients (excluding water) must have product names that indicate the species of livestock and kind(s) of poultry ingredients, e.g., "Beef and Turkey Frankfurter" or "Frankfurter Made from Beef and Turkey. "

Poultry food products are those in which more than 50% of the livestock and poultry products portion consists of poultry. Livestock ingredients at more than 20% of the total poultry and livestock ingredients must have product names that indicate the kind(s) of poultry and species of livestock ingredients, e.g., "Turkey and Beef Frankfurter" or "Frankfurter Made from Turkey and Beef." Such cooked sausage products that contain livestock ingredients at 20% or less of the total poultry and livestock ingredients must have product names that are appropriately qualified to indicate the inclusion of livestock ingredients, e.g., "Turkey Frankfurter—Pork Added" or "Turkey Frankfurter With Pork." (The product names of cooked sausage products that contain no livestock ingredients designate the kind[s] of poultry ingredients, e.g., "Turkey Frankfurter.") Cooked sausage products containing over 50% meat ingredients would carry the red meat legend while those containing over 50% poultry ingredients would carry the poultry legend.

Noodle Chicken Vegetable Dinner or Noodle Chicken Dinner with Vegetables (Canned or in glass jars).

Product must contain at least 6% cooked chicken.

Nugget Labeling

Nuggets are irregularly shaped, usually bite-sized meat and/or poultry products that are usually breaded and deep fat fried and intended to be used as

finger foods. There are a number of different types of nuggets, the labeling for which follows:

1. Products made from a solid piece of meat or poultry may use the term "Nugget" as part of the product name without further qualification (e.g., "Chicken Nugget," "Beef Nugget").
2. Products made from chopped and formed meat or poultry may use the term "Nugget" as part of the product name, provided a qualifying statement describing such process is shown contiguous to the product name (e.g., "Chicken Nugget, Chopped and Formed," or "Beef Nugget, Chopped and Formed").
3. Products made from chopped meat or poultry and containing binders, extenders, and/or water may use the term "Nugget" as a fanciful name, provided a descriptive name immediately follows "Species" or "Kind" nugget (e.g., "Breaded Nugget-Shaped Chicken Patties").
4. Products described in (1), (2), and (3) above that are breaded should be labeled as "breaded" and should be limited to 30% breading.

Oat Fiber

"Oat fiber" should be identified in the ingredients statement as "isolated oat product." It may be used in nonstandardized products and in products, such as, "taco fillings."

Oleomargarine

The Establishment Number may be omitted from the outer container, provided that articles are completely labeled including Establishment Number inside.

See: 9 CFR 317.2(i).

Omelet, Denver or Western Style

Product must contain at least 18% ham with onions and green and/or red peppers.

Omelet, Florentine

Product must contain at least 9% cooked meat and must contain spinach.

Omelets With

Bacon—must contain at least 9% cooked bacon
Chicken Livers—must contain at least 12% cooked liver
Corned Beef Hash—must contain at least 25% corned beef hash
Creamed Beef—must contain at least 25% creamed beef
Ham—must contain at least 18% cooked ham
Sausage—must contain at least 12% dry sausage
Sausage and Cheese (omelet with pepperoni, cheese, and sauce)—must contain at least 9% sausage in the total product

Open Dating

Labels showing further qualifying phrases in addition to the explanatory phrase must submit with the application sufficient documentation to support these additional claims. See 9 CFR 317.8(b)(32) and 9 CFR 381.129(c). Some local authorities require that packaged foods heated and sold hot from industrial catering vehicles be dated with the day the foods were placed in the warming units (e.g., Tuesday, Friday, etc.). When assured by the local authorities that the foods are under a rigid local inspection program, the designations may be approved without an explanatory statement as required by the regulations. To date, only the county of Los Angeles, California, has provided this assurance.

The packing date should be shown on immediate or shipping containers of poultry food products as required by the following regulations: 9 CFR 381.126 and 381.129(c). When meat or poultry products are packed and held in freezer storage for later repacking, the explanatory phrase on repacked product should be in terms of "sell by" or "use before." However, if a "packed on" phrase is desired, the date shown should be that of the original packing of the product.

Ostrich and Other Ratites (Emu)

Products that do not contain 3% of beef, pork, chicken, or turkey cannot contain cure ingredients, i.e., Nitrite, nitrate.

Paella Con Bacalao (SP)

Product must contain at least 35% cooked meat or poultry meat and include seafood and no more than

25% cooked rice. The label must show true product name, e.g., "Beef and Fish with Rice."

Papain

Meat and poultry products that are dipped in a solution containing papain should show in conjunction with the product name a statement, e.g., "Tenderized with a solution of (list ingredients of solution)." Carcasses of animals treated with papain by antemortem injection should be roller branded "Tendered with Papain." Parts not so marked should be labeled as "Tendered with Papain."

See: 9 CFR 317.8(b)(25), 9 CFR 381.120, Enzymes-Proteolytic.

Paprika

Generally, paprika and/or oleoresin of paprika are not permitted in or on fresh red meat products, fresh ground poultry, or fresh poultry sausage. They are permitted under the following conditions:

1. In both red meat and poultry products where such ingredients are acceptable and expected, including Italian Sausage, Salisica, Chorizo, Longaniza, and Hungarian Style products. All requests for additional products should be referred to the Labeling and Consumer Protection Staff to determine their acceptability.
2. On red meat products where their use does not misrepresent the leanness or freshness, e.g., application to a surface layer of fat and not to the muscle tissue. However, the name must be appropriately qualified, e.g., "coated with paprika" or "artificially colored."
3. In or on products where they are expected and the product name discloses this fact, or the product name refers to a component expected to contain the ingredients. Examples include "Beef with Barbecue Sauce," "Beef—Barbecue Flavor," "Chicken Paprikash," "Chicken with Orange Sauce," or similar type products.
4. In fresh whole muscle poultry products, provided their presence is properly described, e.g., "coated with paprika," or "artificially colored," as appropriate.

Partially Cooked

1. Partially cooked bacon—acceptable nomenclature if shrink requirement for fully cooked bacon is not met, must meet requirements for trichinae treatment. Cooking instructions are required.
2. Partially cooked poultry—unacceptable for cooked poultry products.

Partially Defatted (Beef or Pork) Fatty Tissue

These are by-products produced from fatty trimmings containing less than 12% lean meat. These ingredients may be used in meat products in which by-products are acceptable. Products include nonspecific loaves, beef patties, frankfurters with by-products, bologna with variety meats, imitation sausage, potted meat food product, sauces, or gravies. May be used in excess of the amounts of meat necessary to satisfy the standard for only the products listed in the Policy Book. However, in this situation, the PDCB or PDCP must always be declared in the ingredients statement.

See: 9 CFR 319.15(e) 9 CFR 319.29(a).

Partially Defatted Chopped (Beef or Pork) (PDCB, PDCP)

1. Partially Defatted Chopped Beef is not permitted in hamburger, ground, or chopped beef. The School Lunch Program requires that when PDCB is used in products like taco mix, which later may be used in preparing other products (e.g., tacos or patties), the PDCB or PDCP must always be declared in the ingredients statement on the labeling of the taco mix. All Beef or 100% Beef is acceptable as product name.
2. Partially Defatted Chopped may be used in excess of meat necessary to satisfy the standards on only the products listed in the Policy Book. However, in this situation, the PDCP must always be declared in the ingredients statement.

Partially Defatted Cooked (Beef or Pork) Fatty Tissue

This product may be used as an ingredient in the following: beef patties (cooked and uncooked), potted meat food product, sauces, gravies, imitation sausage, and nonspecific loaves. No limit on quantity is made. It is believed to be self-limiting.

The Amount and Labeling of PDCB and PDCP in Food Products

CLASS	FOOD CATEGORY	AMOUNT	LABELING
I.	Beef Patties	No Limit	Beef or Pork, or both
Imitation Sausage		No Limit	Beef or Pork, or both
Nonspecific Loaf		No Limit	Beef or Pork, or both
Potted Meat Food Product		No Limit	Beef or Pork, or both
Patty Mix		No Limit	Beef or Pork, or both
Beef for Roasting		12% of Meat Block	Always must be declared
II.	Chinese Egg Roll and other Chinese Specialties	Up to 12% of the Meat Block	Beef or Pork
Chopped Beef Steak		Up to 12% of the Meat Block	Beef or Pork
Corned Beef Hash		Up to 12% of the Meat Block	Beef or Pork
Fabricated Steaks		Up to 12% of the Meat Block	Beef or Pork
Pepper Steak		Up to 12% of the Meat Block	Beef or Pork
Salisbury Steak		Up to 12% of the Meat Block	Beef or Pork
Luncheon Meat (nonspecific)		Up to 25% of Meat Block	
Pizza Meat Topping		Up to 25% of Meat Block	Beef or Pork
Pizza with Meat		Up to 25% of Meat Block	Beef or Pork
Cooked Sausage, 9 CFR 319.180(b)		Up to 15% of Meat Block	Always must be declared
Pepperoni		Up to 15% of Meat Block	Must be declared
III.	Chili	Up to 25% of Meat Block or larger	As beef or pork, if larger must be declared
Meat Loaf		Up to 25% of Meat Block or larger	As beef or pork, if larger must be declared
Meat Balls		Up to 25% of Meat Block or larger	As beef or pork, if larger must be declared
Meat Fillings for Tacos, Burritos, Enchiladas, Tamales, and other Mexican Foods		Up to 25% of Meat Block or larger	As beef or pork, if larger must be declared
IV.	Corned Beef Hash	Up to 12% of Total Product Formulation	Beef

Note: All percentages as calculated on the basis of the fresh weight of meat content.

Partially Hydrolyzed Whey Protein

An acceptable ingredient name for a binder.

Pastelles (SP)

Product must contain at least 10% fresh meat. Product is always made with pork in Puerto Rico. The label must show the true product name, e.g., "Pork Pastelles."

Pastellillos (SP)

Puerto Rican Style product containing at least 8% cooked meat. Species is part of the product name. The label must show the true product name, e.g., "Pork Pastellillos."

Pastitsio

(Greek for casserole). Product must contain at least 25% fresh meat or 18% cooked meat. A product containing macaroni, ground beef, tomato paste, wine, white sauce, and Parmesan cheese that may be labeled "Greek Style Pastitsio."

Pastrami

Cooked cured beef with spices, generally made from the plate, but other cuts can be used. The product

must be smoked or treated with smoke flavoring. "Pastrami, Water Added" is not permitted, although similar products labeled according to Policy Memo 084A are permitted. The term "Unsmoked Cooked Pastrami" must be used when the product is not smoked or does not contain smoke flavoring. Pastrami may or may not be coated with spices. When product is coated, a qualifier is not required.

Pastrami Jerky

Acceptable name for product processed as pastrami prior to meeting the requirements for jerky.

Pastrami, Turkey

A cured turkey product that is cooked. The product must be smoked or treated with smoke flavoring. The term "Unsmoked Cooked Turkey Pastrami" must be used when the product is not smoked or does not contain smoke flavoring. Cured turkey thigh meat is an acceptable name.

Paté de Foie

Product must contain at least 30% liver. Paté means paste; foie means liver.

See: Foie Gras Products.

Patty Foldover MIT Dressing

Product must contain at least 50% patty.

Patties

Chopped and shaped and similar terms not required on products labeled patties.

1. Paprika not permitted in fresh meat patties.
2. PDCB or PDCP may be listed as beef or pork, except in patties with mechanically separated (species) product and school lunch labeled products.
3. PDBFT and PDPFT permitted. Must show as such in the ingredients statement.
4. Meat patties, with added fat up to 20% of the meat block, from a source other than that shown in the name, show as added (example is Veal Patties, Beef Fat Added), over 20% to be part of the product name, e.g., "Veal and Beef Fat Patties."
5. Ground beef patties, no extenders or water added. Hamburger patties, no extenders or water added. Same requirement as hamburger.
6. Prebroiled beef patties with simulated stripes (patties are deposited on conveyor and prebroiled). Parallel stripes are applied with a solution of caramel coloring and water through parallel spigots. Product name will identify artificial color marks on the label.
7. Antioxidants are permitted in pork or beef patties both raw and cooked.
8. Beef Patties—If beef by-products are added that are not permitted by the standard, the list of ingredients must immediately follow the product name. See: 9 CFR 319.15(c).
9. Pork Patties—The standard for beef patties, 9 CFR 319.15(c), should be applied with the exception if the species is pork.

Paupiette (FR)

Thinly sliced pieces of meat stuffed and rolled. Same standard as "Beef Roulade," which is at least 50% cooked meat.

Peanut Flour

Can only be used in nonspecific products that are not subject to moisture controls.

Pectin

Can be used at a maximum use level of 3% in nonstandardized meat and poultry food products. The common and usual name of the ingredient, regardless of its source, is "pectin" (21 CFR 184.1588).

Pepper

The term "pepper," as used in the Italian sausage regulation, refers to the pungent spices, such as black, white, cayenne, or red pepper. "Paprika" as an optional ingredient is less pungent and is used primarily for its coloring qualities. Bell peppers, chilies, paprika, and cayenne or red pepper are from the capsicum pepper family. These products have specific uses and are recognized by specific names. "Paprika" should not be substituted for "pepper" in a meat or poultry food product.

Pepperoni

A dry sausage prepared from pork or pork and beef. Combinations containing more than 55% beef are called beef and pork pepperoni. Pepperoni made with beef must be called beef pepperoni. Pepperoni must be treated for destruction of possible live trichinae and must have an MPR of 1.6:1 or less. Antioxidants are permitted in pepperoni. The casing, before stuffing, or the finished product, may be dipped in a potassium sorbate solution to retard mold growth. Extenders and binders are not permitted in pepperoni. Hearts, tongues, and other by-products are not acceptable ingredients.

Pepperoni, Cooked

Cooked pepperoni is not an acceptable product name.

Pepperoni with Poultry

Poultry may be added to pepperoni if properly labeled. If the meat block contains 20% or less poultry, the product is labeled "Pepperoni with Turkey (kind) Added." When poultry is over 20% of the meat and poultry block product is labeled "Pork and Turkey (kind) Pepperoni," an MPR of 1.6:1 is applied. If the amount of poultry exceeds that of the meat, the product label reads "Turkey and Pork Pepperoni." This would carry a poultry legend.

Peppers and Cooked Sausage in Sauce

Product must contain at least 20% cooked sausage in total formulation.

Perishable Uncured Meat and Poultry Products in Hermetically Sealed Containers

Establishments seeking approval of label applications for perishable, uncured products that have received a less rigorous heat treatment than traditionally canned product (9 CFR 318 and 381, Subparts G and X, respectively) must submit a sufficiently detailed processing procedure either incorporated on or attached to the FSIS Form 7234-1, Application for Approvals of Labels, Marking or Device. The procedure must include a description of product formulation, method(s) of preparation, cooking and cooling temperatures, type of container, and cooking and handling instructions.

Hermetically sealed containers include glass jars, metal cans, flexible retortable pouches, plastic semi-rigid containers, etc., that are airtight and/or impervious after filling and sealing.

The policy does not apply to raw meat or poultry, cooked or roast beef, cooked poultry rolls and similar products, whole or uncut cured products, or products that are distributed and marketed frozen. However, products containing cured meat or poultry as components in combination with raw vegetables, e.g., pasta salads and other chilled meat/poultry meals or entrees containing raw or partially cooked vegetables, are covered under this policy, provided the above-mentioned procedural attributes are indicative of the manufacturing process.

In addition, an approved partial quality control program (PQCP) is required that must address the critical points in the manufacturing process. As such, the PQCP must contain a detailed description of ingredient storage controls, product formulation and preparation, container filling and sealing, any heat treatment (times/temperatures) applied, including a description of the equipment used, any other treatments applied, cooling procedures (times/temperatures), lot identification procedures; finished product storage conditions, in-plant quality control procedures, and records maintenance procedures. The PQCP must be forwarded to the Processed Products Inspection Division (PPID) for appropriate review and approval before the product label may be used. Guidelines for development of PQCPs for these products may be obtained from PPID upon request.

Pet Food

1. Certified pet food is manufactured under fee-for-service inspection in a facility approved for the manufacture of animal food. Labeling regulations for certified animal food specify that approval is granted by the labeling staff. However, final approvals are not granted since LCPS no longer grants final approvals. Rather, the company should keep a copy of the final label attached to the sketch approval.
2. Most food for animal consumption produced in a federal facility is noncertified. It is not an in-

spected product; therefore, it is an inedible product and does not bear any mark of inspection. The product has to be conspicuously labeled to distinguish it from human food. Additionally, the labeling must be in conformance with 21 CFR Part 501, Animal Food Labeling since animal food labeling is also under the jurisdiction of the Food and Drug Administration.

Pfefferwurst (GR)

Product should conform to sausage standard and contain whole peppercorn. Pork livers, pork stock, and beef blood are not acceptable ingredients.

Phosphated Trimmings in Loaves

Trimmings from preparation of pork cuts, cured with approved phosphates besides other curing ingredients, may be used without limitation in loaves other than meat loaves. When such trimmings are used, phosphates may be listed in the ingredients statement using the term "sodium phosphates" or other applicable generic terms.

Phosphates in Dipping Solutions Containing Proteolytic Enzymes

Phosphates have been approved for use as buffering agents in dry mixtures intended for solutions containing proteolytic enzymes. The phosphates should not exceed 0.1% of the "tenderizing" solution if they are to be considered incidental additives.

Picadillo (SP)

Product must contain at least 35% cooked meat. A Mexican style hash usually made with beef, garlic, onions, vinegar, and raisins. The species should be in the product name, e.g., "Beef Picadillo."

Pickled Products, Dry Packed

Products that are pickled and dry packed should be qualified with the name of the pickle as part of the product name, e.g., "Knockwurst Pickled with Vinegar," or "Knockwurst Pickled." The weight of the package should be the weight of the product less the weight of the pickle that will weep out of the product.

