Comprehensive Reviews in Food Science and Food Safety

Evaluation and Definition of Potentially Hazardous Foods

A Report of the Institute of Food Technologists
for the Food and Drug Administration
of the United States Department of Health and Human Services

December 31, 2001

IFT

Table of Contents

Preface

On September 30, 1998, the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services signed a five-year contract with the Institute of Food Technologists (IFT) to provide scientific review and analysis of issues in food safety, food processing, and human health. Under the terms of the contract, FDA assigns IFT task orders, categorized as comprehensive or abbreviated reviews. IFT assembles Scientific and Technical Panels comprised of experts in the topic area to address the issues. The panels are charged with providing scientific and technical review and analysis, not with setting policy.

This report is IFT's response to Task Order No. 4: Analysis and Definition of Potentially Hazardous Foods. The Background and Scope of Work that FDA provided to IFT are included. In October 2000, IFT assembled a Scientific and Technical Panel. This panel was comprised of experts in food safety and microbiology, including safety in food retail, food service, regulatory affairs, and risk analysis. The panel met in person and via conference calls throughout the year 2000. IFT also assembled a Science Advisory Board to advise IFT on the FDA contract and on the individual task orders.

The Institute of Food Technologists greatly appreciates the efforts of the Scientific and Technical Panels, the Science Advisory Board, the many reviewers, staff and others who made this report possible. Compensation for such an effort pales in comparison to the time, effort and expertise expended.

IFT is especially grateful to the FDA staff for their tremendous cooperation, communication, and assistance at every stage of this project. IFT submits this report to the Agency to contribute to the assessment and development of an operational science-based system to address foods that may require time/temperature control for safety reasons.

Science Advisory Board

Roy G. Arnold, Ph.D.
Executive Associate Dean
College of Agricultural Science
Oregon State University

Lester M. Crawford, Ph.D., D.V.M.
Director
Center for Food and Nutrition Policy
Virginia Polytechnic Institute and State University

Ray A. Goldberg
George M. Moffett Professor of Agriculture
and Business Emeritus
Harvard Business School

Marcus Karel, Ph.D.
Professor Emeritus
Massachusetts Institute of Technology
and Rutgers University

Sanford A. Miller, Ph.D.
Adjunct Professor
Center for Food and Nutrition Policy
Virginia Polytechnic Institute and State University

Martha Rhodes Roberts, Ph.D.
Deputy Commissioner for Food Safety
Dept. of Agriculture
and Consumer Services State of Florida

G. Edward Schuh, Ph.D.
Freeman Chair Professor
Hubert H. Humphrey Institute of Public Affairs
University of Minnesota

Barbara O. Schneeman, Ph.D.
Professor of Nutrition
Department of Nutrition
University of California

Institute of Food Technologists Scientific and Technical Panel

Frank F. Busta, Ph.D.
*Panel Chair and Senior Science Advisor
to the Institute of Food Technologists*
University of Minnesota

Dane T. Bernard, Ph.D.
Keystone Foods

Robert B. Gravani, Ph.D.
Cornell University

Paul Hall
Kraft Foods, Inc.

Merle D. Pierson, Ph.D.
Virginia Polytechnic Institute
and State University

Gale Prince
The Kroger Company

Donald W. Schaffner, Ph.D.
Cook College Rutgers
The State University of New Jersey

Katherine M.J. Swanson, Ph.D.
The Pillsbury Company

Betsy Woodward
Food Labs for Florida Department of Agriculture
and Consumer Services (Retired Chief)

Frank Yiannas
Food Safety and Health
Walt Disney World

Reviewers

Catherine Adams, Ph.D.
Heinz North America

Michael H. Brodsky
Brodsky Consultants

Alfred E. Bugenhagen
New York Department
of Agriculture and Markets

Martin Cole
Food Australia

J. Joseph Corby
New York State Department
of Agriculture and Markets

Carl S. Custer
U.S. Dept. of Agriculture

Michael P. Davidson, Ph.D.
University of Tennessee

Robert Donofrio
NSF International

Michael P. Doyle, Ph.D.
University of Georgia

Russell S. Flowers, Ph.D.
Silliker Labs Group, Inc.

Leonard Wm. Heflich

Bestfoods Baking Company

Leon Gorris
Uniliver

Maureen Olewnik
American Institute of Baking

Virginia N. Scott
National Food Processors Association

John Silliker, Ph.D.
Silliker Labs, Inc.

George Smith
Arkansas Department of Health

Pete Snyder
Hospitality Institute of Technology
and Management

Dan Sowards
Texas Department of Health
Food Safety

William H. Sperber
Cargill, Inc.

Bruce Tompkin
Armour Swift-Eckrich

Edmund A. Zottola, Ph.D.
University of Minnesota

Additional Credits

Food and Drug Administration

Donald M. Kautter, Jr.
Contract Technical Officer
Division of HACCP Programs

Ed Arnold
Contracting Officer

Jeanette Lyon
Consumer Safety Officer
Retail Food and Interstate Travel Team

Institute of Food Technologists

Bruce R. Stillings, Ph.D.
1998-1999 President

Charles E. Manley, Ph.D.
1999-2000 President

Mary K. Schmidl, Ph.D.
2000-2001 President

Phillip E. Nelson, Ph.D.
2001-2002 President

Mark McLellan, Ph.D.
2002-2003 President Elect

Daniel E. Weber
Executive Vice President

Fred R. Shank, Ph.D.
Vice President, Science, Communications
and Government Relations

Jill A. Snowdon, Ph.D.
Director, Department of Science
and Technology Projects

Maria P. Oria, Ph.D.
Staff Scientist

Karen Arcamonte, M.S.
Information Specialist

Kendra Langeteig, Ph.D.
Administrative Assistant

Other Contributors

The Institute of Food Technologists and the Science and Technology Expert Panel express their thanks to the many companies and trade associations that cooperated with this project by sharing research data. While remaining as confidential information to all participants, the data was of great value to this report and we appreciate the efforts of those companies and individuals. In addition, the Institute and the panel appreciate the cooperation of the state governments that, by submitting data and information, contributed to the quality of this report.

Background

(Provided by FDA to IFT)

The June 1940 and 1943 recommendations of the Public Health Service (PHS) for eating and drinking establishments used the term "readily perishable food and drink." The "Food Service Sanitation Manual," issued in 1962 by the PHS first defined the term "potentially hazardous food" (PHF) as any perishable food which consists in whole or in part of milk or milk products, eggs, meat, poultry, fish, shellfish, or other ingredients capable of supporting the rapid and progressive growth of infectious or toxigenic microorganisms. "Perishable Food" was defined as any food of such type or in such condition as may spoil. The 1976 Food Service Sanitation Manual expanded the 1962 PHF definition to include edible crustacea, and food containing synthetic ingredients. Both the 1976 Food Service Sanitation Manual and the 1982 Retail Food Store Sanitation Code clarified that the food must be in a form capable of supporting rapid and progressive growth, and excluded from the definition foods that have a pH level of 4.6 or below; a water activity of 0.85 or less under standard conditions; clean, whole, uncracked, odor-free shell eggs; and food products in hermetically sealed containers processed to prevent spoilage. Whole, shell eggs were later included in the definition of PHF via an interpretation, and subsequently included in the 1993 Food Code definition.

With the advent of the Hazard Analysis Critical Control Point (HACCP) approach to food safety, the root word "hazard" in "potentially hazardous" became inconsistent with the use of the term hazard in HACCP. If an uncontrolled food safety hazard exists, the food is not potentially hazardous; but it *is* hazardous.

Furthermore, scientific understanding and legal enforcement of the term "rapid and progressive growth" was unclear. Scientists questioned what the term really meant out of context, i.e., without a given organism, medium, or conditions of growth. The issue became extremely important when FDA attempted to deal with industry requests to allow pumpkin pies to be stored at room temperature during display at retail.

Beginning in the late 1980s the Food and Drug Administration (FDA) was asked to respond to requests from food processors and manufacturers to evaluate foods which were traditionally considered to be potentially hazardous (requiring time/temperature control) but which were formulated to be nonpotentially hazardous. This end-product condition was achieved not by manipulating the pH or water activity alone, but through a combination of pH or water activity and processing methods or preservatives, and the product was intended to be displayed for sale at room temperature. The vast majority of these requests related to the display of pies, usually pumpkin or sweet potato, for which the pH and water activity were adjusted and preservatives added to control the growth of pathogenic organisms. Other food categories for which FDA is questioned include salad dressings, condiments such as mustard and mayonnaise, chopped garlic-in-oil, garlic-flavored oil, butter (whipped, not whipped, salted, unsalted), margarine, cheeses, filled bakery products (crème vs. cream), stuffed breads such as focaccio.