Pie Filling

Product must contain at least 37% meat. Poultry pie filling must contain at least 18.75% cooked poultry meat.

Pies

Product must contain at least 25% meat. Meat in the gravy may be counted. Poultry pies require at least 14% cooked poultry meat.

Pies, English Style—Australian Style

Product must contain at least 25% meat or meat by-product. Contains gravy and no vegetables with a puff pastry top.

Pimiento (SP)

Refers to allspice, but must be specifically named. It is also known as Jamaica pepper.

Pimiento Sausage

Pimientos permitted when declared in product name as "Pimiento Sausage."

Pinklewurst (GR)

A cooked product that is stuffed in a casing with a diameter of from 1½ to 2 inches and a length of about 10 to 12 inches. It is formulated with beef fat, pork fat, onions, oat groats, water, and sufficient spice to satisfy seasoning requirements.

Piroshki or Pirogi

Product must contain at least 10% cooked meat. A Russian or Jewish dish made of thin rolled dough or pastry that is filled and either steamed, baked, or fried. They resemble small turnovers, pockets, or raviolis.

Pizza

The required meat fill is to be calculated on the stated net weight of the product, not on the formula weight.

See: 9 CFR 319.600 with:

Meat—At least 15% meat
Sausage—At least 12% cooked sausage or 10% dry sausage (e.g., pepperoni)
Poultry—At least 12% cooked poultry meat
Bacon—At least 9% cooked bacon
Chili with Beans—At least 25% chili with beans
Meat Patty Crumble—At least 15% patty crumbles (fresh) or 12% cooked

An antioxidant used in pepperoni or sausage need only be reflected in the ingredients statement as "BHA or BHT added to improve stability."

Pizza, Chicago Style

Acceptable labeling for a product that has been manufactured by first placing the cheese on the crust, then following with the meat and then the sauce. Condimental quantities of a grated cheese may then be placed on the top. The product usually has the deep-dish characteristics. The requirements for pizza as designated in 9 CFR 319.600 and various policies must be met.

Pizza, Combination or Deluxe

Product must meet the requirements for pizza as designated in 9 CFR 319.600. In a combination pizza, e.g., "Sausage and Pepperoni Pizza," the component declared last must be at least 25% of its required level in a pizza containing a single meat component.

Pizza Containing Cheese Substitutes

Meat requirements of 9 CFR 319.600 must be met. Labels that contain cheese in a ratio less than one part cheese to nine parts cheese substitute must contain additional qualifying information (example, Pizza—Sausage, cheese substitute, and cheese; Combination Pizza—Sausage, Pepperoni, Imitation Cheese, and Cheese).

Pizza Dogs

A nonspecific product.

Pizza, Pan Style

Pizza that is marketed in a pan and contains a thick crust.

Pizza Pups

Product has two crusts, filled with a mixture of pork, tomato puree, and condimental substances. The finished article is approximately 8 inches in length, 2½ inches wide with a thickness of ¾ inch. It is a type of pizza. The label must show a true product name, e.g., "Pork and Sauce Filling in a Crust."

Pizza Roll

This is a nonspecific meat food product. When the name appears on a label, there must be a contiguous statement identifying the major components of the product or a complete ingredient listing. There are two major types of pizza rolls. One is a cooked sausage-like meat food product that contains cheese, usually contains peppers and has no water limitation. The second type consists of a roll-shaped dough enclosure with various fillings. A manufacturer of the latter type of product has asserted trademark protection of the term "pizza roll."

Pizza Sausage

Not an acceptable name. Product must be labeled "Sausage for Pizza."

Pizza, Sicilian Style

A thick crust pizza. The crust is usually 50% or greater of the total pizza product.

Pizza Topping Containing Sausage

The sausage portion of cooked pizza topping is permitted to contain up to 10% water and 3.5% binders (9 CFR 319.140). In addition, the application must indicate the sausage portion within the pizza topping formula. However, the ingredients statement of the cooked pizza topping does not have to list "sausage" in its sublisting. There are no restrictions on the amount of seasonings in the sausage portion.

1. Pizza topping must be cooked.
2. In the sausage portion:
   a. Water $<10\%$ of the sausage portion
   b. Binders including TVP $<3.5\%$ of the sausage portion
   c. Seasonings unlimited

3. The ingredients statement of the pizza topping can be arranged in different ways:
   a. Composite, such as, cooked pizza topping (port, water, TVP [. . .], Seasonings [. . .])
   b. Component, such as, cooked pizza topping (sausage made with pork, water, seasonings [. . .], TVP [. . .], water [. . .])

These parameters do not apply when specific sausage products are governed by other regulations, e.g., Italian Sausage. In these situations, the specific regulation (i.e., Italian Sausage) dictates the requirements, such as, the amount of water permitted and binders permitted.

Pizza Topping Mix

A nonspecific product, including those products that indicate the type of meat or poultry in the product name (e.g., Chicken and Pork Pizza Topping or Beef Pizza Topping). Antioxidants are permitted. See 9 CFR 318.7(c)(4). Water, extenders, and binders are acceptable.

Pizza, Word Size

When a pizza has a true product name, e.g., "Combination Sausage and Pepperoni Pizza," the true product name must be prominent, conspicuous, and legible, with all words at least one-third the size of the largest letter in any word of the product name. If on the label, the manufacturer also elects to display elsewhere the word "Pizza" in exaggerated fashion, the word "Pizza" is not considered in the determination of the size of the letters within the true product name.

PFF (Protein Fat Free) Adjusting for Use

Protein Fat Free (PFF) controlled cured pork products with qualifying statements, e.g., "Ham-Water Added," may be used in place of PFF controlled cured pork products without qualifying statements, e.g., Ham, to meet the minimum meat requirements of various products. However, the amounts of the PFF controlled cured pork products with qualifying statements used will need to be increased. For example, if a standard requires a certain amount of Ham and a processor wishes to use "Ham-Water Added," a greater amount of the "Ham-Water Added" will be needed to meet the standard. The magnitude of the additional amount is directly related to the relationship between the respective PFF values.

Example: Ham Salad requires 35% Cooked Ham. "Ham Water Added" will be used in the product formula.

Calculation: Multiply the PFF value for Ham (20.5) by the amount of required Ham (35%). Divide this answer by the PFF value of the product being used to formulate the product. (In this example, PFF value for "Ham-Water Added" is 17.0.)

Answer: $[(0.35 \times 20.5) / 17.0] \times 100 = 42.21\%$ "Ham-Water Added" needed in the formula.

Example: Ham Pie requires 25% Ham based on green weight. "Ham with Natural Juices" will be used in the product formula.

Calculation: Multiply the PFF value for Ham (20.5) by the amount of required ham (25%). Divide this answer by the PFF value of the product being used to formulate the product.

(In this example, PFF value for "Ham with Natural Juices" is 18.5.)

Answer: $[(0.25 \times 20.5) / 18.5] \times 100 = 27.70\%$ "Ham with Natural Juices" needed in the formula.

Adjusting for "Ham and Water Product X% of the Weight is Added Ingredients"

Consider a formulated product that is required to contain at least 50% Cooked Ham. If the processor chooses to use a "Ham and Water Product (HWP)" in which 20% of the weight is added ingredients as the source of the Ham in the formulation, this product contains 80% Ham and 20% added ingredients. Clearly, the processor must use more than 50% HWP in the process. Using 50% HWP would result in only 40% Ham in the finished product, i.e., the added ingredients in the HWP represents 25% of the Ham content. (If it were a 10-pound HWP, there would be 8 pounds of Ham and 2 pounds of added ingredients. $(2 / 8 \times 100 = 25\%)$. Consequently, an additional 25% of HWP is required in the formulation.

The following example may be used to determine the percentage HWP needed to equal Ham:

Ham and Gravy requires 50% Cooked Ham. "Ham and Water Product 20% of Weight is Added Ingredients" will be used in the formulation.

Step 1: Subtract the % added ingredients from 100%. In this example: 1.00 − 0.20 = 0.80)
Step 2: Determine the amount of Ham needed in the formula. (In this example: 50%)
Step 3: Divide the amount of Ham required. Determined in Step 2 by the answer in Step 1. (In this example: (0.50 / 0.80 = 0.625)
Step 4: Multiply the answer in Step 3 by 100. Answer for this example is 62.50% "Ham and 20% Water Product" is needed as the equivalent of 50% Ham.

Plantation

The regulations and policies applicable to "Farm" also apply to plantation.

Point of Purchase Materials

Point of purchase materials that refer to specific meat or poultry products are considered labeling under certain circumstances. When printed and/or graphic informational materials (e.g., pamphlets, brochures, posters, etc.) accompany or are applied to products or any of their containers or wrappers at the point of purchase, such materials and the claims that they bear are deemed labeling and they are subject to the provisions of the Federal Meat Inspection Act and the Poultry Products Inspection Act.

Although the Food Labeling Division (FLD) does not exercise its authority to subject point of purchase materials to specific prior approval (materials shipped with the products from the federally inspected establishment are an exception), point of purchase materials are expected to be in accordance with the federal regulations and all current labeling policies. Upon request, FLD will review and comment on the point of purchase materials submitted to our office. During the review process, promotional materials will be scrutinized for special claims, particularly those related to nutrition, diet, and animal husbandry practices.

Claims related to nutrition and diet must be made in accordance with all current nutrition labeling regulations. Continuing compliance with stated claims will be assured through periodic sampling, as necessary. Claims are expected to be within the compliance parameters identified in the nutrition labeling regulations.

Animal husbandry claims (e.g., the nonuse of antibiotics or growth stimulants) may be made only for products shipped in containers or wrappers labeled with the same animal production claims.

Polish Sausage

A sausage that is cured, cooked, and usually smoked. Pork and pork by-products should comprise at least 50% of the meat and meat by-product ingredients. To have beef as a predominant ingredient, the product name would be "Beef and Pork Polish Sausage." Green peppers are permitted up to 4% in total formulation.

An uncured (fresh), uncooked variety with no more than 3% water also exists. "Fresh" should be used in the name when the product is uncured. When Fresh Polish Sausage is cooked or smoked, then the product name is either "Cooked Fresh Polish Sausage" or "Smoked Fresh Polish Sausage." The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

Polynesian Style Sausage

Product must contain fruit juices, a sweetening agent, and soy sauce.

Polysorbate

Permitted in pickling solutions without declaration.

Pork and Bacon Sausage

Up to 50% bacon permitted provided:

1. Bacon is brought back to green weight before use.
2. Product is trichinae treated.
3. Product name is "Pork and Bacon Sausage."

The standard for "Pork Sausage and/with Bacon" is 10 to 20% bacon, and for "Pork and Bacon Sausage" is more than 20% but not more than 50% bacon.

Pork and Dressing

Product must contain at least 50% cooked pork.

Pork and Dressing with Gravy

Product must contain at least 30% pork.

Pork Cracklings

Product eligible to be labeled as "Pork Cracklings" must be prepared from fatty tissues from which the skin has been detached. If the skin is not removed from the product before rendering, a descriptive name, e.g., "Pork Cracklings, Fried-Out Pork Fat with Attached Skin," must be used.

Pork Fat

Pork fat should be declared as such in the ingredients statement. Clear fatbacks and clear shoulder plates must be declared as "Pork Fat." Pork fat may be declared as pork in the ingredients statement if it contains visible lean and it is used in a standardized product that has a fat limitation.

Pork Jowls

Product may be declared as pork if skinned.

See: Pork Skins.

Pork Sausage

Product identified as pork sausage does not include the use of pork cheeks. When such an item is offered as "Whole Hog," tongues, hearts, and cheeks may be used in the natural proportion as found in the hog carcass. "Fresh" should be used in the name when the product is not cured, cooked, and/or smoked.

Pork Skin Residue After Gelatin Extraction

This material consists of back fat skins from which the gelatin has been extracted by means of soaking the skin in acid and subsequent low temperature cooking for the extraction of gelatin. It is not permitted in sausage but may be used in imitation sausage, potted meat food product, loaves (other than meat loaves), and other nonspecific products.

Pork Skins

Not permitted in salami, bologna, frankfurters, Vienna sausage, and braunschweiger. When packed in vinegar pickle, they are not permitted to be artificially colored. When pork skin, either attached to fat and/or muscle tissue or detached from fat and/or muscle tissue, is used to manufacture meat or poultry products, it must be specifically listed in the formulation on the label approval application form and in the ingredients statement on the label, e.g., "Pork Skins," "Unskinned Pork Jowls," "Unskinned Pork Shoulder Trimming," "Unskinned Pork Fat," and "Unskinned Pork Bellies."

"Detached skin" refers to the portion of skin from which most of the underlying fat is removed, e.g., skin from bacon intended for slicing, skin from closely skinned hams, shoulder cuts, fat backs, etc. If removal of skin portions is incidental to removal of a considerable proportion of underlying fat from ham, shoulder, back, etc., preparatory to rendering such fat, portions of skin so removed should not be regarded as detached skin and may be included with fats and rendered into lard. Ham facings are not regarded as detached skin.

Pork Skins, Fried

When prepared from the skin of smoked pork bellies, it may be labeled as "Fried Bacon Skins," "Fried Bacon Rinds," or "Fried Pork Skins." The kind of skin used must be stated on the labeling records when submitted for label approvals.

Pork Spare Ribs, Center Cut

Center cut pork spare ribs refers to pork spare ribs with the loin portion, the brisket (brisket must be removed at a point that is dorsal to the curvature of the costal cartilages), the tail and two ribs from the shoulder removed, this remaining center section may be further portioned or left in one piece.

Pork Spare Ribs, St. Louis Style

St. Louis Style Spare Ribs are the same as "Pork Spare ribs" except that the sternum and the ventral portion of the costal cartilages are removed with the flank portion. This cut is made at a point in which the sternum and costal cartilages are removed dorsal to the curvature of the costal cartilages. If specified by the purchaser, the diaphragm should be removed.

This anatomical description of the cut must be provided with the information for label approval.

Potato Sausage, Swedish Style or Potato Ring Swedish Style

A cooked or uncooked meat food product with the following requirements:

1. At least 45% meat and no by-products.
2. Water limited to 3% at formulation.
3. Extenders or binders limited to 3.5% of the finished product, except that 2% of isolated soy protein should be deemed to be equivalent of 3.5% of any of the other binders or extenders.
4. Contains at least 18% potato.

Poultry

Cuts of poultry that are not identified in 9 CFR 381.168, Table V, may use the maximum amount of poultry skin permitted for that "kind." For example, "turkey" is listed in the table and may contain up to 15% skin. Therefore, a product identified as "white turkey" can be placed in this category for a maximum of 15% skin.

Poultry Standards

Name	Minimum or Maximum percentage
Poultry a La King	At least 20% poultry meat
Poultry Barbecue	At least 40% poultry meat
Poultry, Breaded	No more than 30% breading
Poultry, Brunswick Stew	At least 12% poultry meat
Brunswick Stew with Poultry	At least 8% poultry meat
Poultry Burgers	100% meat with skin and fat in natural proportions
Poultry Cacciatore	At least 20% poultry meat or 40% with bone
Poultry Cannelloni	At least 7% poultry meat
Poultry Chili	At least 28% poultry meat
Poultry Chili with Beans	At least 17% poultry meat
Poultry Chop Suey	At least 4% poultry meat
Chop Suey with Poultry	At least 2% poultry meat
Poultry Creole with Rice	At least 35% cooked poultry meat and sauce portion. Not more than 50% cooked rice in total product.
Poultry Chow Mein (w/o noodles)	At least 4% poultry meat
Poultry Croquettes	At least 25% poultry meat
Poultry, Creamed	At least 20% poultry meat
Poultry Dinners	At least 18% poultry meat
Poultry Fricassee	At least 20% poultry meat
Poultry Fricassee with Wings	At least 20% poultry meat
Poultry Gizzards and Gravy	At least 35% cooked gizzards
Poultry Hash	At least 30% poultry meat
Poultry Liver Omelet	At least 12% cooked poultry liver
Poultry Meatloaf	At least 65% raw poultry or 50% poultry meat
Poultry Noodle Dinner	At least 15% poultry meat
Poultry Noodle Dinner with Gravy	At least 6% poultry meat
Poultry with Noodles or Dumplings	At least 15% poultry meat or 30% poultry meat with bone
Noodles or Dumplings with Poultry	At least 6% poultry meat
Poultry Paella	At least 35% poultry meat or 35% poultry meat and other meat, no more than 35% cooked rice, must contain seafood
Poultry Parmigiana	At least 40% breaded poultry See: Veal Parmigiana
Poultry Pies	At least 14% poultry meat
Poultry Ravioli	At least 2% poultry meat
Poultry Salad Mix	At least 45% poultry

Poultry Salad	At least 25% poultry See: Salad mix, Poultry
Poultry Soup	At least 2% poultry meat
Poultry Flavored Soup	No minimum requirement (less than 2% poultry meat)
Poultry Spread	At least 30% poultry
Poultry Stew	At least 12% poultry meat
Poultry Stew with Dumplings	At least 8.4% poultry meat (Based on 70% of Stew requirement)
Poultry Subgum	At least 12% poultry
Poultry Tamales	At least 6% poultry meat
Poultry Tetrazzini	At least 15% poultry meat
Poultry Turnover	At least 14% poultry meat
Poultry with Gravy/Sauce	At least 35% poultry meat
Gravy with Poultry	At least 15% poultry meat
Poultry with Gravy and Dressing	At least 25% poultry meat
Poultry with Rice	At least 30% poultry and/or poultry by-products
Poultry Scrapple	At least 30% poultry and/or poultry by-products
Poultry with Vegetables	At least 15% poultry meat

Poultry, Assorted Pieces

The product name "Poultry (Kind) Assorted Pieces" is acceptable and does not require the product to be in natural proportions. In addition, the term "piece" is not the same as the term "part," i.e., a piece does not have to be a whole part, e.g., a breast, thigh, or drumstick.

Poultry Bacon

See: Bacon-like Products.

Poultry Breasts

When poultry breasts with ribs are boned and the resulting product contains portions of the scapula (shoulder) muscles and/or muscle overlying the vertebral ribs, they must be labeled to indicate that fact. Proper names for such products are "Boneless Breast with Rib Meat," "White Chicken Meat," or "White Turkey Meat," or if the skin is left intact, "White Boneless Chicken" or "White Boneless Turkey." Product labeled "Boneless Breast" without further qualification may not contain scapula or rib meat.

Poultry Frankfurters (Similar Cooked Sausages)

Products that contain pork fat must be labeled with pork fat added in the product name.

Poultry Grading (Labeling)

Indicates the quality grades of poultry (U.S. Grade A, B, or C). The shield design contains the letters "USDA," the U.S. grade of the product, and if not shown elsewhere, the class of poultry. Any letter grade on a consumer package or individual carcass indicates the product was graded by a licensed grader of the federal or federal-state grading service, and may not be applied otherwise. Letter grades on bulk packaging or shipping containers only indicate that the product is equal to that particular U.S. Grade.

A. Applying Grademarks to Shipping Containers

All poultry classes and kinds listed in 9 CFR 381.170, except necks, giblets, detached tails, wing tips, skin, and stripped backs (below Grade C) are eligible for grading.

In addition, the following poultry parts may be officially graded:

Boneless, Skinless Breast and Thigh Tenderloin or Boneless Breast without Tenderloin
Boneless Breast Quarters
Breast Quarters with Bone in
Boneless Thigh Halves
Wing Portion or Section Breast Halves
Broiler Turkey or Duck Halves
Split Breast Split Fryers Skinless, bone-in Thighs, Drums and Breasts Boneless Breast

Thigh Bone-in products marinated in a colorless solution

Poultry cuts other than those identified above may not be eligible for grading; therefore, particular attention should be given to the product name when approving labels for various poultry products that include grade marks (e.g., "Thin Breast Fillets, Thigh Strips").

Grade marks on raw poultry parts processed with solutions that may impart color (e.g., injected with a solution of water, salt, butter) or cooked poultry products must include a statement, e.g., "Prepared from Grade A Poultry." The USDA grader in the plant makes the final determination concerning the necessity of the "Prepared from" statement in situations where it is not apparent at the time of label approval that the added solutions have the ability to impart color to the finished poultry product.

Products that may not be grade marked:

Detached Necks
Giblets Packed Separately
Detached Tails
Wing Tips
Stripped Backs
Below C Quality Diced or Shredded Meat

B. Wing Description

The wing is made up of three sections. The section attached to the carcass is the first section. The wing tip is the third section.

C. Grading Backs with Necks

In applying grade standards when necks are packed with backs, follow these steps:

1. When backs are graded as provided for in the standards, the name of the product should read as follows:
   a. Grade A Backs "with necks," or "and necks."
   b. Grade B Backs "with necks," or "and necks."
   c. Grade C Backs "with necks," "Graded backs and necks," or "backs and necks."
2. Necks are to be packed with backs in natural proportions.
3. Necks may or may not be attached to backs. Necks for all officially graded backs are to be free from serious discolorations, feathers, pin feathers, and accumulations of blood and/or excess water.
4. A neck, front, or hind portion of back, when removed from birds that meet the stated quality, may be used to achieve exact weights. Only one of these portions may be used per package. Scraps of backs or necks may not be used.
5. Labels for packages with portions must indicate which portions, e.g., first (1st) portion, 2nd portion, 1st and 2nd portions, 2nd and 3rd portions, etc.

D. Pressure Sensitive Stickers and Tape

1. Inserts or pressure sensitive stickers with the grademark must have plant number.
2. Grademarks on pressure sensitive tape should not be used on consumer packages.
3. Inserts with the grademarks are not to be used inside opaque bags.

Poultry Hindquarters

The term "hindquarters" on labels for single cut poultry items is an acceptable alternative to the recognized terminology "Leg Quarter" specified in the regulations. The use of the term "Hindquarters" requires only a specified class of poultry to be considered a true product name, e.g., "Chicken Hindquarters." Either term refers to a poultry thigh and drumstick, with a portion of the back attached.

Poultry Hindsaddles

Poultry hindsaddles are connected poultry leg quarters (the rear of the bird). The product name "Poultry Hindsaddles" may be used alone on the product's label if the product is not intended for retail sale. In contrast, the name "poultry hindsaddles" on the label of a product intended for retail sale must be accompanied by a fully descriptive name (e.g., "Poultry Hindsaddles, Connected Leg Quarters").

Poultry Parts

Specific net weight packages for poultry parts, usually those containing legs or wings, include a single part, e.g., a drumstick or thigh, to make the stated weight. The name on the label must reflect this practice, e.g., "Chicken Legs—Chicken Thigh added to

make weight." The single part must be cut at the joint. Wing tips are not permitted as added parts.

Poultry Products

In poultry products where "meat" appears in the product name, e.g., "White Meat Chicken Roll," and "Dark Meat Turkey Loaf," skin and attached fat are permitted in greater than natural proportions. However, the ingredients statement must have the poultry skin or poultry fat listed. When skin and attached fat appear in the ingredients statement, their placement should be in the correct order of predominance and determined by the amount present over the permitted natural proportions.

Poultry Products Containing Meat Ingredients—Labeling

Poultry products containing meat in amounts that exceed 20% of the total meat and poultry product portion of the poultry product must be descriptively labeled to indicate the presence of the meat ingredients, e.g., "Chicken and Beef Stew or Stew made with Chicken and Beef".

Poultry products containing meat ingredients in amounts at 20% or less of the total meat and poultry product portion of the poultry product must have names that are qualified to indicate the presence of the livestock ingredients, e.g., "Chicken Stew—Beef Added."

However, poultry products that do not meet specified minimum poultry ingredient requirements because meat ingredients are replacing any part of the required poultry ingredients must be descriptively labeled to indicate the presence of meat ingredients, e.g., "Turkey and Pork Chop Suey."

Poultry Products with Other Than Natural Proportions of White and Dark Poultry

Poultry products containing white and dark chicken or turkey of a distinguishable nature and in quantities other than natural proportions of white to dark meat must bear a qualifying statement identifying the types of poultry meat used in conjunction with the kind of poultry in the product name. The poultry block of white and dark meat (excluding products labeled as "Mechanically Separated [Kind of Poultry]") solely determines the usage of the terms "white and dark," "dark and light," "white," "dark," etc., in the product name. Ground poultry (excluding the skin) that bears the terms "white/light," "dark," "breast," "thigh," etc., in the product names is also considered as part of the poultry block for determining the usage of terms "white and dark," "dark and light," "white," "dark," etc. However, products labeled as "Mechanically Separated (Kind)" do not have any bearing on the use of terms "white," "light," or "dark" in the product name since "Mechanically Separated (Kind)" is an indistinguishable paste-like product that is considered a separated standardized poultry food product ingredient.

Additionally, products with mixture of distinguishable poultry (white or dark) and "Mechanically Separated (Kind)" can not bear claims of "all white," "pure breast," "100% dark," or similar terms. In this situation, the poultry portion of the product contains at least two separated poultry ingredients, one of which is "Mechanically Separated (Kind)."

See: 9 CFR 381.117(c), Table 1.

Poultry Puffs

Product must contain at least 15% cooked poultry meat. Chicken or Turkey Puffs are classified as hors d'oeuvres and must show a true product name, e.g., "Breaded Chicken and Rice Balls."

Poultry, Raw Solution

Unless addressed by other regulations and policies, water- and/or oil-based solutions may be added to raw poultry and poultry parts at various levels with an appropriate qualifying statement to the product name.

The statement must include terms adequate to inform the consumer of the amount and manner of the addition and include the common or usual names of the ingredients in their proper order of predominance (e.g., "Injected with up to 12% of a solution of water, salt, and phosphates"). Other similar designations will be considered on their merits. The statement must be contiguous to the product name and printed in a style and color as prominent as the product name. The statement of the manner and amount of addition must be one-fourth the size of the most prominent letter in the product name. The ingredients of the solution can be printed one-eighth the size of the most prominent letter of the product name.

Terms like "Basted," "Marinated," "For Flavoring," and similar terms contemplated within the provisions of Section 9 CFR 381.169 of the poultry products inspection regulation cannot be used if the amount of the solution added is more than needed to baste, marinate, or flavor the product. Bone-in poultry and poultry parts are limited to 3% as prescribed by the regulations. Boneless poultry is limited to 8% to use these terms.

Poultry Roast

May be formulated with up to 10% liquid without a qualifying statement. If more than 10% liquid is used, the name must be qualified with a statement, e.g., "containing up to x% . . ."

Poultry Salami Products

Poultry sausages prepared to resemble salami and offered to consumers as a salami should bear product names as follows:

1. "(Kind) Salami" should be the product name when the moisture-to-protein ratio in the finished product does not exceed 1.9:1. This product resembles a dry salami made from red meats.
2. "Cooked (Kind) Salami" should be the product name when the product is cooked and the moisture-to-protein ratio is above 1.9:1. This product resembles "cooked salami" made from red meats.

Poultry Sausage

Sausage products made from poultry must be labeled to indicate kind, e.g., "(Chicken) Sausage," "(Turkey) Bologna," etc. Products containing more than one kind of poultry or red meat must declare the added ingredient in the product name, e.g., "Chicken Bologna, Beef Added" and "Turkey Franks, Chicken Hearts Added" per Policy Memo 029 dated September 4, 1981. The basic sausage standards, per meat 9 CFR 319.140, also apply to poultry, except for added water and fat.

Poultry Skin

When determining the amount of poultry skin allowed, refer to 9 CFR 381.168. If the specific part is not identified in this part, use the figure for boneless kind.

Poultry Tenders and Poultry Tenderloins

A "(Kind) Tender" is any strip of breast meat from the kind of poultry designated. A "(Kind) Tenderloin" is the inner pectoral muscle that lies alongside the sternum (breast bone) of the kind indicated.

Poultry Wing Sections—(Kind)

Wing Sections is an acceptable designation for a product consisting of equal proportions of the parts of a wing. It may be and is usually used for equal proportions of wing portions and drummettes.

Premier Jus or (Oleo Stock)

The product obtained by rendering at low heat the fresh fat of heart, caul, kidney, and mesentery collected at the time of slaughter of bovine animals. The raw material does not include cutting fats. Premier Jus is not an acceptable name unless accompanied by the term "Oleo Stock."

Pressure Sensitive Labels

Labels applied to packages should be of the self-destructive type and must adhere to the packages under all conditions of use.

Pressure Sensitive Stickers

Pressure sensitive stickers are a means for manufacturers to use existing labeling material by covering inaccurate and/or misleading labeling information with corrected text or used as a promotional tool, e.g., a starburst encircling sweepstakes terminology. A pressure sensitive sticker must be the type that destroys the underlying label or package if removed, or be self-destructive.

Temporary label approval is not required when the entire label including the pressure sensitive sticker is truthful, not misleading, and the product is not misbranded. Corrected test on the pressure sensitive sticker can cover mandatory or nonmandatory information.

Labeling bearing pressure sensitive stickers falls under the provisions of the generically approved labeling regulations 9 CFR 317.5 or 9 CFR 381.133, which indicates the conditions for use of final label-

ing without prior Washington approval. Companies need to create and maintain records of all final labeling, otherwise known as generic approvals.

Consistent with the rules on generic labeling, sketch labeling approval is required for the entire label when pressure sensitive stickers contain special claims (quality, nutrient content, health, negative, geographical origin, other claims, such as natural, animal production, such as, "no antibiotics administered," breed claims, etc.), guarantees, foreign language, or a change of the nutrition facts serving size.

Primal Parts and Subprimal Meat Cuts

Red meat carcasses, primals, subprimals, or cuts can be labeled as follows:

1. as the species of origin, e.g., beef tenderloin bearing the simple product name of "beef,"
2. as species without identifying the primal or subprimal when certain terms associated with various sizes are part of the product name, i.e., chop, cutlet, steak, fillet, filet roast, strips, etc., e.g., "Beef Steak,"
3. as species and primal or subprimal cut, e.g., "Veal Shoulder Blade Steak," and
4. as species, coin name (butt, cala, daisy, picnic, etc.) and primal or subprimal cut. The species and coin name are not appropriate as a complete product name since it is missing the primal or subprimal cut, e.g., the phrase "pork picnic" is incomplete without "shoulder."

Recent editions of the *Uniform Retail Meat Identity Standards* (URMIS), published and distributed by the National Livestock and Meat Board, and *The Meat Buyers Guide*, published by the Na-tional Association of Meat Purveyors, may be used to identify recommended names. These guides have been prepared through extensive review and analysis of the most recent edition of *Institutional Meat Purchase Specifications (IMPS)* and in cooperation with the U.S. Department of Agriculture, Agriculture Marketing Service (AMS) and public and industry associations.

Prime Rib of Beef or Standing Beef Rib Roast for Prime Rib

These products do not have to be derived from USDA prime grade beef.

Principal Display Panel, Alternate

The determination as to whether or not a panel is an alternate principal display panel should be based on whether or not the panel is likely to be displayed, presented, shown, or examined under customary conditions of sale. If the intent of the panel cannot be determined and demonstrated, and if it has the appearance of a principal display panel, the presence of three or more mandatory labeling features should serve to characterize the panel as an alternate principal panel. As such, any remaining mandatory features required to be placed on a principal display panel must be also included.

Product Names

1. A product standard should only be applied if the product name is the same as that described by the standard in the regulations or in the Policy Book. For example, the product, "Beef, Cheese and Vegetables in a Crust," would not be required to meet the standard for a "turnover" in the policy book.
2. Products such as "Pizza Pouches" must meet the standard for pizza since they are named "Pizza" even though they are not traditional type pizza. Furthermore, cheese may not be substituted for meat in products named "Pizza."

Product Name Qualifiers

Product name qualifiers have no sizing requirements other than appearing contiguous to the product name and being prominent and conspicuous. Examples of product name qualifiers are "Smoked Flavor Added," "Made in Sheboygan," and "Colored with Paprika." Examples of phrases that are not product name qualifiers are "Water Added," and "Containing up to X% of a Solution." Such phrases are actually part of the product name and do have particular sizing requirements.

Product Name Qualifiers in Secondary Products

Product name qualifiers, e.g., "binders added," are not required on secondary products with labeling, with the exception of the statement "Calcium Propionate Added to Retard Spoilage of Crust" on pizza labeling. Secondary products are those meal-like products that

contain a multi-ingredient meat or poultry component, e.g., "Lemon Pepper Seasoned Chicken Breast with Rib Meat, Binders Added in 'Lemon Pepper Chicken Breast with Vegetable Medley.'" The characteristics of the meat or poultry added ingredients, are disclosed in the ingredients statement.

Product of U.S.A.

Labeling may bear the phrase "Product of U.S.A." under one of the following conditions:

1. If the country to which the product is exported requires this phrase, and the product is processed in the U.S., or
2. The product is processed in the U.S. (i.e., is of domestic origin). This entry cancels Policy Memo 080 dated April 16, 1985.

Prosciutto

Italian for ham, dry cured. The product name "Prosciutto" is acceptable on labeling to identify a dry-cured ham.

Prosciutto, Cooked

The product name "Cooked Prosciutto" is acceptable on labeling to identify a dry-cured Prosciutto ham that is cooked.

Prosciutto Cotto, Cooked Ham

The product name "Prosciutto Cotto, Cooked Ham" is acceptable on labeling to identify a regular pickle-cured cooked ham. Prosciutto Cotto is the Italian name for cooked ham.

Protective Coverings (Meat) Processed or Prepared Product

Immediate containers, e.g., bags, cardboard cartons, tray packs, and film bags, enclosing processed or prepared product can be considered protective coverings and exempt from the marking and labeling requirements if placed in a shipping container that meets all mandatory labeling requirements of an immediate container. This does not exempt the mandatory identification and marking that is specifically required on the immediate container of cooked beef (9 CFR 318.17). In addition, the shipping container must be clearly marked "Packed for Institutional Use" or an equally descriptive statement of intended limited distribution. Unlabeled product may not be removed from shipping containers for further distribution nor displayed or offered for sale.

Unprocessed Meat Cuts—Transparent film bags enclosing individual meat cuts in an unprocessed state can be considered protective coverings and exempt from the marking and labeling requirements if placed in a shipping container that meets all mandatory labeling of an immediate container. These unlabeled meat cuts may only be removed from the shipping container for resale and further distribution to retailers, hotels, restaurants, and similar institutions if the product itself or the film bag bears a clearly legible official mark of inspection and the establishment number.

Protective Coverings (Poultry)

Under provision of the Poultry Products Inspection Act, protective coverings may be exempt from labeling requirements for immediate containers. Under certain circumstances, some protective coverings are considered immediate containers; under different circumstances, they are regarded only as protective product coverings.

When plastic film bags, cardboard cartons, etc., are used for protecting poultry sold for export or to institutions, e.g., hotels, restaurants, and hospitals (where the contents are consumed on the premises), they are exempt from the mandatory labeling of immediate containers, provided the shipping container meets all the labeling requirements for an immediate container. Such product may not be diverted to retail channels and displayed for sale or be sold to household consumers unless they bear all labeling features required for immediate containers.

See: 9 CFR 381.65(p).

Pudding

Nonspecific product.

Pulled Pork

Refers to pork removed from bones by hand or by mechanical means. The meat must retain its natural striated muscle fiber structure, i.e., it can be shred-

ded, chunked, etc., but may not be ground, chopped, or comminuted.

Quality Grade Terms and Subjective Terms on Labels

Terms designated as grades of meat, i.e., prime, choice, select, good, etc., may only be used on red meat that has been officially graded. However, the Food Labeling Division (FLD) will take no action to rescind currently approved labels that contain the word "select." Labels for new or reformulated products or new product lines will be approved in accordance with the policy for grading terms described above.