The FDA reviewed these requests regarding pumpkin pies, evaluated challenge studies, and issued opinions allowing or disallowing the display or sale of these pies at ambient temperature, based on the Food Code definition of potentially hazardous food. Although the FDA reviewed the data based on the pathogen of concern for each product, written, specific criteria for the challenge testing were lacking. There is a need for such criteria and for on-site verification that the products are manufactured as claimed. This concern was discussed at the 1996 Conference for Food Protection (CFP) meeting and the CFP subsequently recommended that FDA work with a third party to develop a standard that would address the issue.

In August 1996, NSF International Inc. (NSF), an American National Standards Institute (ANSI)–accredited organization, decided to develop a standard that would address these requests by industry and sought the FDA's participation in a Joint Committee to create new NSF Standard #75, Nonpotentially Hazardous Foods. The FDA has participated in the development of the draft Standard. Draft Standard #75 is being pilot tested by NSF and the document is available for review.

This draft Standard includes a protocol to determine if a food meets the Food Code definition of potentially hazardous. That protocol calls for subjecting the food to predescribed laboratory testing and sets forth the lab methods including inoculation procedures, organisms to be tested, and pass/fail criteria for defining rapid and progressive growth.

In February 2000, the American Bakers' Association (ABA) presented to FDA for review its Protocol for Establishing the Shelf Stability of Pumpkin Pie, a voluntary industry program for manufacturing pumpkin pies to be retailed without refrigeration. ABA based its protocol on the assumption that a pie that is cooked adequately, cooled promptly, and packaged, while minimizing the opportunity for contamination after cooking, is nonpotentially hazardous because pathogens are absent after cooking. It does not address an inoculation or microbial testing protocol. Defining "rapid and progressive growth" is a non-issue under the ABA protocol, since controls are based on industry research that shows that surviving spore formers, after cooking, cannot grow due to barriers in the pie formulation.

Current Policy

FDA's current policy is reflected in the 1999 Food Code, Paragraph 1-201.10 (B) (61) definition of "potentially hazardous food" that describes food that requires temperature control as one that supports the rapid and progressive growth of infectious or toxigenic microorganisms, the growth and toxin production of *Clostridium botulinum*, or, in raw shell eggs, the growth of *Salmonella Enteritidis*. The definition further describes types of foods that are and are not included. Recognizing the need to update and revise the definition, FDA submitted an Issue to the 2000 CFP meeting, asking CFP to address the proposal. CFP referred the Issue to committee for study.

In the CFP Issue, FDA stated that modern food technology makes the determination of whether a food is a PHF very difficult. There is no standardized methodology for what constitutes "laboratory evidence." There are concerns about the slow growth (as opposed to "rapid and progressive growth") of low-dose pathogens in food. Foods that have been historically recognized as not being PHF are now in question, particularly produce items such as lettuce and tomatoes. Foods that are PHF are known to have caused human illness because pathogens are able to grow and multiply to levels that cause infections in humans or produce toxins in the food. Such microbiological hazards must be controlled

through the application of critical limits for pH, a_W, time, and temperature.

The FDA's proposed new definition defines the acceptance criterion for a PHF as being less than a 1 log increase of a pathogen when the food is stored at 24 °C (75 °F) for a period of time that is 1.3 times the shelf life as determined by the manufacturer.

The temperature of 24 °C (75 °F) was selected because it is a temperature at which mesophyllic and psychrotrophic pathogens will demonstrate growth and is commonly used for testing in laboratory settings.

The time frame of 1.3 times the expected shelf life is to allow a reasonable period for storage at the food establishment and at home following purchase. The National Institute for Standards and Technology (NIST) Handbook 130 considers a "reasonable period for consumption to be one-third the approximate total shelf-life of the perishable food." Reference: NIST, Handbook 130, 1998 Edition issued November 1997.

In determining whether a food supports microbial growth, FDA believes that the whole food, its individual components, and interfaces of components must be tested. Individual components, such as toppings or fillings, may have significantly different pH or water activity levels and each needs to be evaluated to determine if it is capable of supporting growth.

FDA's use of the term "potentially hazardous food" is intended to define food that must be kept cold or hot because the food has the necessary intrinsic factors to SUPPORT THE GROWTH of pathogens. The two terms do not imply whether or not the foods have initial loads of bacteria, become contaminated with bacteria, or are adulterated.

Scope of Work (As Assigned by FDA to IFT)

Independently, and not as an agent of the Government, the Contractor shall furnish the necessary materials, services, facilities, and otherwise do all things necessary for or incidental to the performance of the work set forth herein.

The Contractor shall review the scientific literature, shall consult with academic experts, and shall consider the requirements of other governmental bodies to address the following specific questions:

1. The Contractor shall review what criteria or definitions are used by industry, trade organizations, regulatory bodies (foreign and domestic) and others to determine which foods must be temperature-controlled for safety, including pass/fail criteria that are used, organisms of public health significance that are used as indicators, and whether the term "rapid and progressive growth" is used. What is/are the scientific basis/criteria used for such determinations? The Contractor shall evaluate the validity of the scientific basis upon which those criteria or definitions are based. Are there alternative words or phrases that are used by industry, trade groups, and others in lieu of the term "potentially hazardous"?

2. The Contractor shall do an in-depth review of the 2 approaches previously outlined in the Background (NSF and ABA) plus other possible alternative approaches and protocols that address potentially hazardous foods. Describe the advantages and disadvantages of each approach.

3. Based on the information obtained for Items 1 and 2, the Contractor shall provide evaluations and recommendations as to the best science-based framework for defining foods that need time/temperature control(s) for safety. The Contractor shall evaluate and provide options that may be used in addressing foods that should be included in the definition (foods that need time/temperature controls for safety) and foods that should be excluded; incorporating information on whether the food matrix supports growth, pathogenic organisms that are associated with the specific foods, expected storage conditions, shelf life, and potential storage abuse.

4. Based on the information obtained for Items 1 and 2, the Contractor shall review, evaluate and provide recommendations as to the best science-based framework for determining the effectiveness of processing technologies that formulate a food so that it is nonpotentially hazardous. Are processing technologies or mathematical models sufficient, or are biological challenge tests needed, and why? Describe the advantages and disadvantages of each approach considered. For approaches that rely on microbiological challenge testing, the Contractor shall review and evaluate what indicator organism(s) and laboratory testing procedures can be used to validate that a food or food commodity is not potentially hazardous.

5. The Contractor shall demonstrate and critique the systems and frameworks developed by the contractor for Items 1, 2, 3, and 4 by applying them to the following list of food groups in order to determine whether the foods are or are not potentially hazardous and the justification as to the conclusion:
- Salad dressings
- Condiments such as mustard and mayonnaise
- Chopped garlic-in-oil, garlic-flavored oil
- Butter (whipped, not whipped, salted, unsalted)
- Margarine
- Cheeses
- Filled bakery products (crème vs. cream)
- Vegetable-stuffed breads, such as focaccio

Comprehensive Reviews in Food Science and Food Safety

Evaluation and Definition of Potentially Hazardous Foods

A Report of the Institute of Food Technologists for the Food and Drug Administration of the United States Department of Health and Human Services, December 31, 2001

Executive Summary

The current definition of "potentially hazardous foods" (PHF) is articulated in the United States Public Health Service/Food and Drug Administration (FDA) Food Code—a model code for adoption by states or counties overseeing operations providing food directly to the consumer. Many professionals and professional societies involved in food protection share concerns about the limitations and cumbersome nature of the FDA regulations. Both the NSF International (NSF) and the American Bakers Association (ABA) are attempting to address some of these issues by developing protocols to assess the safety of specific types of food held at ambient temperature. In light of these issues, an IFT panel of experts was charged by FDA to review the current Food Code definition and propose a framework to determine if, based on scientific information, a food needs time/temperature control for safety. The panel did not address the following items in the report because they were not included in the FDA charge: issues related to policy and implementation of the proposed framework; food products that do not require time/temperature control for safety but may be hazardous if they contain pathogens with a low infectious dose; and time/temperature control considerations to prevent spoilage.