Letter Grades A, B, C, which are designated grades for poultry, may only be used on poultry (whole birds and parts) that are officially graded and may not be used on red meat. Although poultry grade terms (U.S. Grade A, etc.) are not allowed to be used on red meats, the terms prime, choice, and select may be used on poultry (whole birds or parts) that are equivalent to U.S. Grade A. The use of a possessive, e.g., XYZ's Prime, does not relieve a company of this requirement. The use of quality grade terms on further processed meat and poultry products will be evaluated on a case-by-case basis to determine if they wrongly imply that the meat or poultry used in these products has been graded.

Terms that are subjective in nature, e.g., but not limited to, fancy, finest, super, supreme, ultimate, premium, greatest, best, old fashioned, home style, hotel style, deluxe, special, famous, and old time, may be used unqualified on labels for meat and/or poultry products. The term "selected" as well as other terms, will be considered individually by the Labeling and Consumer Protection Staff, again to determine if these terms wrongly imply that the meat or poultry has been graded.

Quiche Products

The term "Quiche" does not have to be qualified to indicate it is a custard cheese pie. However, when characterizing ingredients, e.g., bacon, ham, chicken, onion, etc., are used either alone or in combination, the ingredients should be either clearly identified as part of the product name or prominently displayed elsewhere on the principal display panel (PDP) of the label (e.g., Bacon Quiche, Ham and Onion Quiche, etc.). Similarly, the characterizing ingredients in Quiches bearing fanciful names should be identified as part of the product name or highlighted elsewhere on the PDP (e.g., Quiche Bercy, made with ham and wine). Since "Quiche Lorraine" is widely recognized, the characterizing ingredients do not have to be identified as a part of the product name or elsewhere on the PDP.

Meat and poultry quiches must contain at least 8% cooked meat or poultry and sufficient cheese so that the combined total at least comprises 18% of the finished product. Quiche Lorraine must contain cooked bacon and/or ham and the only cheeses required are Swiss and/or Gruyere.

If other characterizing ingredients (excluding cheese), e.g., onions, peppers, olives, etc., are used in addition to the meat or poultry ingredient in Quiche Lorraine or in any other quiche, the combination of these other characterizing ingredients and the meat or poultry ingredients must comprise at least 8% of the total product, and the cooked meat or poultry portion must be at least 5% of the total product.

Ranch

The regulations and policies applicable to "Farm" also apply to ranch.

Ravioli (Meat)

This product must contain at least 10% meat.

Rehydrated Dehydrated Vegetables

Rehydrated dehydrated vegetables acceptable as name. The specific vegetable must be identified in the ingredient statement.

Relleno de Papa (PR)

This product must contain 8% cooked meat. A Puerto Rican product that must show a true product name, e.g., "Potato Balls with Beef," or "Potato Dough with a Beef Filling."

Rendered Beef Fat Tissue Solids

The solid remains of a fat extraction process from beef that was ground and rendered by a high temperature (180°F) continuous wet rendering system.

Rework

Rework is allowed in unlimited quantities when added to like product. However, if breaded/battered rework is added to similar products, the rework is limited to 2%.

Rice and Meat

The product must contain at least 12% meat.

Roasted

The term "roasted" may be used to describe products that have been subjected to cooking methods that result in a roasted appearance.

Rolls

Six uses exist for the term "Roll" in conjunction with names for meat food products:

1. Items consisting of a solid piece of meat, e.g., "Boned Veal Rib," formed and tied as a roll and usually offered with seasonings.
2. Chopped meat in combination with condiments, also formed and processed. It can be and often is offered in the fresh meat state.

   Water is not an ordinary or usual ingredient in these two "Meat Roll" items. If water is an ingredient in these products, then a statement indicating the addition of a solution has taken place must appear contiguous to the product name wherever it appears on the label.
3. "Sausage Rolls" have similar formulas and water limitations to cooked sausage. The finished product may contain up to 10% added water, is in roll shape, and is Cooked, or Smoked and Cured (species) Roll Sausages.
4. Nondescriptive rolls, e.g., "Pizza Roll," "Pickle Roll," "Relish Roll," etc., contain meat with cheese, peppers, pimentos, relishes, and other similar materials. An ingredients statement is required as a part of the product name on the basis of instructions in 9 CFR 317.2(c)(1) and (2), and 317.2(e).
5. Product made from meat and water that has been chunked, ground, chipped, wafer-sliced, etc., and formed into a roll containing a plant protein product or other binder could be labeled as a "Meat, Water, and Textured Vegetable Protein Roll." The same size lettering should be used for the product name.
6. Product made from meat that has been chunked, ground, chipped, wafer-sliced, hydroflaked, etc., and formed in a roll containing a plant protein product or other binder should be labeled as "Beef and Textured Vegetable Protein Roll" or "Beef and Soy Protein Concentrate Roll."

Rolls, Poultry

Only natural proportions of skin to the whole carcass or designated part may be used. If skin is in greater than natural proportions, the name must be qualified with the term "Skin Added."

See: 9 CFR 381.159.

Romano Cheese

Label must show "kind" of milk, e.g., (Caprino), "Romano Cheese made with Goat's Milk;" (Pecornia), "Romano Cheese made from Sheep's Milk;" or (Vaccino), "Romano Cheese made from Cow's Milk." The words in parenthesis are not required to be shown.

Rumaki

This product must contain at least 50% chicken livers. An hors d'oeuvre or appetizer. Rumaki is a combination of chicken livers, water chestnuts, and bacon.

Salad—Freeze Dried Ham

Antioxidants have been permitted in Freeze Dried Ham at a level of 0.01%, based on total weight of the ham.

Salad—German Style Potato Salad with Bacon

Requires at least 14% cooked bacon.

Salad Mix, Poultry

Product must contain at least 45% cooked poultry.

Salads

Standards for salads:

- Meat salads must include at least 35% cooked meat or meat food product (e.g., corned beef, ham). Ingredients, e.g., "Ham water added" or "Corned Beef and water product" may be used if the formula is adjusted to account for the amount of added substances. Example: If 85% of a meat food product is meat, then 35% required meat divided by 0.85 equals 41% required meat food product in the salad.
- Cobb Salad contains lettuce and chicken or turkey. The other ingredients that may be found include bacon, hard cooked eggs, tomatoes, Roquefort or other blue cheese or dressing. The product name must include the poultry component(s) and also identify any meat ingredient when present about 2%, e.g., "Bacon and Chicken Breast Cobb Salad."
- Caesar Salad is an acceptable product name and normally contains cheese, meat, or poultry pieces and may contain other vegetables.
- Ham and Cheese Salad must contain at least 25% cooked ham.
- Macaroni with ham or beef must contain at least 12% cooked meat.
- Poultry Salad must contain at least 25% cooked poultry (natural proportions of skin and fat).
- Chopped Egg and Ham Salad must contain at least 12% ham. Chopped egg and bacon salad must contain at least 12% bacon (9% fully cooked bacon).
- Vegetable and/or fruit with poultry must contain at least 25% cooked poultry.

Cracker meal, breadcrumbs, and similar ingredients may be included in meat or poultry salads up to 2% of the total formula. If more than 2% is used, a product name qualifier is required. Modified food starch and textured vegetable protein cannot be substituted for cracker meal and breadcrumbs in salad products.

Salami

A dry sausage that requires an MPR of 1.9:1 or less. Extenders and binders are permitted. It may be cooked to shorten drying period.

Salami, Beef

A cooked, smoked sausage, usually mildly flavored, in a large casing, containing coarsely ground beef. Cereals and extenders are permitted. May contain fat. Product does not have to be labeled cooked.

Salami, Cooked

The product "Salami" must be labeled to include the word "Cooked," regardless of the type and size of its packaging, unless it is one of the following:

1. A salami with a moisture protein ratio of no more than 1:9 to 1;
2. "Genoa salami" with a moisture protein ratio of no more than 2.3:1;
3. "Sicilian salami" with a moisture protein ratio of no more than 2.3:1;
4. Labeled, as . . . ,
   a. Kosher Salami,
   b. Kosher Beef Salami,
   c. Beef Salami,
   d. Beer Salami, and
   e. Salami for Beer.

Pork skins are not a permitted ingredient in cooked salami.

Salami, Cotto

A mildly flavored cooked, cured sausage, in a large casing, usually containing coarsely ground beef and pork. The product contains whole or visible pieces of peppercorns. It is cooked in dry heat.

Salami, Hard

A dry sausage with an MPR of 1.9:1. It is made with beef and pork and seasoned with garlic. Less highly flavored but usually more heavily smoked than Italian Salami. It is tied with loops or twine that gives a scalloped appearance.

Salami, Italian

This kind of dry salami is usually prepared in the San Francisco area and is easily distinguished by its covering of a white mold. This salami consists of about 80% finely chopped pork, to which a small

amount of pork fat may be added. Nonfat dry milk can comprise 3½% of the finished product. The remainder consists of chopped beef, seasoning, salt, and curing agent. The product should have an MPR not in excess of 1.9:1 to ensure the fat content and dryness properties associated with a "dry salami."

Salchichon (SP)

This term, means "Large Sausage." This term may only be used for large casing sausage products that are 3 inches in diameter or more. Label must show a true product name.

Salisbury Steak

Finished product must contain at least 65% meat. Fat is limited to 30%. Other requirements are:

1. It is an unbreaded cooked product.
2. The meat block may contain 25% pork, with the remainder beef. Or, the meat block may contain up to 12% partially defatted chopped beef and pork.
3. Extenders are permitted up to 12%. When isolated soy protein is used, 6.8% is the equivalent of 12% of the other extenders. Those extenders include, but are not limited to, cereal, bread-crumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
4. Meat by-products are not permitted. Beef heart meat is permitted.
5. Permitted liquids include, but are not limited to, water, broth, milk, cream, skim milk, and reconstituted skim milk (9 parts water to 1 part NFDM).
6. Product not cooked that conforms to the above may be labeled "Patties for Salisbury."

Salisbury Steak, Turkey

Product must contain at least 55% turkey meat in natural proportions (light and dark) or 65% turkey with skin and fat in natural proportions (skin 10%, turkey meat 55%). Maximum amount of binders and extenders is 12%.

Salpicao

A smoked sausage. The label must show a true product name, e.g., "Smoked Sausage." No more than 3% water can be added at formulation.

Salsiccia (IT)

A fresh pork sausage, highly spiced, in which paprika is permitted. It is a rope-style sausage made of finely cut pork trimming.

Salt as a Cure

Dry processed hams, pork shoulders, and bacon are ordinarily cured with mixtures that contain mostly salt along with sugar and nitrates plus nitrites. However, some processors use salt alone in preparing their products. The salt in contact with the meat provides the desired cured color, taste, and necessary product protection.

Salt is an acceptable cure when used singly in the curing and salt equalization of dry processed hams, pork shoulders, and bacon. The cured products must have a 10% brine concentration.

Samosa

This product originated in India, although it is also associated with Pakistan. It resembles a "Meat Turnover" and consists of a spiced vegetable and meat mixture in a dough crust. At least 25% meat is required. Label must show a true product name, e.g., "Beef Turnover."

Samples

Free samples included along with the meat and poultry food products are not to be included in the net weight statement, and the ingredients do not need to be identified in the ingredients statement as long as the ingredients appear on sample package.

Sandalwood

According to FDA regulations, Red Saunders (red sandalwood) is not an acceptable ingredient in meat and poultry products. It is a permitted coloring and flavoring agent in alcoholic beverages only. In contrast, white or yellow sandalwood is acceptable in meat and poultry products as a flavoring agent in an amount that is "sufficient for purpose." White or yellow sandalwood extract may be labeled as "sandalwood extract" or "flavoring."

Sandwich, Closed

Product must contain at least 35% cooked meat and no more than 50% bread. Sandwiches are not amenable to inspection. If inspection is requested for this product, it may be granted under reimbursable Food Inspection Service.

Typical "closed-faced" sandwiches consisting of two slices of bread or the top and bottom sections of a sliced bun that enclose meat or poultry, are not amenable to the federal meat and poultry inspection laws. Therefore, they are not required to be inspected nor bear the marks of inspection when distributed in interstate commerce.

Sandwich, Open

Must contain at least 50% cooked meat. Sandwiches are amenable only if they are open-faced sandwiches. Product must show a true product name, e.g., "Sliced Roast Beef on Bread."

This regulatory policy in no way alters the department's present policy with respect to caterers who include meat sandwiches in their dinners.

Sandwiches (Meat or Poultry as Components of "Dinner Products")

Dinners containing a sandwich type product, e.g., a frankfurter, hamburger, or sliced poultry meat with a bun, are amenable and subject to inspection.

Santa Fe Style

Acceptable for products that contain chilies with corn or beans and one of the following ingredients: cheese (jack, cheddar, Mexican Style, or fresh goat), bell pepper, onion, garlic, tomatoes, tomatillos, cumin, oregano, or cilantro. The beans should be either black, kidney, navy, pink, pinto, red, or white beans or an indigenous variety.

Sarno

A dry smoked sausage that is air dried. The label must show a true product name, e.g., "Smoked Sausage." Coarsely chopped beef, pork, and garlic are not permitted.

Satay

This term refers more to a preparation method than to the nature of a finished product. Satay can be made from chicken, beef, lamb, pork, and other food items, and prepared in two ways:

1. Meat is cut into 1-inch cubes, then dipped into a spicy sauce, skewered, and roasted over an open fire (similar to "Kabobs" except with no vegetables or fruit). Label must show a true product name, e.g., "Beef Cubes on Stick."
2. Meat is cut into 1-inch cubes, then dipped into a spicy sauce and canned. Label must show a true product name, e.g., "Beef Cubes in Spicy Sauce."

Sauce with Meat or Meat Sauce

Product must contain at least 6% ground meat.

Sauerbraten (GR)

"Sauerbraten" must contain at least 50% cooked beef. "Gravy with Sauerbraten" must contain at least 35% cooked meat. Sauerbraten is cooked beef in a vinegar-flavored sauce. The beef is marinated in vinegar sauce, then separated from the sauce and partially cooked, and put back in the sauce and cooked completely.

Sauerkraut Balls with Meat

Product must contain at least 30% meat or meat food product.

Sauerkraut with Franks and Juice

Product must contain at least 20% franks.

Sausage Classification

1. Fresh Sausage—Made of fresh, uncured meat, generally cuts of fresh pork, and sometimes beef. Its taste, texture, tenderness, and color are related to the ratio of fat to lean. Trimmings from primal cuts, e.g., pork, loin, ham, and shoulders are often used. When ice or water is used to facilitate chopping and mixing, it is limited to a maximum of 3% of the total formula. It must be kept under refrigeration and thoroughly

cooked before serving. Bratwurst is in this class. Binders and extenders are permitted in fresh sausages except where regulations do not permit the use of such ingredients, i.e., 9 CFR 319.140 (Pork Sausage), 9 CFR 319.142 (Beef Sausage), 9 CFR 319.144 (Whole Hog Sausage), and 9 CFR 319.145 (Italian Sausage).
See: 9 CFR 319 Subpart E.

2. Uncooked smoked sausage—Has all the characteristics of fresh sausage except it is smoked, producing a different flavor and color. It must be thoroughly cooked before serving. "Smoked Pork Sausage" is included in this class. If it is a mixture of pork and other meats, regardless of size, it must be treated for trichinae.
See: 9 CFR 319 Subpart F.
3. Cooked sausages and/or Smoked sausages—These products are chopped or ground, seasoned, cooked, and/or smoked. Added water is limited to 10% of the finished product. Meat byproducts may be used when permitted by standard. Cure is required for particular sausages, e.g., wieners or Polish sausage. These sausages come in various shapes and sizes, e.g., short, thin, long, and chub. Cotto salami, liver sausage, and cooked weisswurst are included in this category. Wieners, bologna, knockwurst, etc., are also in this class but are further distinguished by a fat and moisture limitation.
See: 9 CFR 319.180.
4. Dry and Semidry sausages—Dry sausages may or may not be characterized by a bacterial fermentation. When fermented, the intentional encouragement of a lactic acid bacteria growth is useful as a meat preservative as well as producing the typical tangy flavor.

   The meat ingredients, after being mixed with spices and curing materials, are generally held for several days in a curing cooler. Afterward, the meat is stuffed into casings and is started on a carefully controlled air-drying process. Some dry sausage is given a light preliminary smoke, but the key production step is a relatively long, continuous air-drying process.

   Principal dry sausage products are salamis and cervelats. Salamis are coarsely cut, and cervelats are finely cut with few exceptions. They may be smoked, unsmoked, or cooked. Italian and French dry sausage are rarely smoked; other varieties usually are smoked.

   Dry sausage requires more production time than other types of sausage and results in a concentrated form of meat. Medium-dry sausage is about 70% of its "green" weight when sold. Less dry and fully dried sausage range from 80% to 60% of original weight at completion.

   Semidry sausages are usually heated in the smokehouse to fully cook the product and partially dry it. Semidry sausages are semisoft sausages with good keeping qualities due to their lactic acid fermentation.

   Although dry and semidry sausages originally were produced in the winter for use in the summer and were considered summer sausage, the term "summer sausage" now refers to semidry sausages, especially Thuringer Cervelat.

Sausage Containing Cheese

Sausages may contain cheese under the following conditions:

1. If there is a standard for that particular sausage, it must be met as though it contained no cheese.
2. The cheese must characterize the product and appear as part of the product name. Example "Italian Sausage with Cheese."

Sausage, Liquid Added

Sausages containing fluid ingredients that are expected such as fruit and juice and vinegar, are permitted at any level as long as the product is descriptively labeled. The sausage portion of the product, however, must meet any applicable standard. Vinegar is an expected ingredient in chorizos, and the name does not have to indicate its presence.

Sausage, Shelf-Stable

Dry sausage must have a Moisture Protein Ratio (MPR) of 1.9:1 or less, unless an MPR is cited under Moisture Protein Ratio.