Definitions

The IFT panel searched domestic and international regulations and guidelines for terms similar to PHF and associated requirements. Most states have adopted the FDA Food Code definition of PHF. The U.S. Department of Agriculture identifies criteria for shelf-stable products, such as Moisture Protein Ratio, pH, or a_w. Australia, Canada, and the United States use the term PHF in their food safety regulations. Other regulatory entities have temperature control requirements, but do not use the term PHF. While temperature requirements for chilled foods are identified, other regulations for temperature control generally do not present guidelines or a framework to determine which foods fall into the "chilled" category. Rather, specific reference is made to the need of temperature control to protect public health. Some products that need to be temperature controlled for safety are identified. These products generally have a history of association with illness in the absence of temperature control.

It is the opinion of the panel that the current FDA Food Code definition of PHF foods is complex and causes some in the food safety community to limit consideration of factors to only pH and a_w. This limitation results in the inclusion of many foods as PHF when, in fact, they are not. Many foods that meet the current definition can be hazardous if pathogens are present at infectious levels. Conversely, many products with pH and a_w above the levels identified in the current Food Code definition have been safely stored at ambient temperatures (for example, white bread, certain cheese spreads, some fermented sausages) due to other science-

based reasons. Control of all relevant pathogens must be addressed and should not be restricted to *Clostridium botulinum* and *Salmonella* Enteritidis. The term "rapid and progressive" in Section a in the Food Code is no longer appropriate. Current production, processing and packaging technologies, extended shelf life products, distribution systems, and consumer-use practices have altered this paradigm. Pathogen growth need not be rapid but only progressive; the amount of growth that may present a hazard is specific to the organism, the food, and other factors. "Scientific evidence" to determine whether a food needs time/temperature for safety should include laboratory and modeling evidence, and literature.

The panel recommends the development of a simplified definition, with an interpretive guide, to strengthen the regulatory focus on appropriate foods by (1) providing detailed, scientifically based examples of products that can be stored safely without temperature control; and (2) avoiding misclassification of safe foods. The agency might consider adopting a term for defining foods that require time/temperature control for safety such as "temperature controlled for safety" (TCS). This term accurately describes both what is required—temperature control with time implied—and why it is required—safety. A definition of TCS foods might be considered such as "foods that require time/temperature control to limit pathogen growth or toxin formation that constitutes a threat to public health."

Factors that influence microbial growth

The need for time/temperature control is primarily determined by (1) the potential for contamination with and survival of pathogenic microorganisms of concern, and (2) the potential for subsequent growth and/or toxin production. The following list of factors may be considered when determining whether a food requires time/temperature control during storage, distribution, and handling at retail and in food service to assure consumer protection. Care should be taken when analyzing multicomponents foods because measurements of pH, redox potential, antimicrobials, or a_w may not reflect the actual value in a microenvironment or at the interface among the different components. In these cases, the parameters should be measured at the interface areas of the food, as well as in any potential microenvironment.

Moisture content

The water requirements of microorganisms are defined in terms of the water activity (a_w) of the food or environment. The a_w of a food describes, among other factors, the availability of water to facilitate growth of microorganisms. In foods, it ranges from 1.00 (for example, meats) to 0.1 (for example, crackers). The a_w can be manipulated in foods by a number of means, including addition of solutes, physical removal of water, or bind-

ing of water to various macromolecular components in the food. Microorganisms generally have optimum and minimum levels of a_w for growth depending on other growth factors in their environments, such as the solute. Also, a_w may be used in combination with other factors to control pathogens in certain food products.

pH and acidity

Increasing the acidity of foods, either through fermentation or the addition of weak acids, has been used as a preservation method since ancient times. Most foods such as meat, fish, and vegetables are slightly acidic, while most fruits are moderately acidic. A few foods such as egg white are alkaline. Organic acids are more effective as preservatives in the undissociated state. Buffering capacity also must be considered. For certain foods, titratable acidity is a better measure of the microbiological stability. It is well known that groups of microorganisms have a pH optimum, minimum, and maximum for growth in foods. The pH can interact with other factors such as a_w, salt, temperature, redox potential, and preservatives to inhibit growth of pathogens and other organisms. Based on a comprehensive review of the literature, the panel concluded that a pH of 4.6 is appropriate to control spore-forming pathogens, and a pH of 4.2 is appropriate to control vegetative pathogens.

Nutrient content

The abundance of nutrients in most foods is sufficient to support the growth of a wide range of foodborne pathogens.

Biological structure

Plant and animal derived foods have biological structures that may prevent the entry and growth of pathogenic microorganisms. Several factors may influence penetration of these barriers and potentially allow the growth of microbial pathogens.

Redox potential

Redox potential is a measurement of the ease by which a substance gains or loses electrons. Eh for growth of aerobes is +500 to +300 mV; facultative anaerobes is +300 to –100 mV; and anaerobes is +100 to less than –250 mV. Values of Eh for foods can be highly variable. Although Eh measurements could possibly be used in combination with other factors to evaluate the potential for pathogen growth, limitations such as low accuracy of measurements make it a rather difficult and variable factor that could result in erroneous conclusions.

Antimicrobials

Antimicrobials include naturally occurring plant-based antimicrobials (for example, essential oils, tannins, glycosides) and animal-based antimicrobials (for example, lactoferrin, lysozyme). Some food processing forms antimicrobial compounds (for example, Maillard compounds, smoke condensates, bacteriocins). In addition, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination. Added antimicrobial compounds can have an interactive or synergistic inhibitory effect with other parameters of the formulation, such as pH, a_w, presence of other preservatives, types of food constituents, presence of certain enzymes, processing temperature, storage atmosphere, and partition coefficients.

Competitive microflora

Metabolic products produced by microorganisms growing in food may limit (by antagonistic interactions) or induce (by synergistic interactions) the growth of particular species, creating an as-sociation or succession. Dominance of particularly metabolically active organisms occurs as a dynamic process. Antagonistic processes usually include competition for nutrients, competition for attachment/adhesion sites (space), unfavorable alterations of the environment, or a combination of these factors. Growth stimulating mechanisms also exist and must be considered when the hurdle concept is used to control microorganisms in temperature-sensitive foods.

Atmosphere

Gases inhibit microorganisms by two mechanisms: direct toxic effects that can inhibit growth and proliferation (carbon dioxide, ozone, and oxygen), or modification of the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment (nitrogen). Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. Technologies used to inhibit the growth of microorganisms include modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage.

The major safety concern in extending shelf life of foods by MAP or related technologies is the loss of sensory cues to spoilage provided by bacterial growth, that is, a food could have acceptable organoleptic quality, but be unsafe. By combining antimicrobial atmospheres with other techniques, hurdle technology strategies may be generated that can further enhance food quality and safety.

Time/temperature

Time parameters define the growth of a microorganism and, consequently, determine a product's microbial shelf life and safety. Shelf life is the time period from when the product is produced until the time it is intended to be consumed or used. Several factors determine shelf life, ranging from organoleptic qualities to microbiological safety. For the purpose of this report, the key consideration is the microbiological safety of the product. Under certain circumstances, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question. The lag time and generation time of a microorganism depend on temperature; therefore, for a specific food product, the shelf life or use period required for safety may vary depending on the temperature at which the product is stored.

Microorganisms have a minimum, maximum, and optimum temperature for growth and/or toxin production. Temperature has a dramatic impact on both the generation time of an organism and its lag period. Growth rate increases with increasing temperature up to the optimum, thereafter declining rapidly, until the temperature maximum is reached. The relationship between temperature and growth rate varies significantly across groups of microorganisms. The lag period and growth rate of a microorganism are influenced not only by temperature but by other intrinsic and extrinsic factors as well.

Storage and holding conditions

Some key factors addressed were storage/holding temperature, the time/temperature involved in cooling of cooked items, and the relative humidity to which the food or packaging material may be exposed. Time and temperature are integral and must be considered together. Foods that have been cooked or reheated and are served or held hot may require appropriate time/temperature control for safety. Cooling food too slowly may permit growth of spore-forming pathogens. Consequently, for certain foods specific

times and temperatures for rapid cooling are prescribed for safety.

The relative humidity of the storage environment may alter the a_w of the food. Foods that depend on a certain a_w for safety need to be stored in an environment that does not markedly change this characteristic. Product should be held under conditions where the environment does not alter the a_w of the product in an unfavorable way.

Processing steps

Low-acid canned foods in a hermetically sealed container do not require temperature control for safety. However, less processed foods in less robust packaging, for example a baked product cooled and packaged under conditions that do not allow recontamination, may be safe and stable at room temperature until consumed. Scientifically sound criteria for determining whether foods require time/temperature control for safety should consider (1) processes that destroy vegetative cells but not spores (when product formulation is capable of inhibiting spore germination); (2) post-process handling and packaging conditions that prevent reintroduction of vegetative pathogens onto or into the product before packaging; and (3) the use of packaging materials that while they do not provide a hermetic seal, do prevent reintroduction of vegetative pathogens into the product.

Intended end-use

A food product that does not require time/temperature control for safety at one point in the food production may require such control at another point, depending on its intended use. For example, a thermally processed food that is hot-filled into its final packaging may not require refrigeration if spore-forming pathogens are not capable of outgrowth but may require refrigeration once the food item is removed from its original packaging.

Product history

There are foods, such as white bread, that have a long history of safe storage use at ambient temperatures yet have formulations, pH, and a_w that would designate them as TCS foods. For a product to be identified as non-TCS based on history and traditional use, the intrinsic and extrinsic factors affecting microbial growth need to have been and remain constant. Product history, alone, should not be used as the sole factor in determining whether or not a food needs time/temperature control for safety, unless a valid scientific rationale is provided.

Interaction of factors

Although there is a long-standing recognition of interactions and the hurdle technology effect of inhibitory factors, the current definition of "potentially hazardous foods" considers pH and a_w only as individual independent factors. The panel believes that pH and a_w interactions must also be taken into consideration. Models that address pH/a_w interaction are available. Models including other factors such as atmosphere and preservatives have also been published. However, a general model for foods that covers all of these interactions does not currently exist. Nevertheless, evaluation of the need for time/temperature control for safety could consider data from microbial growth models that are based on the interaction of only pH and a_w. Individual companies have shown that predictive pathogen growth models for a particular food that incorporate preservative effects can be useful tools in reducing the need for extensive challenge testing and risk assessment.

The pathogens of concern and appropriate control processes that inactivate those pathogens differ for each category of foods. The panel listed such pathogens and control processes in Table 1.

Effects of Processing Technologies

Establishment of traditional thermal processes (for example, canning, pasteurization, baking, and cooking) for foods has been based on two main factors: (1) knowledge of the thermal inactivation kinetics of the most heat-resistant pathogen of concern for each specific food product; and (2) determination of the nature of heat transfer properties of the food system. The validity of a thermal process must be confirmed by an inoculated challenge test conducted on the product under actual plant conditions using surrogate microorganisms as biological indicators to mimic pathogens. Thus, the two factors described above, which are well established for thermal processes, should be used for establishing and validating scheduled new thermal processes based on thermal effect on microorganisms, such as microwave heating.

For other preservation processes not based on heat inactivation, key pathogens of concern and nonpathogenic surrogates need to be identified and their significance evaluated.

NSF and American Bakers Association

Both the ABA and the NSF testing protocols suffer from significant weaknesses that hamper their usefulness in determining whether a food can be safely stored at room temperature. The NSF protocol takes an overly stringent approach, whereas the ABA protocol is sometimes overly permissive. Two major significant differences between the two protocols are (1) the consideration (or lack of consideration) of the process the food did or will undergo, and (2) the selection of organisms used or not used to inoculate the food. The panel developed a general protocol for microbiological challenge testing. Table 2 presents a comparison of the features of these protocols.

Development of a Framework

Based on the criteria used by industry, government, and trade organizations; survey data collected by the panel; available scientific literature; and the panelists' experience on this subject, a framework was developed to facilitate the determination of whether or not a food needs time/temperature control for safety.

Figure 1 describes the proposed framework to determine whether a food needs time/temperature control for safety.

Before proceeding with Step 1 of the evaluation process, the evaluator needs to make a succinct review of the food product in question. If the food may already be held hot or cold for safety reasons, there is no need not proceed any further. Also, product history in combination with a valid scientific rationale that justifies such safe history of use may be used as criteria to designate a food as non-TCS food.

The panel concluded that the appropriate scientific evidence on pH, water activity, and pH/a_w interaction exists to allow for the evaluation of a food. Two pH/a_w tables were designed. If heat or process technologies alternative to heat are applied, then effectiveness needs validation. For some products, and specially combination products, the analysis of pH and a_w may be inaccurate. Consequently, for these products the pH and a_w would not be considered as controlling factors without supporting data.

If the determination indicates that a food may be a TCS in the table, an analysis may be performed to assess the microbial risk of holding the product at ambient temperature. A comprehensive description of the product as part of this analysis is compiled. If historical information regarding product safety is considered, it should be provided with a sound scientific rationale. In addition

to the usual factors, time of expected storage and display might also be considered. If the duration of storage and/or display is less than that needed for microbial growth and/or toxin production, adequate control may be achieved through a variety of time and temperature combinations. Under certain circumstances, time alone at ambient temperatures can be used to control product safety. The USDA Pathogen Modeling Program v. 5.1 could be used, with appropriate interpretation, to assist in the determination of pathogen growth. Unless used conservatively, it is often more appropriate to use them in combination with challenge testing. In the absence of an appropriate and validated prediction model, a challenge test alone could be used. If the hazard analysis indicates the product is a non-TCS, the product can be stored at room temperature. If the product is identified as a TCS, the evaluator can either decide to modify the product, change the processing and handling it undergoes, control pathogen growth with time/temperature, or revisit the commercial feasibility of the product.

The panel's framework on time/temperature control of foods for safety was critiqued by applying it to a variety of foods. Each step of the framework has been described as it applies to the food under consideration. Most of the data on the individual foods were from industry studies submitted to the panel.

Summary

In summary, the panel introduced a new approach for evaluating foods that may need time/temperature control for safety. This framework was based on scientific data from peer-reviewed publications that were further evaluated by the panel. The panel recognizes that the implementation of its approach in the field may not be an easy task. For example, although some of the considerations introduced in the proposed framework require careful evaluation and assessment by an expert microbiologist, this report does not attempt to propose who would be responsible for deciding the time/temperature status of a food. The panel also did not address the implications of the framework at the retail level. The panel believes, however, that in light of the complexity of the food systems and the confusion over the interpretation of the term "potentially hazardous foods," a science-based framework such as the one proposed here would be a more accurate, comprehensible, and clear alternative to the current definition and application of the term.

Table 1—Pathogens of concern and control methods for various product categories.

Product Category (examples of possible foods for evaluation)	Pathogens of concern	Types of process control[1] (alone and in combination)
Meats and poultry (fermented sausage)	Clostridium botulinum[5] and Clostridium perfringens, Salmonella spp., enterohemorrhagic Escherichia coli, Camplylobacter jejuni, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes	Time/temperature, pH, a_w, preservatives, moisture protein ratio, fermentation, heat processing
Fish and seafood (smoked fish)	Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, C. botulinum[5], L. monocytogenes, Salmonella spp., Shigella spp., S. aureus	Time/temperature, harvest site control, fermentation, pH, a_w, water-phase salt, preservatives, drying, salting
Fruits and vegetables (peeled carrots)	Salmonella spp., Shigella spp., enterohemorrhagic E. coli, L. monocytogenes, Bacillus cereus, C. botulinum[5], Y. enterocolitica	Production control (Good Agriculture Practices), time/temperature, cooking, preservation techniques
Cereal grains and related products (fresh pasta, focaccia)	Salmonella spp., S. aureus, B. cereus, C. botulinum[5]	Cooking, a_w, pH, preservatives, time/temperature
Fats, oils & salad dressings	S. aureus[2], Salmonella spp.[2], B. cereus[2], C. botulinum[2] (garlic-in-oil)	pH, a_w, salt
Butter and margarine (light salted butter)	S. aureus, L. monocytogenes, Y. enterocolitica	Production/raw ingredient quality control, moisture droplet size in the water-in-oil emulsion, water phase salt, a_w
Sugars and syrups (light maple syrup)	C. botulinum[3,]	a_w, acidification (light syrups)
Eggs and egg products (merengue)	Salmonella spp.[4], L. monocytogenes[4]	Production control, cooking/pasteurization, time/temperature
Milk and milk products (yoghurt)	Salmonella spp.[4], L. monocytogenes[4], enterohemorrhagic E. coli[4], S. aureus[4], B. cereus (cells[4] and spores[5]), C. botulinum (cells[4] and spores[5]), Campylobacter jejuni[4]	Production control, time/temperature, cooking/pasteurization, a_w, preservatives
Cheese and cheese products (Natural Swiss cheese)	Salmonella spp.[4], L. monocytogenes[4], enterohemorrhagic E. coli[4], S. aureus[4], Shigella spp.[4], C. botulinum (cells[4] and spores[5])	Production control, moisture content, a_w, pasteurization, preservatives, pH
Combination products (cheese with veg. pieces, pumpkin pie, stuffed pastry)	Variable, based on raw materials and processing	Variable, based on raw materials and product

[1]Good Manufacturing Practices would help in reducing the hazards. For meats, poultry, and fish and seafood products the Hazard Analysis Critical Control Point principles should be implemented as a control system.
[2]A pH > 4.0 and a_w ~ 0.92 in salad dressings and mayonnaise would preclude the growth of pathogens of concern.
[3]Only a concern in light syrups and can be controlled by acidification.
[4]In pasteurized products, all pre-processing vegetative pathogens would be controlled.
[5]Only a concern in anoxic environments.