Nonrefrigerated, semidry, shelf-stable sausage must have an MPR of 3.1:1 or less and a pH of 5.0 or less, unless commercially sterilized or unless an MPR is cited under Moisture Protein Ratio. Alternately, nonrefrigerated, semidry, shelf-stable sausages are those that:

1. are fermented to a pH of 4.5 or lower (or pH may be as high as 4.6 if combined with product water activity no higher than 0.91),
2. are in an intact form or, if sliced, are vacuum packed,
3. have internal brine concentration no less than 5%,
4. are cured with nitrite or nitrate, and
5. are smoked with wood.

Sausage, Rework

This term applies to a fully or partially processed product (excluding uncooked trimmings) rerouted for reasons other than unwholesomeness or adulteration (i.e., emulsion residue, product breakage, slicing operations, smoked meats, returns, etc.) and intended for inclusion in cooked sausage, loaves, and similar products. Rework may be used provided it does not adulterate the product, violate its standard of composition, change the order of predominance of ingredients, or perceptibly affect the normal characteristics of the product. Rework is subject to the following restrictions:

1. Cooked sausage, meat loaves may be used in similar products without limitation.
2. Except in products covered by section 9 CFR 319.180, pieces of cooked and/or smoked meat may be used without limitation if properly identified in the ingredients statement.
3. Pieces of uncooked, cured pork from primal parts may be used without limitation if properly identified in the ingredients statement.
4. Sausage products in edible collagen casings may be used in similar finely comminuted products without limitation and need not be peeled.
5. Finished cooked sausage in natural casings may be used in similar finely comminuted products without limitation, except sausages in bungs, middles, beef rounds, bladders, or stomachs, which must be stripped of the casings before use. Also, natural casings of any type that break during the stuffing operations should not be included in emulsions.

Sausage Type Products with Fruits and Vegetables

Sausage type products that contain unexpected ingredients that significantly alter the character of the product may be descriptively labeled as (characterizing ingredient) Sausage, e.g., "Cherry Pecan Sausage," "Wild Rice Sausage," or other equally descriptive names, e.g., "Sausage with Wild Rice."

The sausage portion of fresh sausage products must meet any applicable standards, including fat and added water limitations, moisture/protein ratios, and use of binders and extenders prior to the addition of any characterizing ingredient(s). For cooked, smoked, or dry sausages, the finished sausage type product must meet the sausage standard prior to the addition of any characterizing ingredients.

The unexpected ingredient must be present in sufficient quantity or form to characterize the sausage type product in flavor, texture, or other sensory attributes. However, there are no minimum use levels.

This policy applies to products containing unexpected food ingredients, e.g., fruits and vegetables, e.g., cherries, pecans, tomatoes, etc., that change the character of the product by the addition of unique flavor and other sensory characteristics. The policy does not apply to imitation products, i.e., products formulated to resemble in taste, texture, color, etc., the traditional sausage products, but which are nutritionally inferior. Sausages containing cheese are addressed in Policy Memo 010, and Potato Sausages are addressed in Policy Memo 011.

Sausage with Sauerkraut in Sauce

Product must contain at least 40% sausage.

Sausage (Species)

(Species) sausages identified in 9 CFR 319.141, 319.142, 319.144, and 319.160 of the meat inspection regulations may be cooked, cured, or smoked (or any combination), but must comply with the standards before being processed if the product name is to include "(species) sausage." For example, fresh beef sausage identified in 9 CFR 319.142, which is cured and cooked may be labeled "cured, cooked beef sausage." Prior to this processing, these products could not contain more than the 3% water permitted by the standard.

Cooked cured sausages or smoked cured sausages containing up to 10% added water in the finished product and prepared from one species may be labeled as "cooked cured sausage," "smoked sausage," "cooked cured sausage made with (species)," or "smoked sausage made with (species)."

Semidry and dry sausages made from a single species may be labeled "(species) sausage," e.g., "beef sausage."

This policy does not apply to cooked sausages identified in section 9 CFR 319.180 of the meat regulations.

Scalloped Potatoes and Ham

Product must contain at least 20% cooked ham.

Scalloped Potatoes and Sausage

Product must contain at least 20% cooked sausage.

Scalloped Potatoes Flavored with Sausage

Product must contain at least 3% sausage.

Scalloppini

Product must contain at least 35% cooked meat or poultry meat. Thin slices of cooked veal, sometimes beef or poultry, seared or fried. Label must show a true product name, e.g., "Veal Scalloppini" or "Chicken Scalloppini."

Schickenwurst (GR)

The product is made of two parts, one of which is an emulsion prepared from pork and beef cuts. The second component consists of chunks of ham measuring from 2 to 3 inches in size. The two parts are mixed, stuffed into large casings, and smoked while being cooked. The final product appears as a luncheon sausage with large pieces of red ham meat held together by a light pink binder. The ham sections comprise at least 50% of the product, and the item has a distinct smoked flavor. This product is very similar in appearance to the product sold as "Ham Bologna."

Scrambled Eggs with Bacon

Product must contain at least 10% cooked bacon.

Seaweed

The term is not an acceptable ingredient declaration. There are many types of seaweed; some are not as safe.

Select or Higher

This phrase has been extended to retail beef products that have been officially graded prime, choice, or select.

Shepherds Pie (With or Without Vegetables)

Product must contain at least 25% meat in total formulation. Shepherds Pie is a meat food product consisting of chopped, minced, or cubed beef or lamb, seasoned with gravy or sauce, with or without vegetables, and baked with a covering layer or surrounding border of seasoned mashed potatoes. The label must show a true product name, e.g., "Beef Shepherds Pie."

Shipping Containers

A mark of inspection and a handling statement are required on all shipping containers. Safe Handling Instructions are required with all other required features only when the shipping container is also the immediate or primary container.

See: 9 CFR 316.13.

Shu-Mai

Product must contain at least 10% meat. A Chinese product that resembles a dumpling. It is similar to a meat ravioli. The label must show a true product name, e.g., "Pork Dumpling."

Signature Line

It is not necessary to include the term "General Office" in signature lines on labels used by companies with multiple plant operations. A zip code should appear following the address.

See: 9 CFR 317.2(g)(1), 9 CFR 381.122.

Sloppy Joe

A coined name that must be qualified by a true product name, e.g., "Barbecue Sauce with Beef." The meat content depends on the name of the product. Heart meat and tongue meat can be used but not to satisfy the minimum meat requirement.

Smoke

For imported Canadian products, e.g., bacon, which is physically smoked during processing, the word "Smoke" is acceptable in the ingredients statement. Although not required or customary, smoke can also appear in the ingredients statement of domestically produced products that are physically smoked. If included in the ingredients statement, smoke should appear as the last item.

Smoke Flavoring

The use of smoke flavoring (natural or artificial) in a component of a meat or poultry food product, e.g., ham in a ham salad, does not require that the product name be qualified to indicate the presence of the smoke flavoring. However, the smoke flavoring must be declared in the ingredients statement on the meat or poultry product labels.

Secondary product—When meat and extender product is produced using a meat product in which smoke flavoring is added, the secondary product name does not have to be qualified with a phrase as "smoke flavoring added."

When smoke flavor (natural or artificial) has been directly added to a product as part of a seasoning mix, the presence of the smoke flavor must be identified in a qualifying statement to the product name, such as the following:

1. "Chicken soup smoke flavor added," and in the ingredients statement.
2. "Beef soup smoke flavor."
3. If a product is simply sprayed with liquid smoke, it must be labeled "smoke flavoring added."

Smoked Products

The guidelines for approving labels for products prepared with natural smoke and/or smoke flavor (natural or artificial) are as follows:

1. Meat or poultry products that have been exposed to smoke generated from burning hardwoods, hardwood sawdust, corn cobs, mesquite, etc., may be labeled as "Smoked" or with terms, e.g., "Naturally Smoked" to indicate that the traditional smoking process is used.
2. Meat or poultry products that have been exposed to natural liquid smoke flavor that has been transformed into a true gaseous state by the application of heat or transformed into vapor by mechanical means, e.g., atomization, may be labeled "Smoked."
3. Meat or poultry products may be labeled "Smoked" if natural liquid smoke flavor is applied by spraying, dipping, liquid flooding, or similar processes prior to or during heat processing. In such cases, the natural liquid smoke flavoring must be transformed into a true gaseous state by the heat of processing. If a product is simply sprayed with liquid smoke it must be labeled "smoke flavoring added."
4. Meat or poultry products to which smoke flavor (natural or artificial) has been directly applied to the exposed product surface, e.g., massaging or margination, or incorporated into the product by such means as injection, must be labeled to identify the smoke flavor as part of the product name, e.g., "Ham-Natural Smoke Flavor Added," and in the ingredients statement.
5. Meat or poultry products that are smoked, as provided for in (1), (2), and (3) above and also treated with smoke flavor as described in (4), may only be labeled "Smoked" or with terms, e.g., "Naturally Smoked," if it is clearly disclosed that the product is also treated with smoke flavor. The presence of the smoke flavor must be identified as part of the product name, e.g., "Smoked Ham-Smoke Flavoring Added" and in the ingredients statement.
6. Product may be labeled as "hickory smoked" only if the plant provides the inspector with appropriate certification that such sawdust or wood for smoking is 100% hickory.

Smoked Thuringer Links

A cooked smoked sausage made with pork only.

Snacks (Hors d'Oeuvres)

Product must contain at least 15% cooked meat or 10% cooked bacon. The label must show a true product name, e.g., "Liver Paté on Toast."

Sodium Alginate

This is added as a binder in "Taquitos." Approval may be given for use at a level of less than 1% with

.25% of calcium citrate to stabilize a pizza sauce or pizzas heated in household toasters.

Sodium alginate when used as glue to seal burrito and burrito-like products is acceptable, if declared in the ingredients statement, or if a statement such as "sealed with sodium alginate" appears at the end of the ingredients statement.

Sodium Benzoate

Sodium Benzoate is not an acceptable ingredient for meat and poultry products, except in oleomargarine. It is accepted as an incidental additive when it is a part of a product prepared under FDA rules, e.g., sauces, gravies, and similar substances.

Sofrito with Pork

This is a sauce containing 6% smoked pork.

Soppresate (IT)

This is an acceptable name for a dry salami with an MPR of 1.9:1. This is an Italian salami that is lightly flavored with garlic and, generally, hotly seasoned with paprika and black or red peppers. It is smoked to varying degrees depending on regional tastes.

Sorbitol

This is only permitted in 9 CFR 319.180 products, cured pork products, dried beef, kielbasa, and products similar to kielbasa. Do not approve when used in other products.

Souffle (Species) or (Kind)

Product must contain at least 18% cooked meat or poultry meat.

Soujouk (TK)

This is a Turkish sausage made from beef that is very dry and highly spiced with an MPR of 2.04:1. The product is usually flattened or resembles a dry salami or ring bologna. The label must show a true product name, e.g., "Dried Beef Sausage."

Soup

1. Soups that declare meat stock in the product name are meat food products and should contain at least 25% meat stock with an MPR of not less than:

   a. Condensed soup—67:1
   b. Ready-to-eat—135:1
   c. Beef Bouillon—67:1 and at least 50% beef stock

2. Soups made with meat should contain not less than:

   a. Condensed soup—4% cooked meat
   b. Ready-to-eat—2% cooked meat

3. Soups containing smoked meats should contain not less than:

   a. Condensed soup—4% smoked meat
   b. Ready-to-eat—2% smoked meat

4. Soups made with cooked sausages should contain at least 4% cooked sausage.

Soup Products

Bean & Ham Shank—When soup is made from ham shanks, they must be shown in the true product name, e.g., "Bean and Ham Shank Soup."

Blood—Product must contain at least 1% blood and be made under inspection.

Chowders—Follow standard for soups.

Consommé—A broth cooked with vegetables and then strained. Must have an MPR of 135:1.

Consommé Instant—Dehydrated, not amenable.

Cream—Condensed cream soups may be made from various creams, whole milk, or dry milk powder. The amount of cream, whole milk, or dry milk powder should provide a minimum of .45% butterfat to the final product. Examples:

1. A cream containing 18% butterfat should make up the product formulation; this provides .45% butterfat to the product formulation.
2. Dry milk powder containing 27% butterfat should make up 1.67% of the product formulation.

Dried Meat Soup Mixes—Not amenable.

Italian Style Minestrone—Soup must contain zucchini. Identify meat in the true product name.

Pepper Pot—Soup must contain at least 20% scalded tripe.
Petite Marmite (FR)—A soup made with meat, chicken, and vegetables.
Scotch Broth—Soup must contain at least 3% mutton in a thick mutton broth.
Vegetable—Vegetable soups made with soup stock are not considered amenable.

Souse

This is a nonspecific product that can be made with all pork by-products. The ingredients statement directly follows the product name.

Southern Fried

Southern fried poultry cuts or patties are breaded and fried. This is not geographical.

Southwestern Style

An acceptable identification for products containing any five of the following types of food ingredients: beans (kidney beans, black beans, pinto beans, red or pink beans), corn, chili peppers, bell peppers, cheddar cheese, cilantro, onions or onion powder, cumin, oregano, garlic or garlic powder, paprika, chili powder, either mesquite smoked, or mesquite smoke flavor added.

Soy Protein Products

Whenever soy flour, defatted soy grits, soy protein concentrate, isolated soy protein, and similar products are used as ingredients of meat and poultry products, they must be called by their common or usual name (e.g., soy flour, soy protein isolate, etc.).

Two percent isolated soy protein is equivalent to 3.5% binders.

If these products are textured, then "textured" should also be included in the name. We allow the use of the term "textured vegetable protein" when the textured soy products are mixed with spices, colorings, enrichments, etc., and the ingredients of the textured vegetable protein are listed parenthetically. "Vegetable Protein Product" is an acceptable declaration for a soy product fortified in accordance with Food and Nutrition Service regulations. The ingredients of the VPP must be listed parenthetically.

Spaghetti

Sauce with meatballs	Must contain at least 35% cooked meatballs
Sauce with meat	Must contain at least 6% meat
with meatballs	Must contain at least 12% meat
with meatballs & sauce	Must contain at least 12% meat
with meat and sauce	Must contain at least 12% meat
with franks and sauce	Must contain at least 12% franks

Spaghetti Sauce with Meat Stock

This spaghetti sauce consists mainly of tomatoes with seasoning. Product must contain 5% fresh beef and 12.5% concentrated meat stock.

Spaghettios in Cheese Sauce with Ground Beef

Product must contain at least 12% meat.

Spanish Rice with Beef

Product must contain at least 20% cooked beef.

Speckwurst

Product should conform to sausage standard (9 CFR 319.140) without the use of by-products. Chunks of fat are usually present.

Starch

Starch, wheat starch, and cornstarch are synonymous in meaning. When "Vegetable Starch" is used as a designation, it refers to the starchy materials derived from any vegetable source, e.g., potatoes, peas, etc. Tapioca starch cannot be declared as "starch."

See: Tapioca Product.

Steak, Chinese Pepper

Product must contain at least 30% cooked steak. A Chinese dish usually served with rice. Beef steak is cut in thin strips, browned, and added to a sauce.

Vegetables are also added to the sauce; green pepper strips are always used, and other vegetables may include celery, onions, scallions, red pepper, bean sprouts, tomatoes, or water chestnuts.

Steak, Country Style

This term is popular in the southern region of the country. It resembles a "Gravy and Swiss Steak" product. Characteristics of this product are:

1. It is prepared from the steak portions of beef (usually from the round) and braised.
2. The meat is mechanically "tenderized" and floured prior to browning.
3. The meat is browned by sautéing or oven browning, but not flame browned nor cooked in water.
4. When a true product name is shown as "Gravy and Beef Steak," at least 35% cooked steak must be used.
5. When a true product name is shown as "Beef Steak with Gravy," at least 50% cooked steak must be used.

Steak, Pepper

Product must meet the standard for "Fabricated Steak" in 9 CFR 319.15(d) and contain green and/or red peppers.

Sticks

There are three types of meat or poultry sticks:

1. Meat Sticks, which are an extended "patty-like" product and are usually breaded. No more than 10% extenders and 30% breading are permitted. When whole egg, tomato, and nonfat dry milk are used, they must appear as added ingredients in the true product name, e.g., "Breaded Meat Stick—Nonfat Dry Milk Added."
2. The infant finger food type of sticks is usually packed in jars. It conforms to the sausage standard and must show a true product name, e.g., "Meat Stick".
3. Nonspecific dry or semidry sticks that do not meet the sausage standard must be followed by the ingredients statement. If products meet the sausage standard, they may be identified as "Smoked Sausage."

Stroganoff, Meatball

Product must contain at least 45% cooked meatballs. Sauce portion should comply with the Stroganoff Sauce standard.

Stroganoff Sauce

The sauce must contain at least 10% sour cream or a combination of at least 7.5% sour cream and 5% wine, or 2% sour cream, 2½% wine, and 9½% whole milk.

Stroganoff Sauce with/and Beef

Product must contain at least 31% beef or 21% cooked beef based on the total weight of the product, with sauce portion complying with the stroganoff sauce standard.

Stroganoff Sauce with/and Meatballs

Product must contain at least 31% cooked meatballs. Sauce portion should comply with the stroganoff sauce standard.

Stromboli (IT)

Product is not considered a traditional sandwich. Minimum meat requirement is 25% fresh or 18% cooked meat. The label must show a true product name, e.g., "Pepperoni and Cheese Wrapped in Dough."

Stuffed Cabbage with Meat in Sauce

Product must contain at least 12% meat or at least 8% cooked poultry.

Stuffed Peppers with Meat in Sauce

Product must contain at least 12% meat or at least 8% cooked poultry.

Sukiyaki

Product must contain at least 30% beef. Sukiyaki consists of cut up vegetables, e.g., mushrooms, leeks, and celery, which are cooked briefly with thin slices of beef and soy sauce.

Sulfiting Agents

The presence of sulfiting agents (sulfur dioxide, sodium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite, and potassium metabisulfite) must be declared on the label if their concentration in the finished meat or poultry food product is 10 ppm or higher. However, some finished meat and poultry food products may be comprised of multiple separable components, e.g., potatoes or apple cobbler in frozen dinner. For these products, if a separable component contains 10 ppm or more sulfiting agents, the sulfiting agents must be declared even though the total product contains less than 10 ppm of sulfiting agents. When sulfiting agents are required to be declared under conditions described above, their declaration should be according to the following:

1. Sulfiting agents should be declared by their specific name or as "sulfiting agents."
2. Declaration should be in the ingredients statement in order of predominance or at the end of the ingredients statement with the statement "This Product Contains Sulfiting Agents" (or specific name[s]).
3. When the total product contains less than 10 ppm, but a separable component contains 10 ppm or more, the sulfiting agent must be declared as part of the component according to (1) and (2) above.

Summer Sausage

Product may be a semidry or cooked sausage. Meat by-products and extenders are permitted.

Swiss Steak

Swiss Steak and Gravy—Contains not less than 50% cooked beef.

Gravy and Swiss Steak—Contains not less than 35% cooked beef.

Product labeled "Swiss Steak" must be floured or dusted before searing, or may have flour added to gravy.

Sweet and Sour Pork, Beef, or Poultry

Product requires at least 25% meat or poultry meat, or 18% cooked meat or poultry meat. Product also requires sufficient traditional sweet and sour ingredients (fruit, fruit juices, vinegar, etc.) to impart sweet and sour characteristics.

Szechwan Style

Acceptable identification for any product containing one item from three of the four groups below.

1. Soy sauce.
2. Spring onions, scallions, or leeks.
3. Garlic, ginger, ginger root.
4. Chili, Szechwan peppercorn, Chili oil.

Taco

Product must contain at least 15% meat.

Taco Filling

Product must contain at least 40% fresh meat. The label must show true product name, e.g., "Taco Filling with Meat," "Beef Taco Filling," or "Taco Meat Filling."

Taco Filling, Kind

Product must contain at least 40% raw poultry meat.

Tags, Tissue Strips, Brands

When tags, tissue strips, brands, etc., are used to apply ingredients statements on sausages and other products in casings or link form, the only additional marking required is the official inspection legend. However, if other features are added, e.g., the product name, all applicable required labeling features are required.

See: 9 CFR 316.10.

Tallow

Acceptable product name for the meat food product consisting of rendered beef fat or mutton fat or both.

Tamales

Product must contain at least 25% meat. Tamales prepared with meats other than beef and/or pork must include them in the product name, e.g., "Chicken Tamale" or "Chicken and Beef Tamale."

See: 9 CFR 319.305.

When inedible wrappings are used, they must be indicated:

a. In the product name, e.g., "Beef Tamale Wrapped in Corn Husk."
b. As a qualifier to the product name, e.g., "remove parchment paper prior to eating," or
c. As information in the preparation instructions, e.g., "remove the inedible covering prior to serving." The wrapper cannot be included as part of the net weight.

Filling—must contain at least 40% beef.
Pie—must contain at least 20% fresh meat. Filling must be at least 40% of the total product.
(kind)—must contain at least 6% poultry meat.
(kind) with sauce or gravy—must contain at least 5% poultry meat.
(species)—must contain at least 25% meat.
(species) with sauce or gravy—must contain at least 20% fresh meat.

If by-products are used, their presence must be included in the product name.

Tapioca Product

Tapioca flour can be used as a binder in some products in which "starchy vegetable flour" is permitted, as long as it is declared as tapioca flour.

Tapioca starch can be used as a binder in some meat and poultry products in which "vegetable starch" is permitted as long as it is declared as "tapioca starch or food starch." Tapioca starch cannot be declared as "starch."

Taquitos

A Mexican dish requiring at least 15% meat. Cooked meat product is cut into strips or shredded and placed in center of tortilla. The tortilla is then rolled around the filling.

Tasajo Salted Beef (SP)

MPR not to exceed 2:1. Product is stitch pumped and cured in salt brine for 72 hours or more after which it is dried with circulated warm air for a period of at least 20 days. If the item is dipped in a tallow mixture, a statement must be shown contiguous to the product name identifying the constituents of the dipping mixture.

Teawurst or Teewurst

A cooked or uncooked product processed with or without curing and cold smoked 2 to 5 days. It is ground or coarsely chopped and is characterized by a soft spreadable texture. Typical meat ingredients include pork, beef, pork bellies, and bacon. Fresh pork bellies may be used in place of pork fat and bacon.

Tempura

A Japanese dish consisting of shrimp, fish, vegetable, meat, poultry, etc., each dipped in an egg batter and deep fried. The label must show true product name, e.g., "Chicken Tempura," "Pork Tempura," etc.

Teriyaki, Meat or Poultry

Cubes or slices of meat or poultry meat that have been marinated in a sauce containing soy sauce, some kind of sweetener, and usually ginger, garlic, or wine. When the marinated product is combined with additional sauce, the product name must reflect the sauce; for example, "Beef Teriyaki with Sauce."

See: Teriyaki Products.

Teriyaki Products

Meat and poultry teriyaki products are not required to be cooked, provided a prominent statement is on the principal display panel informing the consumer that the product is not cooked. Example: "Ready to Cook," "Raw," and "Ready to Bake."

Tetrazzini, Poultry or Beef

Product must contain at least 15% cooked poultry or cooked beef. Made with diced cooked poultry or meat in a rich cream sauce containing sherry. This is added to cooked spaghetti or noodles in a casserole. Usually topped with breadcrumbs or grated cheese.

Textured Vegetable Protein (Textured Vegetable Protein Product) for Cooked Meat and/or Poultry Meat

If the cooked meat and/or poultry meat to TVP ratio exceeds 9:1, then the TVP is declared by its common or usual name in the ingredients statement only.

If the cooked meat and/or poultry meat to TVP ratio is less than 9:1 but at least 7:1, the label must contain a qualifying phrase contiguous to the product name, e.g., "Chicken Salad, Textured Vegetable Protein Added."

If the cooked meat and/or poultry meat to TVP ratio is less than 7:1, the TVP must be shown in the product name, e.g., "Chicken and Textured Vegetable Protein Salad."

Textured Vegetable Protein (TVP) Products—Fresh Meat or Poultry Meat Ratios

The following guidelines and labeling requirements have been established regarding use of TVP in products other than patties and pizza toppings.

If the ratio of fresh meat or poultry meat to TVP is greater than or equal to 13:1, the TVP product is not considered to be characterizing or deceptive, e.g., 40% fresh meat: 3% textured soy flour = 13.3:1, and the TVP only needs to be shown in the ingredients statement.

If the ratio of fresh meat or poultry meat to TVP product is less than 13:1 but greater than or equal to 10:1, the TVP is characterizing and must be shown contiguous to the product name, e.g., "Hot Dog Chili Sauce made with Beef Textured Vegetable Protein added."

Thai Style

Acceptable identification for products containing at least five of the following: basil, chilies or chili products, cilantro, coconut or coconut products, coriander, cumin, fish sauce, galangal, garlic, ginger, green onions, jasmine rice, lemon grass, peanuts or peanut products, rice noodles, shallots, or soy sauce.

Thuringer

Usually classed as a "Semidry" sausage with an MPR of 3.7:1. It is usually smoked and complies with the following factors:

1. Pork fat as such may comprise up to 10% of the total ingredients.
2. Heart meat (Beef or Pork) may comprise up to 50% of meat ingredients.
3. Tongue meat (Beef or Pork) may comprise up to 10% of meat ingredients.
4. Cheek meat (Beef or Pork) may comprise up to 50% of meat ingredients.
5. No binders or extenders are allowed.
6. "Cooked Thuringer" can contain up to 10% added water.
7. Acceptable product names for uncooked Thuringer include "Beef Summer Sausage—Thuringer Cervelat" and "Summer Sausage—Thuringer Cervelat."

Tips

Is the subprimal of the beef round and is often referred to as the "Sirloin Tip." If the term "Tips" is used for other than from the "Sirloin Tip," it must be qualified as to the specific part of the primal such as "Beef Ribs Tips."

Titanium Dioxide

When Titanium Dioxide is used in poultry salads, a qualifying phrase should appear under the product name stating that the product has been "Artificially Whitened" or "Artificially Lightened."

Tocino

Spanish word for salt pork or bacon. Except in Puerto Rico, must show and use true product name in English, e.g., bacon, salt pork.

Tocino, Poultry

A fanciful name for a tocino product made from poultry. The fanciful name must be followed by a true descriptive product name, e.g., "Chicken Tocino, Sliced, Marinated, Cured Chicken Thigh Meat."

Tocino (Filipino or Philippine Style)

The thinly sliced piece of meat taken from either the hind leg or shoulder portion of the pork carcass. The product is treated with salt, sugar, and nitrite and/or nitrates, with optional ingredients of ascorbic acid, spices, monosodium glutamate, and phosphates. Acceptable color agents are annatto, beet powder, and paprika that must be shown as "artificially colored." A true product name must be shown on the label, e.g., "Sliced Marinated Cured Pork Shoulder Butt, followed by the solution statement."

Tocopherol

May be listed as "Tocopherol (Vitamin E)" on the label but not "Vitamin E (Tocopherol)." Tocopherol and vitamin E are not synonyms. Also, acceptable in rendered or unrendered fat.

Tomato and Bacon Spread

Product must contain at least 25% cooked bacon.

Tongue Trimmings

Labeling terminology for the various kinds of tongue and cheek trimmings should be as follows:

1. "(Species) tongue trimmings" should be used to identify all tissues except cartilage and bone that are obtained by converting long-cut to short-cut tongues. This conversion is done by making a transverse cut anterior to the epiglottis, removing the soft palate and epiglottis, and cutting through the hyoid bone. Approximately 1½ inches of the bone is left with the tongue. "(Species) tongue trimmings" may also be used to identify salivary glands, lymph nodes, and fat from which the muscle tissue has not been removed.
2. "(Species) salivary glands, lymph nodes, and fat (tongue)" must be preceded by the name of the species from which derived. Tongue meat should not include any tissues described in paragraph 2.
3. Trimmings from the tongue itself should be identified as "tongue meat" preceded by the name of the species from which derived. Tongue meat should not include any tissues described in numbers 1. and 2. above.
4. Trimmings with fat from tongue is an acceptable ingredient in cooked sausage products covered under section 9 CFR 319.180 of the regulations. Lymph nodes and salivary glands are not acceptable ingredients.

Topping, (Species) or (Kind)

Topping is an acceptable product name for a nonspecific product containing the species or kind indicated as well as various other ingredients. The ingredients statement must follow the product name.

See: Pizza Topping Mix.

Tortellini with Meat

Product must contain at least 10% meat.

Tortilla with Meat

Product must contain at least 10% meat. Tortilla is a thin, flat unleavened masa cake that is baked on both sides.

Tostada with Meat

Product must contain at least 15% meat. A tortilla is usually topped with refried beans, meat, cheese, and fresh vegetables.

Touristenwurst

A semidry type of sausage. The MPR must not exceed 3.7:1.

"Tropic Cure" Pork Products

Pork products when ready for shipment from the official establishment must have a moisture protein ratio not in excess of 3.25:1, and a salt content not less than 6%.

Truffles

Meat food product, e.g., "Liver Paté with Truffles" or "Sandwich Spread with Truffles" would be expected to be prepared with at the least 3% truffles. Labels of product containing less than 3% truffles should indicate the amount of truffle content in the name, e.g., "Liver Paté with 2% truffles." If the name does not feature truffles and they are mentioned only in the list of ingredients, we have no minimum requirement, provided the illustration does not show truffles.

Turkey Braunschweiger

The product name must be shown on the label as "Turkey Liver Sausage." No by-products other than liver are permitted in the product.

Turkey Chops

Turkey chops are prepared by cutting the frozen breast into slabs with each cut being made perpen-

dicular to the long axis of the keel bone (sternum). The larger slabs are split in half through the center of the sternum, resulting in two individual servings of meat with a piece of bone on one side and a thin layer of skin on the other. The smaller pieces at each end of the breast are left intact as individual servings. The word steak is unsuitable because a turkey steak is boneless by definition.

Turkey Ham Products Containing Added Water

Product otherwise conforming to the standard for turkey ham under section 9 CFR 381.171 of the poultry products inspection regulations but weighing more than the original weight of the turkey thigh meat used prior to curing should be descriptively labeled as follows:

1. The product name must include in addition to "Turkey Ham," words that specify the amount of the additional substances, e.g., "and % Water," "With % Water Added," or "Turkey Ham and Water Product % of Weight is Added Ingredients." (The ingredients of the added solution may be incorporated into the product name, e.g., "Turkey Ham and Water Product % of Weight is Added Water, Salt, Dextrose, Sodium Phosphate, and Sodium Nitrite.") The blank is filled in with the % determined by subtracting the original weight of the turkey thigh meat from the weight of the cooked finished product. "Turkey Ham and 12% Water" is an example.
2. In retail and nonretail size packaging, the qualifying statements described in 1. above must be shown in lettering that is either not less than three-eighths inch in height or is at least one-third the size of the letters used in the product name and in the same color and style and on the same background as the product name. Full length of the product labeling is not required.
3. The "Turkey Ham" portion of the product name must be qualified with the statement "Cured Turkey Thigh Meat" in the manner described in 9 CFR 381.171(e). This may be affected by using an asterisk as long as there is no type or other designs between the total product name and the qualifying statement. Other means of qualifying "Turkey Ham" will be evaluated based on clarity. Alternatively, the total name as described in numbers 1. and 2 above may be qualified with a statement that includes "Cured Turkey Thigh Meat" and the amount of added water, e.g., "Cured Turkey Thigh Meat and 12% Water." The statement should be presented in the manner described in 9 CFR 381.171(e).
4. The product name should be further qualified with the statement(s) required by section 9 CFR 381.171(f) and any other statements required in Part 381. A product complying with the standard for Turkey Ham, containing added water, and descriptively labeled as stated above, must be produced under a Partial Quality Control (PQC) program approved by the Processed Products Inspection Division (PPID) prior to the use of the approved label.

Turkey Ham and Water Products Containing Binders

Turkey ham products containing added water and binders must be labeled as "Turkey Ham and Water Products" X% of weight is added ingredients as described in Policy Memo 057a to provide freeze/thaw stability and reduce purge in packages. The binders that are acceptable for use in cured pork product can be used in these turkey ham products. The binders must be used in accordance with 9 CFR, Sections 9 CFR 319.104(d) and 424.21(c). Where several limits are listed, depending upon the cured pork product, the maximum amount permitted in the regulation is acceptable.

Turkey Ham Products Containing Ground Turkey Thigh Meat (Labeling)

Small amounts of ground turkey thigh meat may be added as a binder in turkey ham products as defined in 9 CFR 381.171 without declaration, provided the ground turkey thigh meat is made from trimmings that are removed from the turkey thighs during the boning and trimming process. The amount of ground turkey thigh meat that may be used can represent no more than the amount that was trimmed and in no case more than 15% of the weight of the turkey thigh meat ingredients when formulated. Products containing any ground turkey thigh meat not removed during the boning and trimming processes or products containing more than 15% ground turkey thigh meat must be labeled to indicate

the presence of the ground turkey thigh meat, e.g., "a portion of ground turkey thigh meat added." The provision in the regulations, 9 CFR 381.171(f) regarding the required use of terminology, e.g., "Chunked and Formed," "Chopped and Formed," and "Ground and Formed," will continue to be followed.

Turkey Loaf, Cured, Chopped (Canned)

May contain seasonings, cures, and no more than 3% water at formulation. Binders and extenders are not permitted.

Turnovers

Product must contain 25% meat or 14% poultry meat. Similar to pies except the dough is folded. Cheese may be substituted for meat or poultry meat in an amount not to exceed 50% under the conditions outlined below:

1. Cheese must be part of the product name, e.g., "Beef and Cheese Turnover" or "Chicken and Cheese Turnover."
2. Imitation Cheese, substitute cheese, cheese food, and cheese spreads are not acceptable replacements for cheese.

Tzimmes

The true product name is "Beef and Vegetables" (or similar wording) when at least 50% beef is present in the product. "Vegetables with Beef" (or similar wording) is acceptable when at least 35% raw beef is used.

Ukranian Sausage

A dry sausage made from lean pork and/or veal chunks, containing large amounts of garlic that dominates the flavor. It is cooked and smoked at high temperatures and then air dried. The water activity (Aw) of the finished product should not exceed 0.92 or a moisture/protein ratio 2.0:1 or less.

Variety Meats in Franks

Cooked sausages with variety meats (by-products) identified in 9 CFR 319.180(b) must contain not less than 15% red skeletal meat based on total meat block weight. The meat block includes meat, meat by-products, and if applicable, poultry.

Variety Packs, Hors D'Oeuvres

Whenever FDA regulated products are included as a part of a variety pack bearing the legend (e.g., seafood hors d'oeuvres included with meat and poultry hors d'oeuvres), the labeling information must still be reviewed to assure accuracy. FDA regulated products that are found mislabeled should be corrected according to the policies of the FDA before the label can be approved.

Veal and Peppers in Sauce

Product must contain at least 30% cooked veal.

Veal Birds

Product is similar to a turnover made with meat and no more than 40% stuffing. Categories of products are as follows:

1. Veal Birds—At least 60% veal
2. Veal Birds Beef Added—At least 60% veal and beef of which 20% may be beef
3. Veal and Beef Birds—At least 60% veal and beef of which up to 50% may be beef
4. Veal Birds (made from patties)—Birds made from patties should bear a true product name descriptive of patty used, e.g., "Veal Birds made with Veal Patties—Beef Added." The patty portion should contain 70% meat.

Veal Cordon Bleu (FR)

The standard requires at least:

1. 60% veal;
2. 5% ham, Canadian bacon, or cooked cured pork loin; and
3. Cheese (either Swiss, Gruyere, Mozzarella, or pasteurized processed Swiss)

If the product is breaded, it must be shown in the product name. When the product is made with other than solid pieces of meat, "Chopped and Formed" must be shown contiguous to the product name. Beef is not permitted in this product.