Table 2—Summary of comparison of NSF, ABA and expert panel protocols to determine if a food requires time/temperature control for safety.

Item	ABA	NSF	Panel's Alternative Protocol
Type of product	Pumpkin pie	Four groups: bread with vegetables and cheese pre-bake, filled post-bake, filled pre-bake, toppings. Traditional and other products excluded.	Any food product proposed to be stored outside temperature control.
Consideration of process	Yes (Good Manufacturing Practices, [GMP's], baking temperature, cooling, and packaging)	No	Yes. Additional information for validation of process also required.
Microorganisms tested	Aerobic Plate Counts (APC), *Staphylococcus aureus*, coliforms, salmonellae	*Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, Salmonella spp., *S. aureus*, *Clostridium perfringens* depending on pH and a_w	Organisms should be selected based on history of safety, formulation, storage atmosphere environment and packaging of the food.
Inoculation type	None (indigenous only)	Composite of 5 strains of each organism. Each composite inoculated into the product separately.	Composite of multiple strains of each organism. Each composite inoculated into the product separately.
Inoculation method	Not applicable	Prescribed in phosphate buffer.	Prepared in system that mimics the product: Previously mixed with buffer or water, directly added to product, aseptically injected, mixed powder product, or lyophilized, depending on the product.
Inoculum preparation	Not applicable. before or after inoculation.	Aerobes cultured in tryptic soy broth, *C. perfringens* cultured in fluid thioglycolate broth.	Cultures grown in suitable media under either optimal or food-adapted conditions. Spores are washed and heat-shocked
Inoculum	Not applicable	Each unique component and each unique interface between components at both internal and external surfaces.	Each component, and each unique interface position between components, but only where the organisms of concern would survive the process or be reintroduced post-processing.
Inadvertent product modification	Not applicable	Addition of the inoculum in buffer has a potential to change product water activity.	Additional measurements of a_w should be taken to insure that inoculation technique does not influence product a_w.
Inoculum technique	Not applicable	No consideration for relative component weights given when splitting inoculum between components.	Not applicable
Sampling	Two: within 24 h of packaging, and at end of shelf life	One to ten: depending on intended shelf life.	A minimum of 5 times over shelf life, times including zero time.
Pass criteria	No pathogens detected, APC less than 1,000 CFU after bake, and less than 100,000 CFU at end of shelf life	Less than 1 log CFU increase for any pathogen by the end of the study and not to exceed 1 log CFU for any pathogens at two consecutive time points before the end of the study.	Depending on the pathogen, less than 1 or 4 log increase at any point in shelf life for vegetative pathogen(s) of concern and no detectable toxin at the end of the shelf life for toxin-forming microbes.
Other tests pH, a_w	Oxidation/Reduction potential,	pH, a_w	pH, a_w, pH/a_w interaction.
Process	Process is considered by use of natural inoculum. food after processing.	Process is not considered, since pathogens are inoculated into the study.	Process should be considered in the selection of appropriate microbes for use in the challenge. Data to validate the process should be provided.
Methods	Association of Official Analytical Chemistry/Bacteriological Analytical Manual	Compendium of Methods for the Microbiological Examination of Foods	Any reproducible, validated method is acceptable.
Duration of study	The study lasts until the use by date, which is calculated by multiplying 1.3 times the sell by date.	The study lasts 1.3 times the time the products will be out of temperature control.	The study should last for at least the shelf life of the product, but 1.3 times the intended shelf life is recommended.
Spoilage	Addressed indirectly with APC	Not applicable	Testing of inoculated sample for background bacteria.
Replication	6 samples at beginning and 6 at end of one production run	3 lots, 2 samples/lots, over shelf life	Minimum of 3/sampling time unless this is a revalidation study or control sample (less samples are needed).
Anaerobes	Only an O/R potential measurement is made, no microbial tests are done.	*C. perfringens*	*C. botulinum* itself is used, with toxin production as the definitive measure of safety.
Microbial growth modeling	Not applicable	Not applicable	Properly validated growth models can be used alone or in combination with microbial challenge studies.
History of safe use	Not applicable	Not applicable	A long history of safe use can be considered in combination with appropriate scientific rationale instead of challenge studies.

Figure 1—Framework for determining if time/ temperature is required for safety

The food in question may already be held hot or cold for safety reasons. In this case, and if there is no desire for ambient temperature storage, an analysis using this framework is not needed. If the need to control the temperature of the product for safety reasons is unknown, a review of the food, its ingredients, and general methods of preparation should precede the evaluation of the food. If the food, as described, has a substantial and extensive history of safe use without time/temperature control, and there is enough scientific rationale that supports such safe history of use, then the food may continue to be classified as not requiring temperature control for safety, or non-TCS (see also Chapter 3, section 4.2.).

If there is no known history of safe use, proceed with Step 1.

The panel's framework on time/temperature control of foods for safety was critiqued by applying it to a variety of foods. Each step of the framework has been described as it applies to the food under consideration. Most of the data on the individual foods were from industry studies submitted to the panel.

In summary, the panel introduced a new approach for evaluating foods that may need time/temperature control for safety. This framework was based on scientific data from peer-reviewed publications that were further evaluated by the panel. The panel recognizes that the implementation of its approach in the field may not be an easy task. For example, although some of the considerations introduced in the proposed framework require careful evaluation and assessment by an expert microbiologist, this report does not attempt to propose who would be responsible for deciding the time/temperature status of a food. The panel also did not address the implications of the framework at the retail level. The panel believes, however, that in light of the complexity of the food systems and the confusion over the interpretation of the term "potentially hazardous foods," a science-based framework such as the one proposed here would be a more accurate, comprehensible, and clear alternative to the current definition and application of the term.

Step 1—Was the food treated to destroy vegetative cells of potential pathogens and packaged to avoid recontamination? If yes, position your product in Table A according to its pH and water activity (a_w). If not, position your product in Table B according to its pH and a_w.

Table A—Control of spores: Product treated to control vegetative cells and protected from recontamination.

Critical a_w values	Critical pH values		
	4.6 or less	> 4.6 to 5.6	> 5.6
0.92 or less	Non-TCS	Non-TCS	Non-TCS
> 0.92 to .95	Non-TCS	Non-TCS	?
> 0.95	Non-TCS	?	?

Table B—Control of vegetative cells and spores: Product not treated or treated but not protected from recontamination

Critical a_w values	Critical pH values			
	< 4.2	4.2 to 4.6	> 4.6 to 5.0	> 5.0
< 0.88	Non-TCS	Non-TCS	Non-TCS	Non-TCS
0.88 to 0.90	Non-TCS	Non-TCS	Non-TCS	?
> 0.90 to .92	Non-TCS	Non-TCS	?	?
> 0.92	Non-TCS	?	?	?

Step 2—If the food is classified as a non-TCS food according to Step 1 above, it may be stored and held safely without regard to time or temperature. If the need for time/temperature control is questionable, the food should be held either hot or cold for safety, or subjected to a product assessment as the next step in determining the appropriate classification.

Science Advisory Board

Roy G. Arnold, Ph.D.
Executive Associate Dean
College of Agricultural Science
Oregon State University

Lester M. Crawford, Ph.D., D.V.M.
Director (formerly), Center for Food and Nutrition Policy
Virginia Polytechnic Institute and State University

Ray A. Goldberg
George M. Moffett Professor of Agriculture and Business Emeritus
Harvard Business School

Marcus Karel, Ph.D.
Professor Emeritus
Massachusetts Institute of Technology and Rutgers University

Sanford A. Miller, Ph.D.
Adjunct Professor
Center for Food and Nutrition Policy
Virginia Polytechnic Institute and State University

Martha Rhodes Roberts, Ph.D.
Deputy Commissioner for Food Safety
Dept. of Agriculture and Consumer Services State of Florida

G. Edward Schuh, Ph.D.
Freeman Chair Professor
Hubert H. Humphrey Institute of Public Affairs
University of Minnesota

Barbara O. Schneeman, Ph.D.
Professor of Nutrition
Department of Nutrition
University of California

Institute of Food Technologists Scientific and Technical Panel

Frank F. Busta, Ph.D.
Panel Chair and Senior Science Advisor to the Institute of Food Technologists
University of Minnesota

Dane T. Bernard, Ph.D.
Keystone Foods

Robert B. Gravani, Ph.D.
Cornell University

Paul Hall
Kraft Foods, Inc.