Veal that has been injected with water and phosphates and used for Veal Cordon Bleu should be labeled "Veal Roll Cordon Bleu" or other descriptive names as appropriate.

Veal Drumstick, Breaded

May not contain more than 15% water or more than 10% extenders.

Veal Fricasse

Must contain at least 40% meat.

Veal Parmigiana

The following categories of products exist:

1. "Breaded Veal Parmigiana" is the product name for a solid piece of veal that is breaded and topped with cheese and tomato sauce. Breaded cooked veal must represent 40% of the finished product.
2. "Breaded Veal Parmigiana, Chopped and Formed Beef (or Beef Fat) Added" is the product name for chopped veal with up to 20% beef and/or beef fat added that is formed, breaded, and topped with cheese and tomato sauce. The chopped and formed beef added statement is shown one-third the size of "Veal" contiguous to the product name. Breaded cooked patty must represent 40% of the finished product.
3. "Breaded Veal Parmigiana made with Veal Patties, Beef (or Beef Fat) Added" is the product name for a veal patty containing at least 70% fresh meat (in unbreaded patty) of which 20% may be beef or beef fat. The patty is breaded, topped with cheese and tomato sauce. The entire qualifying statement in the product name is to be shown one-third the size of "Veal" contiguous to product name. The breaded cooked patty represents 40% of the finished product.
4. Breaded Veal and Beef Patty Parmigiana. The patty may be prepared in proportions as governed by 9 CFR 317.2(f)(1)(v) of the regulations; the minimum meat patty requirement is 50%. If the product is breaded, the name must reflect this fact. The cheese component of the product does not have to be shown in the name of the product. A specific kind of cheese is not required, although Romano, Mozzarella, and Parmesan are the usual types used. No specific spelling of the word "Parmigiana" is required. Name applies to a "Cooked Product Assembled, Ready to Heat and Eat."

The labeling of Veal Parmigiana made from a veal patty should include veal patty in the product name, e.g., "Breaded Veal Parmigiana made with Veal Patties" or "Breaded Veal Patty Parmigiana." The ingredients of the veal patty do not have to be part of the product name.

Veal Patties

Up to 20% beef and/or beef fat of the meat block permitted. Beef and/or beef fat must show in the true product name, e.g., "Veal Patties, beef added" or "Veal Patties, beef fat added." Beef and/or beef fat in excess of 20% of the meat block must show as "Veal and Beef Fat Patties."

Veal Scaloppini

Veal and sauce type product that must contain at least 35% cooked sliced veal.

Vegetable Declaration on Labels

1. The use of the terms onion, garlic, celery, and parsley should mean fresh, frozen, or canned.
2. Processed onion or garlic must be qualified in a manner, e.g., "dried" or "dehydrated onion" or may be shown as "onion flakes" or "powdered."
3. It is usually not necessary to show vegetables as whole, diced, sliced, granulated, powdered, or pureed; however, whenever the name of the vegetable is necessary to describe a food, then the name of the vegetable should be modified to show the form of its degree of processing.
4. Onion or garlic juice to which water has been added should be noted, e.g., onion juice with water added.
5. Celery seed may be listed as a spice.
6. Celery salt should be shown as celery salt.
7. Oil of celery may be listed as a flavoring.

Vegetable Extract

The source must be identified i.e. "soy," "corn," and "beet."

Vegetable Gum

Declare common or usual name of each vegetable gum, e.g., Guar Gum.

Vegetable Pie with Meat

"Species" meat must contain 12% meat on a raw basis. "Kind" poultry must contain 7% cooked poultry.

Vegetable Stew with Minimum Meat Content

Meatballs	12% meat
Meat	12% meat
Meat Sauce or Gravy	6% meat
Sauce and Meat	12% meat
Poultry	6% cooked poultry meat

Vienna Sausage, Packed in Beef Broth

Product must contain 80% sausage to be in compliance prior to inclusion in can. Broth component to have a MPR of not more than 135:1. A manufacturer holds trademark rights to the terms "Vienna" and "Vienna Beef."

Vinegar

Product must contain at least 4 grams of acetic acid per 100 cubic centimeters (approximately 4% acetic acid). This strength is referred to as 40 grain vinegar. Cider vinegar, which during the course of manufacture has developed an excess of acetic acid over 4%, may be reduced to a strength of not less than 4%. Cider vinegar so reduced is not regarded as adulterated, but must be labeled as to its nature as "diluted" or "water added" cider vinegar. However, when vinegar of any concentration (not less than 4% acetic acid) is used in a food product, the only labeling requirement is "vinegar." Statements like "diluted" or "water added" are not required.

Vinegar Pickle

Sausage in vinegar pickle is approved with the understanding that sausage is completely covered with pickle and that the pickle has a pH level not higher than 4.5.

Water Base Solution in Red Meat in Meat Products

Solutions intended to impart flavor (not extend the product) may be added in any amount to uncooked, cured and uncooked, uncured red meat products including those that have been chunked, ground, wafer sliced, etc., and formed/shaped. Whenever an uncooked, cured red meat product is injected, massaged, tumbled, etc., with a flavoring or seasoning solution, the product name must be qualified with a statement indicating that the addition of a solution has taken place, e.g., "Containing 6% of a Solution," "Injected with up to 12% of a Flavoring Solution." The qualifier must appear contiguous to the product name whenever it appears on the label. The ingredients of the solution may accompany the qualifier or appear in locations prescribed for ingredient statements.

For products marinated (i.e., soaked, steeped, massaged, tumbled, or injected in order to improve taste, texture, tenderness, or other sensory attributes, such as color or juiciness) and identified as "marinated," the solution added is limited to 10%. The qualifying statement must include the percentage of solution contained in the product, e.g., "Marinated with up to 8% of a Solution of Water, Salt, and Sugar."

In situations where it has been customary to coat a product by rubbing, spraying, or dipping water mixed with seasonings, flavorings, etc., onto the surface of the meat, the qualifying statement describing this treatment does not have to include the amount and a partial quality control program is not needed. If, however, these components are incorporated into the meat by excessive rubbing, massaging, or tumbling, a qualifying statement indicating the composition and the amount of any solution absorbed is needed as described herein. An approved partial quality control program is also needed.

The addition of an enzyme solution to meat products is limited to 3% of the raw meat product (green weight) by the meat inspection regulations, 9 CFR 318.7(c)(4). If a product is treated with an enzyme solution and a flavoring solution, separately or in one step, both treatments must be separately identified on the label, e.g., "Tenderized with Papain," and "Marinated with up to 7% of a Solution." No particular order is required for these qualifying statements. Combined tenderization/marination solutions are limited to 10% of the raw meat product (green weight).

For all products, the qualifying statement must be at least one-fourth the size of the largest letter in the product name. If the ingredients of the solution accompany the qualifier, they must appear in print at least one-eighth the size of the largest letter in the product name. Product name labeling prominence guidelines are found in Policy Memo 087A.

For uncooked products, the percent added substances for the label statement is determined by subtracting the fresh (green) weight of the article from the weight of the finished (total) product, i.e., after injecting, marinating, etc., dividing by the weight of the fresh article, and multiplying by 100.

In all situations where the percentage of a solution is disclosed, a partial quality control (PQC) program for the addition of solutions must be approved before the label can be used regardless of the amount of solution added.

Since the meat inspection regulations (9 CFR 319.101 and 102) allow uncooked corned beef brisket to contain 20%, and uncooked corned beef round and other cuts to contain 10% of a curing solution above the weight of the fresh, uncured (green weight) product without disclosure, the above labeling scheme does not apply until these levels are exceeded. If these levels are exceeded, the total amount of added solution, not just the level above compliance, must be indicated in the format described for other uncooked, cured products. Similarly, the labeling scheme does not apply to uncooked cured pork trimmings or uncooked cured pork products that are not labeled to indicate the presence of hams, loins, shoulders, butts, picnics, or cured pork made from parts not covered by the cured pork products regulation (9 CFR 319.104) until more than 10% added substance is present.

This policy memo does not apply to uncooked cured pork products covered by the cured pork products regulation. The labeling schemes for indicating the presence of added substances in these products are outlined in the meat inspection regulations (9 CFR 319.104 and 105) and FSIS Directive 7110.2 (Rev. 1). The percentage of the weight of added ingredients is determined as described above.

Water—Declaration

The use of water must be declared in the ingredients statement of all products with the exception of the following:

1. The water added to lactic acid starter culture (.05% or less) for the purpose of rehydration.
2. The water added to products that are freeze-dried or sprayed-dried.

Water in Canned Sausage

Water, not to exceed 8% of the total product weight, may be used in the preparation of precooked pork sausage links intended for canning. The amount of water used is for the purpose of replacing that which is lost during the processing operation that takes place prior to canning. The weight of the sausage at the time of canning should not exceed the weight of the fresh uncured meat ingredients plus the weight of the curing ingredients and the seasoning ingredients.

Water-Misted and Ice-Glazed Meat and Poultry Products

When meat or poultry products are water-misted or ice-glazed, the net weight of the product may not include the weight of the water or ice. An acknowledgment to this effect must be indicated on the label application form. A prominent and conspicuous statement must appear on the principal display panel adjacent to the product name, describing that the product is protected with a water mist or ice glaze (e.g., "Product Protected with Ice Glaze").

If the manufacturer can show that a water or ice glaze is sublimed from the unpackaged product during freezing so as not to compromise the integrity of the product's formulation or the standard with which it must comply, the labeling of the product need not bear the statements identified above.

Because the regulatory standard 9 CFR 319.15 precludes the addition of water, hamburger, ground beef, and chopped beef patties cannot be ice-glazed and, if there is evidence of an ice-glaze on such patties subsequent to freezing, they must be labeled appropriately to being sold in commerce, e.g., as "beef patties." However, water-misting of formed hamburger, ground beef, or chopped beef patties just prior to freezing individual patties is permitted if (1) the water applied in misting acts as a processing aid to prevent shrinkage of the patties, and (2) the misted water sublimes from the surface of the patties during the freezing process such that the weight of the patty exiting the freezer does not exceed the

green weight of the patty just prior to water-misting and freezing.

Weisswurst

An acceptable name for fresh sausage. It is usually made of pork or veal and must be thoroughly cooked before eating. It is of German origin, which means White Sausage, similar to Bratwurst. Weisswurst with milk should be labeled "Kalbsbratwurst." Weisswurst with milk and eggs should be labeled "Bockwurst."

Welsh Rabbit Sauce with Cooked Ham

Product must contain at least 20% cooked ham in the total formulation.

Wheat Gluten

Acceptable for use to bind fresh meat cuts, e.g., boneless loins, boneless legs, and livers together, so that they may be cooked and sliced without falling apart. The amount used should not exceed 2% of the weight of the total product. The product name should be qualified by the phrase "Wheat Gluten Added."

Wheat gluten is not acceptable for use with chunked and/or chopped specific products as roasts, rolls, and reformed meat cuts.

Acceptable in nonspecific products and homestyle meat loaves within the prescribed limits of other extenders and binders.

Whole Hog Sausage

Must contain all primal parts of a hog. Hearts and tongues, in natural proportions, are permitted ingredients in whole hog sausage when declared in the ingredients statement. Other meat by-products are not permitted in whole hog sausage.

See: 9 CFR 319.144.

Wiener Schnitzel (GR)

A veal cutlet prepared by dipping in egg, flour, and bread crumbs and frying to a golden brown.

Wild Boar

Products prepared from wild boar from feral swine are amenable and subject to the meat inspection regulations.

"Wild Boar" is an acceptable label term for a product, provided the words "Wild Boar" are directly followed by the statement "Meat from Feral Swine." The statement "Meat from Feral Swine" must appear prominently on the principal display panel as described in 9 CFR 317.2(d)(1)(2) and (3). If the statement "Meat from Feral Swine" does not directly follow the term "Wild Boar," then an asterisk may be included with the term "Wild Boar" and the statement "Meat from Feral Swine" should appear prominently elsewhere on the principal display panel. "Wild Boar from Feral Swine," "Wild Boar Meat from Feral Swine," "Wild Boar (by-product) from Feral Swine," are also acceptable product names.

In order to obtain approval for a product label bearing the name "Wild Boar from Feral Swine," or similar acceptable names, a statement describing and verifying the following physical and environmental characteristics typical of wild boar is required: color patterns, e.g., white stripes or spots, longer bristly hair coat, elongated snout with visible tusks, a "razorback" body shape, and wild boar males that are uncastrated. (We acknowledge both males and females under the term "Wild Boar.") The purchased hogs should be obtained from a nonrestrictive environment that permits foraging for uncultivated feed, natural selection, and breeding and farrowing without confinement. A letter should be submitted with "Wild Boar from Feral Swine" labels describing the environment where such swine live and their method of capture or entrapment. These same criteria would also apply to imported "Wild Boar Meat from Feral Swine" and arrangements should be made through foreign programs for slaughter and export from approved establishments.

In multi-ingredient products, e.g., "Beans in Sauce with Wild Boar," the "Wild Boar" part of the product name must be followed by an asterisk and a statement "(Meat or meat by-product) from Feral Swine" must appear somewhere on the principal display panel. The ingredient wild boar, wild boar meat, or wild boar by-product, must be listed as "Wild Boar ([Meat or meat by-product] from Feral Swine)" in the ingredients statement in its proper order of predominance.

Wing Sections

First wing section is described as the wing drummette. Second wing section is described as the wing portion.

Wing Sections is an acceptable wing term for both wing drummette and wing portion when in natural proportions.

With Natural Juices (Poultry)

The term "With Natural Juices" may be used with poultry products to indicate the presence of cooked out juices derived solely from the liquid normally associated with the poultry prior to cooking. If liquids have been added to the poultry prior to cooking, natural cannot be used.

Wraps

A ready-to-eat meat/poultry food product that may contain vegetables and seasoning ingredients and is wrapped in a dough-based component, e.g., tortilla. The product name must bear the kind or species, e.g., "Ham Wraps." The minimum meat or poultry requirement is 2% cooked meat or 2% cooked poultry meat.

Yearling

The term "yearling" (e.g., yearling beef) may be used to describe an animal of either sex that is too old to be classified as a calf or lamb but less than 2 years of age. The company is required to segregate carcasses and provide product identification to ensure that no commingling occurs between qualifying and nonqualifying products.

The terms "Yearling Ovine," "Yearling Mutton," and "Yearling Sheep Meat" are acceptable product names for meat derived from sheep between 1 and 2 years of age. Yearling Lamb is not an acceptable name for this product.

SPECIFICATIONS FOR FRESH AND FROZEN SEAFOODS

Specifications and standards for seafoods are issued by the FDA and the National Marine Fisheries Service (NMFS). The FDA has issued standards while NMFS has issued some minimal criteria for several frozen seafood and seafood products: what they are, what types and styles are available, and so on. Both standards and specifications achieve two objectives: assure product safety and minimize economic fraud. They are described in the following products.

Canned Oysters

Canned oysters is the food prepared from one or any mixture of two or all of the forms of oysters specified above and a packing medium of water, or the watery liquid draining from oysters before or during processing, or a mixture of such liquid and water. The food may be seasoned with salt. It is sealed in containers and so processed by heat as to prevent spoilage.

According to the FDA, the different forms of oysters are prepared from oysters that have been removed from their shells and washed and that may be steamed while in the shell or steamed or blanched or both after removal of the shell. Such oysters include:

1. Whole oysters with such broken pieces of oysters as normally occur in removing oysters from their shells, washing, and packing.
2. Pieces of oysters obtained by segregating pieces of oysters broken in shucking, washing, or packing whole oysters.
3. Cut oysters obtained by cutting whole oysters.

Such oysters can be called "Oysters" or "Cove oysters," species *Ostrea virginica*; "Oysters" or "Pacific oysters," if of the species *Ostrea gigas*; "Oysters" or "Olympia oysters," if of the species *Ostrea lurida*. When the form of oysters used include pieces, the name of the food is "Pieces of ———," the blank being filled in with the name "Oysters" or "Cove oysters," if of the species *Ostrea virginica*; "Oysters" or "Pacific oysters," if of the species *Ostrea gigas*; "Oysters" or "Olympia oysters," if of the species *Ostrea lurida*.

Canned Pacific Salmon

Canned Pacific salmon is the food prepared from one of the species of fish enumerated, prepared in one of the forms of pack specified, and to which may be added one or more of the optional ingredients specified. The food is packed in hermetically sealed

containers and so processed by heat as to prevent spoilage and soften bones.

The species of fish that may be used in this food are:

Oncorhynchus tshawytscha—Chinook, king, spring
Oncorhynchus nerka—Blueback, red, sockeye
Oncorhynchus kisutch—Coho, Cohoe, medium red, silver
Oncorhynchus gorbuscha—Pink
Oncorhynchus keta—Chum, keta
Oncorhynchus masou—Masou, cherry

The optional forms of canned Pacific salmon are processed from fish prepared by removing the head, gills, and tail, and the viscera, blood, fins, and damaged or discolored flesh to the greatest extent practicable in accordance with good manufacturing practice, and then washing. Canned Pacific salmon is prepared in one of the following forms of pack:

- "Regular" consists of sections or steaks that are cut transversely from the fish and filled vertically into the can.
- In preparation, segments of skin or large backbone may be removed. The sections or steaks are so packed that the cut surfaces approximately parallel the ends of the container. A small portion of salmon may be added if necessary to complete the fill of the container.
- "Skinless and backbone removed" consists of the regular form of canned salmon from which the skin and vertebrae have been removed in accordance with good manufacturing practices.
- "Minced salmon" consists of salmon that has been minced or ground.
- "Salmon tips or tidbits" consists of small pieces of salmon.
- "No salt added" consists of canned salmon to which no salt has been added.

One or more of the following optional ingredients may be added: salt; edible salmon oil comparable in color, viscosity, and flavor to the oil that would occur naturally in the species of salmon canned.