Merle D. Pierson, Ph.D.
Virginia Polytechnic Institute and State University (formerly)

Gale Prince
The Kroger Company

Donald W. Schaffner, Ph.D.
Cook College Rutgers
The State University of New Jersey

Katherine M.J. Swanson, Ph.D.
The Pillsbury Company

Betsy Woodward
Food Labs for Florida Department of Agriculture and Consumer Services (Retired Chief)

Frank Yiannas
Food Safety and Health
Walt Disney World

Reviewers

Catherine Adams, Ph.D.
Heinz North America

Michael H. Brodsky
Brodsky Consultants

Alfred E. Bugenhagen
New York Department of Agriculture and Markets

Martin Cole
Food Australia

J. Joseph Corby
New York State Department of Agriculture and Markets

Carl S. Custer
U.S. Department of Agriculture

Michael P. Davidson, Ph.D.
University of Tennessee

Robert Donofrio
NSF International

Michael P. Doyle, Ph.D.
University of Georgia

Russell S. Flowers, Ph.D.
Silliker Labs Group, Inc.

Leonard Wm. Heflich
Bestfoods Baking Company

Leon Gorris
Uniliver

Maureen Olewnik
American Institute of Baking

Virginia N. Scott
National Food Processors Association

John Silliker, Ph.D.
Silliker Labs, Inc.

George Smith
Arkansas Department of Health

Pete Snyder
Hospitality Institute of Technology and Management

Dan Sowards
Texas Department of Health
Food Safety

William H. Sperber
Cargill, Inc.

Bruce Tompkin
Armour Swift-Eckrich

Edmund A. Zottola, Ph.D.
University of Minnesota

Additional Credits

Food and Drug Administration

Donald M. Kautter, Jr.
Contract Technical Officer (formerly)
Division of HACCP Programs

Ed Arnold
Contracting Officer

Jeanette Lyon
Consumer Safety Officer
Retail Food and Interstate Travel Team

Institute of Food Technologists

Bruce R. Stillings, Ph.D.
1998-1999 President

Charles E. Manley, Ph.D.
1999-2000 President

Mary K. Schmidl, Ph.D.
2000-2001 President

Phillip E. Nelson, Ph.D.
2001-2002 President

Mark McLellan, Ph.D.
2002-2003 President Elect

Daniel E. Weber
Executive Vice President (formerly), retired

Fred R. Shank, Ph.D.
Vice President, Science, Communications and Government Relations

Jill A. Snowdon, Ph.D.
Director, Department of Science and Technology Projects (formerly) (currently with SGA Associates)

Maria P. Oria, Ph.D.
Staff Scientist (formerly) (currently with the National Academy of Sciences)

Karen Arcamonte, M.S.
Information Specialist (formerly) (current affiliation unknown)

Kendra Langeteig, Ph.D.
Administrative Assistant (formerly) (current affiliation unknown)

Other Contributors

The Institute of Food Technologists and the Science and Technology Expert Panel express their thanks to the many companies and trade associations that cooperated with this project by sharing research data. While remaining as confidential information to all participants, the data was of great value to this report and we appreciate the efforts of those companies and individuals. In addition, the Institute and the panel appreciate the cooperation of the state governments that, by submitting data and information, contributed to the quality of this report.

Comprehensive Reviews in Food Science and Food Safety

Introduction and Explanatory Notes

The term "potentially hazardous food" (PHF) was developed by the United States Public Health Service during the last half of the twentieth century to regulate perishable food or drink in eating and drinking establishments (see Appendix A). The current definition of PHF is articulated in the United States Public Health Service/Food and Drug Administration (FDA) Food Code (FDA 1999, p 12)—a model code for adoption by states or counties overseeing operations providing food directly to the consumer. As explained in the Background section of the Charge from FDA, the definition of PHF has become outdated and cumbersome. In particular, the word "hazard" in "potentially hazardous" has implications inconsistent with the use of the word in the Hazard Analysis Critical Control Points (HACCP) concept (NACMCF 1998). The term "rapid and progressive growth" in the FDA Food Code definition is also unclear, especially in the absence of specific information on organisms, media, conditions of growth, or new products with extended shelf life. The need for time/temperature control in foods that traditionally required such control has been eliminated in some instances by new formulations and processes. Moreover, no clear-cut, standardized means are specified in the Food Code to determine whether time/temperature control is needed to ensure the safety of a food.

Many professionals and professional societies involved in food protection share these concerns about the limitations and cumbersome nature of the FDA regulations. For example, the 1996 Conference for Food Protection (CFP) recommended that FDA work with a third party to develop a standard that would address this issue. CFP has also referred the issue to a committee (the Potentially Hazardous Foods Definition Committee) for study. In addition, both the NSF International (NSF) and the American Bakers Association (ABA) are attempting to address these issues by developing protocols to assess the safety of specific foods held at room temperature.

The panel collected and reviewed the criteria and/or definitions that industry, foreign and domestic regulatory agencies, trade organizations, and others currently use to determine whether a food should be stored under controlled time/temperature conditions for safety purposes. Criteria such as pass/fail criteria and challenge tests are included in the scope of this report. In addition, the panel reviewed the common pathogenic microorganisms that are used by industry and organizations in challenge studies to determine whether a food needs time/temperature control for safety. The panel reviewed whether the Food Code term "rapid and progressive growth of microorganisms" is commonly used by industry or organizations, and how it is applied to determine if a food can be safely stored at room temperature. The panel also evaluated the scientific basis of criteria used to assess growth of common organisms of public health significance.

The panel also discussed the appropriateness of the Food Code definition/term "potentially hazardous," and proposed the use of an alternative or equivalent term, such as "temperature controlled for safety" (TCS). This terminology serves to avoid confusion with the current definition of "hazard" in the Hazards Analysis Critical Control Points (HACCP) approach, and emphasizes the importance of temperature control as a safety factor.

Before any approach to determine whether a food needs time/temperature control for safety can be accepted, an evaluation of its scientific basis is necessary. Thus, the panel conducted an in-depth review of NSF's Standard 75 "International Standard for Non-Potentially Hazardous Foods" (NSF 2000), and ABA's "Industry Protocol for Establishing the Shelf Stability of Pumpkin Pie" (ABA 2000). The panel identified advantages and disadvantages to each organization's approach.

Based on their review and analysis of state, industry, and organization data and protocols, the panel recommended science-based approaches for defining foods that need time/temperature control for safety as well as those that can be excluded from such control. In developing a framework for determining the need for time/temperature control, the panel considered the following criteria: the presence of pathogens on the foods, the characteristics of foods that support growth of pathogens, expected storage conditions, shelf life, and potential storage abuse. The panel developed a science-based framework for determining the effectiveness of processing and/or formulation technologies that result in a food not requiring time/temperature control for safety. The panel reviewed validation techniques that are suitable for determining the effectiveness of these technologies, including process controls, mathematical models, and biological challenge testing. Advantages and disadvantages of each approach are also included in this report.

The panel used their proposed framework to determine its applicability to a specific example(s) from each of the following product categories: salad dressings, condiments such as mustard and mayonnaise, chopped garlic-in-oil, garlic-flavored oil, butter (whipped, not whipped, salted, unsalted), margarine, cheeses, filled bakery products (crème vs. cream), and vegetable breads, such as focaccia.

The panel did not address the following items because they were not included in the FDA charge:

● Issues related to the implementation of a program to verify compliance with the recommended framework. For example, the panel did not identify who the decision-maker should be or how a retail food store inspector would verify whether a product needs time/temperature control.

- Food products that do not require time/temperature control for safety but may be hazardous (that is, cause disease) if they contain pathogenic microorganisms with a low infectious dose were not included in the scope of this report. These are products such as apple cider contaminated with *Escherichia coli* O157:H7. Because the infectious dose of this microbe is low, a low concentration of *E. coli* O157:H7 in food can cause disease. Thus, preventing pathogen growth through time/temperature control would not control the risk.

- Time/temperature control considerations to prevent spoilage.

References

[ABA] American Bakers Association. 2000 Jan 18. Industry protocol for establishing the shelf stability of pumpkin pie [final version plus executive summary]. Washington (DC): ABA. 18 p. Available from: lsanders@americanbakers.org.