Canned Tuna

Canned tuna is the food consisting of processed flesh of fish of the species enumerated, prepared in one of the optional forms of pack, conforming to one of the color designations, in one of the optional packing media, and may contain one or more of the seasonings and flavorings specified. For the purpose of inhibiting the development of struvite crystals, sodium acid pyrophosphate may be added in a quantity not in excess of 0.5% by weight of the finished food. It is packed in hermetically sealed containers and so processed by heat as to prevent spoilage.

The fish included in the class known as tuna fish are:

Thunnus thynnus (Linnaeus 1758)—Northern bluefin tuna
Thunnus maccoyii (Castelnau 1872)—Southern bluefin tuna
Thunnus alalunga (Bonnaterre 1788)—Albacore
Thunnus atlanticus (Lesson 1830)—Blackfin tuna
Thunnus obesus (Lowe 1839)—Bigeye tuna
Thunnus albacares (Bonnaterre 1788)—Yellowfin tuna
Thunnus tonggol (Bleeker 1851)—Longtail tuna
Katsuwonus pelamis (Linnaeus 1758)—Skipjack tuna
Euthynnus alletteratus (Rafinesque 1810)—Spotted tuna
Euthynnus lineatus (Kishinouye 1920)—Black skipjack tuna
Euthynnus affinis (Cantor 1849)—Kawakawa
Allothunnus fallai (Serventy 1948)—Slender tuna
Auxis rochei (Risso 1810)—Bullet tuna
Auxis thazard (Lacepede 1800)—Frigate tuna

The optional forms of processed tuna consist of loins and other striated muscular tissue of the fish. The loin is the longitudinal quarter of the great lateral muscle freed from skin, scales, visible blood clots, bones, gills, viscera, and from the nonstriated part of such muscle, which part (known anatomically as the median superficial muscle) is highly vascular in structure, dark in color because of retained blood, and granular in form. Canned tuna is prepared in one of the following forms of pack, the identity of which is determined in accordance with the methods prescribed.

Solid or solid pack consists of loins freed from any surface tissue discolored by diffused hemolyzed blood, cut in transverse segments to which no free fragments are added. In containers of 1 pound or less net contents, such segments are cut in lengths suitable for packing in one layer. In containers of more than 1 pound net contents, such segments may be cut in lengths suitable for packing in one or more

layers of equal thickness. Segments are placed in the can with the planes of their transverse cut ends parallel to the ends of the can. A piece of a segment may be added if necessary to fill a container. The proportion of free flakes broken from loins in the canning operation should not exceed 18%.

Chunk, chunks, chunk style consists of a mixture of pieces of tuna in which the original muscle structure is retained. The pieces may vary in size, but not less than 50% of the weight of the pressed contents of a container is retained on a ½ inch mesh screen.

Flake or flakes consist of a mixture of pieces of tuna in which more than 50% of the weight of the pressed contents of the container will pass through a ½ inch mesh screen, but in which the muscular structure of the flesh is retained.

Grated consists of a mixture of particles of tuna that have been reduced to uniform size, that will pass through a ½ inch mesh screen, and in which the particles are discrete and do not comprise a paste.

Any of the specified forms of pack of canned tuna may be smoked.

Canned tuna, in any of the forms of pack specified falls within one of the following color designations, measured by visual comparison with matte surface neutral reflectance standards corresponding to the specified Munsell units of value.

White—This color designation is limited to the species *Thunnus alalunga* (albacore), and is not darker than Munsell value 6.3.

Light—This color designation includes any tuna not darker than Munsell value 5.3.

Dark—This color designation includes all tuna darker than Munsell value 5.3.

Blended—This color designation may be applied only to tuna flakes specified by established requirements, consisting of a mixture of tuna flakes of which not less than 20 percent by weight meet the color standard for either white tuna or light tuna, and the remainder of which fall within the color standard for dark tuna.

Canned tuna is packed in one of the following optional packing media:

- Any edible vegetable oil other than olive oil, or any mixture of such oils not containing olive oil
- Olive oil
- Water

Canned tuna may be seasoned or flavored with one or more of the following:

- Salt
- Monosodium glutamate
- Hydrolyzed protein
- Spices or spice oils or spice extracts
- Vegetable broth in an amount not in excess of 5% of the volume capacity of the container, such broth to consist of a minimum of 0.5% by weight of vegetable extractives and to be prepared from two or more of the following vegetables: beans, cabbage, carrots, celery, garlic, onions, parsley, peas, potatoes, green bell peppers, red bell peppers, spinach, and tomatoes
- Garlic
- Lemon flavoring to be prepared from lemon oil and citric acid together with safe and suitable carriers for the lemon oil that are present at nonfunctional and insignificant levels in the finished canned food. When lemon flavoring is added, a safe and suitable solubilizing and dispersing ingredient may be added in a quantity not exceeding 0.005% by weight of the finished food
- Edible vegetable oil or partially hydrogenated vegetable oil, excluding olive oil, used alone or in combination in an amount not to exceed 5% of the volume capacity of the container, with or without any suitable form of emulsifying and suspending ingredients that has been affirmed as GRAS or approved as a food additive to aid in dispersion of the oil, as seasoning in canned tuna packed in water

The canned tuna can be packed in water or oil.

Fresh Oysters

Oysters, raw oysters, shucked oysters are obtained by shucking shell oysters and preparing in accordance to regulations.

If water, or salt water containing less than 0.75% salt, is used in any vessel into which the oysters are shucked, the combined volume of oysters and liquid when such oysters are emptied from such vessel is not less than four times the volume of such water or salt water. Any liquid accumulated with the oysters is removed. The oysters are washed, by blowing or otherwise, in water or salt water, or both. The total time that the oysters are in contact with water or salt

water after leaving the shucker, including the time of washing, rinsing, and any other contact with water or salt water, is not more than 30 minutes. In computing the time of contact with water or salt water, the length of time that oysters are in contact with water or salt water that is agitated by blowing or otherwise, should be calculated at twice its actual length. Any period of time that oysters are in contact with salt water containing not less than 0.75% salt before contact with oysters, should not be included in computing the time that the oysters are in contact with water or salt water. Before packing into the containers for shipment or other delivery for consumption the oysters are thoroughly drained and are packed without any added substance.

Shell oysters means live oysters of any of the species, *Ostrea virginica*, *Ostrea gigas*, *Ostrea lurida*, in the shell, which, after removal from their beds, have not been floated or otherwise held under conditions that result in the addition of water.

The oysters are drained on a strainer or skimmer that has an area of not less than 300 square inches per gallon of oysters, drained, and has perforations of at least ¼ of an inch in diameter and not more than 1¼ inches apart, or perforations of equivalent areas and distribution. The oysters are distributed evenly over the draining surface of the skimmer and drained for not less than 5 minutes.

Another method of draining is as follow. Liquid from the oysters is removed so that when the oysters are tested within 15 minutes after packing by draining a representative gallon of oysters on a skimmer (see above) for 2 minutes, not more than 5% of liquid by weight is removed by such draining.

Frozen Headless Dressed Whiting

The product described in this part consists of clean, wholesome whiting (silver hake) *Merluccius bilineraris, Merluccius albidus* completely and cleanly headed and adequately eviscerated. The fish are packaged and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

Frozen Halibut Steaks

Frozen halibut steaks are clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and are 2 ounces or more in weight. Each steak has two parallel surfaces and is derived from whole or subdivided halibut slices of uniform thickness that result from sawing or cutting perpendicular to the axial length, or backbone, of a whole halibut. The steaks are prepared from either frozen or unfrozen halibut (*Hippoglossus* spp.) and are processed and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

Frozen Salmon Steaks

Frozen salmon steaks are clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and are 2.5 ounces or more in weight. Each steak has two parallel surfaces and is derived from whole or subdivided salmon slices of uniform thickness that result from sawing or cutting dressed salmon perpendicularly to the axial length, or backbone. The steaks are prepared from either frozen or unfrozen salmon (*Oncorhynchus* spp.) and are processed and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product. The steaks in an individual package are prepared from only one species of salmon.

Species

Frozen salmon steaks covered are prepared from salmon of any of the following species:

Silver or coho (*0. kisutch*)
Chum or keta (*0. keta*)
King, chinook, or spring (*0. tshawytscha*)
Red, sockeye (*0. nerka*)
Pink (*0. gorbuscha*)

Frozen Fish Fillet Blocks

Frozen fish blocks are rectangular shaped masses made from a single species of fish flesh. They are made from fillets or fillet pieces that are either skin-on and scaled or skinless. Blocks processed from skin-on fish flesh should be so labeled. The blocks should not contain minced or comminuted fish flesh. The blocks should not be made by restructuring (reworking) pieces of fish blocks into the shape of a fish block.

Frozen Minced Fish Blocks

Frozen minced fish blocks that are uniformly shaped masses of cohering minced fish flesh. A block may contain flesh from a single species or a mixture of species with or without food additives. The minced flesh consists entirely of mechanically separated fish flesh processed and maintained in accordance with good commercial practice. This minced flesh is made entirely from species that are known to be safe and suitable for human consumption.

Frozen Raw Fish Portions

The product described in this part consists of clean, wholesome, shaped masses of cohering pieces (not ground) of fish flesh. The fish portions are cut from frozen fish blocks, and are packaged in accordance with good manufacturing practice. They are maintained at temperatures necessary for the preservation of the product. All fish portions in an individual package are prepared from the flesh of one species of fish.

Frozen Raw Breaded Fish Sticks

Frozen raw breaded sticks are clean, wholesome, rectangular-shaped unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The sticks are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; are packaged and frozen in accordance with good commercial practice. They are maintained at temperatures necessary for preservation of the product. Frozen raw breaded fish sticks weigh up to and including 1½ ounces; are at least ⅜-inch thick; and their largest dimension is at least 3 times the next largest dimension. All sticks in an individual package are prepared from the flesh of one species of fish.

Frozen raw breaded fish sticks should contain 72% by weight of fish flesh determined by the official end-product method. Fish flesh content may be determined by the on-line method provided the results are consistent with the fish flesh content requirement of 72% by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

Frozen Raw Breaded Fish Portions

Frozen raw breaded portions are clean, wholesome, uniformly shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The portions are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; and are packaged and frozen in accordance with good commercial practice. They are maintained at temperatures necessary for the preservation of the product. Frozen raw breaded fish portions weigh more than 1½ ounces, and are at least ⅜-inch thick. Frozen raw breaded fish portions contain not less than 75%, by weight, of fish flesh. All portions in an individual package are prepared from the flesh of one species of fish.

Frozen Fried Fish Sticks

Frozen fried fish sticks are clean wholesome, rectangular-shaped unglazed masses of cohering pieces (not ground) of fish flesh coated with breading and partially cooked. The sticks are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; are fried, packaged, and frozen in accordance with good manufacturing practices. They are maintained at temperatures necessary for preservation of the product. Frozen fried fish sticks weigh up to and including 1½ ounces; are at least ⅜ of an inch thick; and their largest dimension is at least three times the next largest dimension. All sticks in an individual package are prepared from the flesh of one species of fish.

Frozen fried fish sticks should contain 60% by weight of fish flesh. Fish flesh content may be determined by the on-line method, provided the results are consistent with the fish flesh content requirement of 60% by weight, when verified by the official end-product method.

Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

Frozen Fried Fish Portions

Frozen fried fish portions are clean, wholesome, uniformly shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading and partially cooked. The portions are cut from

frozen fish blocks; coated with a suitable, wholesome batter and breading; are fried, packaged, and frozen in accordance with good manufacturing practices. They are maintained at temperatures necessary for preservation of the product. Frozen fried fish portions weigh more than 1½ ounces and are at least ⅜ of an inch thick. All portions in an individual package are prepared from the flesh of one species of fish.

Frozen fried fish portions weigh more than 1½ ounces and are at least ⅜ of an inch thick. All portions in an individual package are prepared from the flesh of one species of fish.

Frozen fried fish portions should contain 65% by weight of fish flesh. Fish flesh content may be determined by the on-line method, provided the results are consistent with the fish flesh content requirement of 65% by weight, when verified by the official end-product method.

Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors that may affect the relative fish flesh content.

Examination of sample, frozen state:

- Ease of separation refers to the difficulty of separating portions from each other or from packaging material that are frozen together after the frying operation and during the freezing.
- Broken portion means a portion with a break or cut equal to or greater than ½ the width or length of the portion.
- Damaged portion means a portion that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of squares ¼ inch (that is, squares with an area of 1/16 square inch each) to measure the area of the portion affected. Deductions are not made for damage less than 1/16 square inch.

Fresh and Frozen Shrimp

The products are clean wholesome shrimp that are fresh or frozen, raw or cooked. The product forms include types and styles:

Types

1. Chilled, fresh (not previously frozen).
2. Unfrozen, thawed (previously frozen).
3. Frozen individually (IQF), glazed or unglazed.
4. Frozen solid pack, glazed or unglazed.

Styles

1. Raw (uncoagulated protein).
2. Blanched (parboiled), heated for a period of time such that the surface of the product reaches a temperature adequate to coagulate the protein.
3. Cooked, heated for a period of time such that the thermal center of the product reaches a temperature adequate to coagulate the protein.

Market Forms

a. Heads on (head, shell, tail fins on).
b. Headless (only head removed; shell, tail fins on).
c. Peeled, undeveined, round, tail on (all shell removed except last shell segment and tail fins, with segments unslit).
d. Peeled, undeveined, round, tail off (all shell and tail fins removed, with segments unslit).
e. Peeled and deveined, round, tail on (all shell removed except last shell segment and tail fins, with segments shallowly slit to last segment).
f. Peeled and deveined, round, tail off (all shell and tail fins removed, with segments shallowly slit to last segment).
g. Peeled and deveined, fantail or butterfly, tail on (all shell removed except last shell segment and tail fins, with segments deeply slit to last segment).
h. Peeled and deveined, fantail or butterfly, tail off (all shell and tail fin removed, with segments deeply slit to last segment).
i. Peeled and deveined, western (all shell removed except last shell segment and tail fins, with segments split to fifth segment and vein removed to end of cut).
j. Other forms of shrimp as specified and so designated on the label.

Frozen Raw Breaded Shrimp

The FDA has provided the following on the standards for frozen raw breaded shrimp.

Frozen raw breaded shrimp are whole, clean, wholesome, headless, peeled shrimp that have been

deveined where applicable of the regular commercial species, coated with a wholesome, suitable batter and/or breading. Whole shrimp consist of five or more segments of unmutilated shrimp flesh. They are prepared and frozen in accordance with good manufacturing practice and are maintained at temperatures necessary for the preservation of the product.

The food is frozen and tests not less than 50% of shrimp material. The term shrimp means the tail portion of properly prepared shrimp of commercial species. Except for composite units, each shrimp unit is individually coated.

The optional forms of shrimp are:

1. Fantail or butterfly—Prepared by splitting the shrimp; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
2. Butterfly, tail off—Prepared by splitting the shrimp; tail fins and all shell segments are removed.
3. Round—Round shrimp, not split; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
4. Round, tail off—Round shrimp, not split; tail fins and all shell segments are removed.
5. Pieces—Each unit consists of a piece or a part of a shrimp; tail fins and all shell segments are removed.
6. Composite units—Each unit consists of two or more whole shrimp or pieces of shrimp, or both, formed and pressed into composite units.

Prior to coating; tail fins and all shell segments are removed; large composite units, prior to coating, may be cut into smaller units. The batter and breading ingredients are the fluid constituents and the solid constituents of the coating around the shrimp. These ingredients consist of suitable substances that are not food additives. Batter and breading ingredients that perform a useful function are regarded as suitable. Chemical preservatives that are suitable are:

1. Ascorbic acid, which may be used in a quantity sufficient to retard development of dark spots on the shrimp
2. Legal antioxidant preservatives may be used to retard development of rancidity of the fat content of the food

The label should name the food as follows:

- "Breaded fantail shrimp." The word "butterfly" may be used in lieu of "fantail" in the name.
- "Breaded butterfly shrimp, tail off."
- "Breaded round shrimp."
- "Breaded round shrimp, tail off."
- "Breaded shrimp pieces."
- Composite units:

  If the composite units are in a shape similar to that of breaded fish sticks, the name is "Breaded shrimp sticks"; if they are in the shape of meat cutlets, the name is "Breaded shrimp cutlets." If prepared in a shape other than that of sticks or cutlets, the name is "Breaded shrimp _____," the blank to be filled in with the word or phrase that accurately describes the shape, but that is not misleading.

Frozen Raw Scallops

Frozen raw scallops are clean, wholesome, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species. The portion of the scallop used should be only the adductor muscle eye that controls the shell movement. Scallops should be washed, drained, packed, and frozen in accordance with good manufacturing practices and are maintained at temperatures necessary for the preservation of the product. Only scallops of a single species should be used within a lot.

Frozen Raw Breaded Scallops and Frozen Fried Scallops

Frozen raw breaded scallops are:

1. Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading;
2. Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation; and
3. Composed of a minimum of 50% by weight of scallop meat.

Frozen fried scallops are:

1. Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading;
2. Precooked in oil or fat;
3. Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation; and
4. Composed of a minimum of 50% by weight of scallop meat.

North American Freshwater Catfish and Catfish Products

The descriptions apply to products derived from farm-raised, or from rivers and lakes, North American freshwater catfish of the following common commercial species and hybrids thereof:

1. Channel catfish (*Ictalurus punctatus*)
2. White catfish (*Ictalurus catus*)
3. Blue catfish (*Ictalurus furcatus*)
4. Flathead catfish (*Pylodictis olivaris*)

Fresh products will be packaged in accordance with good commercial practices and maintained at temperatures necessary for the preservation of the product. Frozen products will be frozen to 0°F (−18°C) at their center (thermal core) in accordance with good commercial practices and maintained at temperatures of 0°F (−18°C) or less.

The product may contain bones when the principal display panel clearly shows that the product contains bones.

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Handbook of Meat, Poultry and Seafood Quality
Edited by Leo M. L. Nollet

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