[FDA] Food and Drug Administration. 1999. Food Code: 1999 recommendations of the United States Public Health Service, Food and Drug Administration. Springfield (VA): U.S. Dept. of Commerce, Technology Administration, National Technical Information Service. Report nr PB99-115925. Chapter 1, Part 1-201.10(B)(61). p 12. Available from: <http://www.foodsafety.gov/%7Edms/foodcode.html>.

[NACMCF] National Advisory Committee on Microbiological Criteria for Foods. 1998. Hazard analysis and critical control point principles and application guidelines. J Food Prot 61:762-75.

[NSF] NSF International. 2000 Nov. 10. Non-potentially hazardous foods. Ann Arbor (MI): NSF International. Report nr ANSI/NSF 75-2000. 12 p.

Current and Proposed Definitions of "Potentially Hazardous Foods"

1. Regulations review

The IFT panel searched domestic and international regulations and guidelines for terms similar to the FDA Food Code definition of "potentially hazardous foods" (PHF) and associated requirements with a focus on the scientific basis for these definitions. Australia and Canada, like the United States, use the term "potentially hazardous foods" in their food safety regulations (ANZFA 2001a, CFIS 2001ab, FDA 1999). Other regulatory entities have temperature control requirements, but do not use the term PHF. While temperature requirements for chilled foods are identified, other regulations for temperature control generally do not present guidelines or a framework to determine which foods fall into the "chilled" category. Rather, specific reference is made to the need for temperature control to protect public health. Lists of products that need to be temperature controlled for safety are sometimes included. These products generally have a history of association with illness in the absence of temperature control. A summary of regulations used by agencies in the United States, Australia, Canada, the United Kingdom, and the European Union follows.

1.4. Food and Drug Administration

The following is the definition used in the FDA Food Code (FDA 1999, pt 1-201.10[B][61]):

(a) "Potentially hazardous food" means a food that is natural or synthetic and that requires temperature control because it is in a form capable of supporting:

(i) The rapid and progressive growth of infectious or toxigenic microorganisms;

(ii) The growth and toxin production of *Clostridium botulinum*; or

(iii) In raw shell eggs, the growth of *Salmonella* Enteritidis.

(b) "Potentially hazardous food" includes an animal food (a food of animal origin) that is raw or heat-treated; a food of plant origin that is heat-treated or consists of raw seed sprouts; cut melons; and garlic-in-oil mixtures that are not modified in a way that results in mixtures that do not support growth as specified under Subparagraph (a) of this definition.

(c) "Potentially hazardous food" does not include:

(i) An air-cooled hard-boiled egg with shell intact;

(ii) A food with an a_w value of 0.85 or less;

(iii) A food with a pH level of 4.6 or below when measured at 24 °C (75 °F);

(iv) A food, in an unopened hermetically sealed container, that is commercially processed to achieve and maintain commercial sterility under conditions of nonrefrigerated storage and distribution; and

(v) A food for which laboratory evidence demonstrates that the rapid and progressive growth of infectious or toxigenic micro-organisms or the growth of *S.* Enteritidis in eggs or *C. botulinum* cannot occur, such as a food that has an a_w and a pH that are above the levels specified under Subparagraphs (c)(ii) and (iii) of this definition and that may contain a preservative, other barrier to the growth of microorganisms, or a combination of barriers that inhibit the growth of microorganisms.

(vi) A food that does not support the growth of microorganisms as specified under Subparagraph (a) of this definition even though the food may contain an infectious or toxigenic microorganism or chemical or physical contaminant at a level sufficient to cause illness.

1.2. United States Department of Agriculture (USDA)

USDA/Food Safety and Inspection Service (FSIS) Food Standards and Labeling Policy Book (USDA 1996) identifies criteria for a "shelf-stable" product. Criteria include product specific Moisture Protein Ratio (MPR) such as: "dry sausage with Moisture Protein Ratio (MPR) = 1.9:1, semi-dry sausage with MPR = 3.1:1 with a pH = 5.0, or commercially sterilized. Alternatively, nonrefrigerated, semi-dry shelf stable sausages are those that are fermented to a pH of 4.5 or lower (or 4.6 combined with a a_w of less than 0.91); are in an intact form or, if sliced, are vacuum packed; have an internal brine concentration of no less than 5%; are cured with nitrite; and are cured smoked with wood." With the full implementation of HACCP, 9 C.F.R. § 417.4 (2001), establishments are required to have records validating their critical limits to control hazards. The MPR criteria provide an alternative approach to pH and a_w alone that recognizes the effectiveness of combined multiple controls. A product processed in the retail environment and therefore not covered under the USDA/FSIS HACCP rule should meet these same requirements for shelf stability and have records documenting control of hazards.

1.3. State regulations

Most states have adopted the FDA Food Code definition of "potentially hazardous foods" or the previous FDA/AFDO Retail Code or FDA Food Service Code, which do not state a specific a_w or pH value, but use a general definition that has been interpreted by the FDA as including a_w = 0.85 and pH = 4.6. The state of Washington, and a county within that state, King County Seattle, have adopted a modified requirement of pH = 4.6 and a_w = 0.90 (Wash. Admin. Code § 246-215-010).

A possible explanation for the adoption of a higher a_w limit follows. Toxin production by *Staphylococcus aureus* under anaerobic conditions is limited by an a_w of 0.92. Under aerobic conditions, toxin production is generally inhibited at a_w < 0.90 (Baird-Parker 1990). However, studies in pure culture have demonstrated toxin production at a_w = 0.88 adjusted with glycol (Stewart and

others 2001). Additional studies in food systems are necessary to validate the effectiveness of a_w 0.90 as an effective control.

1.4. International regulations

1.4.1. Australia. In July 2000, the Australia New Zealand Food Standards Council adopted three Food Safety Standards: Interpretation and Application (Standard 3.1.1), Food Safety Practices and General Requirements (Standard 3.2.2), and Food Premises and Equipment (Standard 3.2.3) into the Australia New Zealand Food Standards Code. These standards will replace existing State and Territory regulations. Standard 3.2.2 defines "potentially hazardous food" as "food that has to be kept at certain temperatures to minimize the growth of any pathogenic microorganisms that may be present in the food or to prevent the formation of toxins in food" (ANZFA 2001a). The regulations further define specific temperature requirements for a food business to receive, store, display, or transport a "potentially hazardous food" (for example, ≤ 5 °C [41 °F] or ≥ 60 °C [140 °F]). Alternatively, it requires that "the food business demonstrate that maintenance of the food at a temperature for the period of time for which it will be so maintained, will not adversely affect the microbiological safety of the food" (ANZFA 2001a). The standard also requires specific times and temperatures for cooling cooked "potentially hazardous foods."

Australia's Priority Classification System for Food Businesses" (ANZFA 2001b) provides further discussion of high, medium, and low risk foods as follows:

- High risk foods are foods that "may contain pathogenic microorganisms and will normally support formation of toxins or growth of pathogenic microorganisms." Examples are raw meat, fish, oysters, poultry, milk, tofu, fresh filed pasta, meat pies, frankfurts, salami, cooked rice, and lasagne.
- Medium-risk foods are foods that "may contain pathogenic microorganisms but will not normally support their growth due to food characteristics; or food that is unlikely to contain pathogenic microorganisms due to food type or processing but may support formation of toxins or growth of pathogenic microorganisms." Examples are fruits and vegetables, orange juice, canned meats, pasteurized milk, dairy products, ice cream, peanut butter, and milk-based confectionery.
- Low-risk foods are foods that "are unlikely to contain pathogenic microorganisms and will not normally support their growth due to food characteristics." Examples are grains and cereals, bread, carbonated beverages, sugar-based confectionery, alcohol, and fats and oils.

1.4.2. Canada. The Canadian Food Inspection Agency (CFIA) *Food Retail and Food Services Regulation* defines "potentially hazardous food" as "food in a form or state which is capable of supporting the growth of pathogenic microorganisms or the production of toxins" (CFIS 2001a). This definition which is similar to some of the provisions in the FDA Food Code is expanded in the CFIA Food Retail and Food Services Code as follows: "any food that consists in whole or in part of milk or milk products, eggs, meat, poultry, fish, shellfish (edible mollusca and crustacea), or any other ingredients, in a form capable of supporting growth of infectious and/or toxigenic microorganisms. This does not include foods which have a pH of 4.6 or below and foods which have an a_w of 0.85 or less" (CFIS 2001b). The Canadian Code further interprets potentially hazardous foods in its Appendix A (CFIS 2001b), which extrapolates interpretative questions from the "Guidelines for Production, Distribution, Retailing and Use of Refrigerated Prepackaged Foods with Extended Shelf Life" (Health Canada, Health Protection Branch 1992 Mar 1; Guideline No. 7). The Canadian Code's a_w limit is based on control of *S. aureus* growth. As explained in Chapter 3, however, toxin is inhibited at a higher value even under optimum conditions. Conversely, lower pH values are

required in some situations to control *Salmonella* spp. for an extended period of time.

1.4.3. United Kingdom. The United Kingdom does not use the term "potentially hazardous food" but identifies foods that require temperature control in the *Food Safety (Temperature Control) Regulations,* (1995) SI 1995/2200. These regulations require "Chill holding" at ≤ 8 °C (46 °F) for "any food … which is likely to support the growth of pathogenic microorganisms or the formation of toxins." Foods considered likely to fall into this category include the following:

- Dairy products, such as soft or semi-hard cheeses ripened by molds and/or bacteria, and dairy based desserts, unless the pH is < 4.5;
- Cooked products such as meat, fish, eggs, milk, hard and soft cheese, rice, pulses, and vegetables;
- Smoked or cured fish;
- Smoked or cured ready-to-eat meat which is not ambient shelf-stable;
- Prepared ready-to-eat foods such as prepared vegetables, salads;
- Uncooked or partly cooked pastry and dough products such as pizzas, sausage rolls, or fresh pasta.

Time-related exemptions from temperature control are provided for the following products:

- "(a) cooked pies and pasties containing meat, fish or any substitute for meat or fish or vegetables or cheese or any combination thereof encased in pastry into which nothing has been introduced after baking and sausage rolls which are intended to be sold on the day of their production or the next day;
- "(b) uncut baked egg and milk pastry product, e.g., custard tarts and Yorkshire curd tarts intended for sale within 24 hours of production."

General exemptions from chill holding requirements are given to "foods which, for the duration of their shelf life, may be kept at ambient temperatures with no risk to health." A food business must provide "well founded scientific assessment of the safety of the food at the specified temperature and shelf-life" for products recommended to be held above 8 °C (46 °F). Regulations do not articulate data requirements, rather they stipulate that assessments should be done by a "competent laboratory," either in-house, for large businesses, or through independent laboratories.

These regulations recognize the influence of processing and time. For example, baking destroys vegetative cells and dehydrates exterior surfaces. The potential for growth of pathogenic spore formers exists, but time is used to control this hazard. The panel questions whether there is adequate scientific basis to support a time of one day of safety at ambient temperature for the time/related exemptions.

1.4.4. European Union. The European Union *Hygiene of Foodstuffs E(876)* specifies that "Raw materials, ingredients, intermediate products and finished products likely to support the growth of pathogenic microorganisms or the formation of toxins must be kept at temperatures which would not result in a risk to health" (The Council of the European Communities 1993). Specific times, temperatures, or other factors are not identified, therefore no parameters require justification.

2. Critique of FDA's "potentially hazardous foods" definition

The panel reviewed the current FDA Food Code definition for "potentially hazardous foods" (PHF) (see section 1.1) and the history of its development. The original concept, and that used elsewhere in the world, acknowledges that certain foods (for example, meat, poultry, milk products, eggs, and other high a_w, neutral products) require time/temperature control to maintain safety. These products have a well documented history of causing foodborne illness outbreaks when subjected to temperature abuse;

therefore, time/temperature control is essential to protect the public health. However, many products with pH and a_w above the levels identified in the current Food Code definition have been safely stored at ambient temperatures (for example, white bread, certain cheese spreads, some fermented sausages) due to science-based factors other than pH and a_w. It is the opinion of the panel that the current definition of PHF is complex and causes some in the food safety community to limit consideration of factors to only pH and a_w. This limitation results in the inclusion of many foods as "potentially hazardous foods" when, in fact, they are not.

The term "potentially hazardous food," in its current usage, causes considerable confusion. This definition (which limits or prescribes consideration of factors other than pH and a_w) is narrower than what the term implies in that temperature control alone cannot provide product safety. Many foods that meet the current definition can be hazardous if pathogens are present at infectious levels. For example, temperature control will not prevent outbreaks caused by *Escherichia coli* O157:H7 or *Salmonella* spp. in juices with pH of less than 4.6. Conversely, certain fermented sausages have pH and a_w levels higher than those in the definition, yet have a well documented history and validation of safety at room temperature. In addition, the food safety community does not generally make use of the term PHF; other terms, such as high-risk food, are used.

The panel recommends use of a simplified definition, with an interpretive guide, to strengthen the regulatory focus on appropriate foods by (1) providing detailed, scientifically based examples of products that can be stored safely without temperature control; and (2) avoiding misclassification of safe foods. The panel also proposes the use of the term "temperature controlled for safety" (TCS) foods in place of PHF. This term accurately describes both what is required: temperature control with time implied, and why it is required: safety. The TCS term avoids confusion with the term "hazard" as it is applied in HACCP. It also avoids the inclusion of foods that do not require time/temperature control for safety, and avoids confusion related to products that present a risk to consumers where the risk is not controlled by storage or holding temperature (for example, *E. coli* O157:H7 in fruit juice). The term "temperature controlled for safety" is a more accurate reflection of the true concept behind the current definition for PHF. The use of both terms, TCS and PHF, during the transition can facilitate migration from one term to the other. Other terms considered by the panel (and the rationales for their exclusion) follow:

- Temperature Sensitive Food or Temperature Controlled Food (the safety aspect is not explicit)
- Microbiologically Unstable Food (does not articulate safety concerns and does not identify the control strategy)
- Time/Temperature Sensitive Food (more cumbersome than the term proposed, does not articulate safety, and time can be included in a simplified definition)
- High-Risk Products (can be confused with risk assessment efforts and does not identify control strategy)
- Temperature Safe Food
- TempSafe Food

The agency might consider adopting a term for defining foods that require time/temperature control for safety such as "temperature controlled for safety" (TCS). The panel suggests using a definition for TCS foods such as "foods that require time/temperature control to limit pathogen growth or toxin formation that constitutes a threat to public health."

As part of the charge, the panel also reviewed the current Food Code definition 1-201.10 (B) (61) (see section 1.1. of this chapter) and has the following observations relative to the scientific basis for the definition:

Section a

The term "rapid and progressive" in Section a in the Food Code is no longer appropriate. The term was originally used at a time when shelf life of most foods was relatively short and the concern was growth of pathogens occurring in hours rather than days. Current production, processing and packaging technologies, extended shelf life products, distribution systems, and consumer-use practices have altered this paradigm. Therefore, microbial growth need not be rapid to present a threat to public health in some food products. Progressive growth of pathogens to levels that present a threat to public health or levels that produce toxin are the key issues. The amount of growth required to present a threat to public health is specific to the organism, the food, and other factors discussed subsequently in this report. Removing the subjective requirement for "rapid" growth removes the need to specifically address *Clostridium botulinum* in the definition. Formation of hazardous levels of any toxic substance through microbial growth is unacceptable. Specifics related to *C. botulinum* control and *Salmonella* Enteritidis in eggs may be more appropriately covered in the recommended interpretive guidelines.

Section b

The food items listed in Section b of the Food Code definition have been linked to foodborne illness. Time/temperature abuse has been a contributing factor for most of the products listed in this section.

Section c

The pH and a_w values in Section c are problematic. The actual values that restrict growth vary with different acidulants for pH, humectants for a_w, and other properties of the food under consideration. Technically, an a_w of 0.85 is inappropriately low as a general a_w minimum because most pathogens are inhibited at values well above 0.86 and *S. aureus* toxin formation (the true hazard) is restricted at higher a_w values (see Chapter 3). Conversely, a pH of 4.6 may not control the growth of certain pathogens with some acidulants within the intended "use time." There is no scientific basis to single out *C. botulinum* and *Salmonella* Enteritidis in this section. Control of all relevant pathogens must be addressed. The term "laboratory evidence" currently used in the Food Code definition is unnecessarily restrictive in describing potential documentation for demonstrating safe storage. Supporting documentation should be expanded to include validated modeling programs in addition to laboratory evidence. The use of the term "scientific evidence" should be modified to include laboratory, literature, and modeling evidence. Section c(vi) in the Food Code adds to confusion that is not necessary if the term PHF is replaced with the more descriptive term TCS. The concept of refrigeration is already captured under temperature control in the term "temperature controlled for safety," and therefore, no further explanation on storage temperature conditions for hermetically-sealed containers would be needed.

References

[ANZFA] Australian New Zealand Food Authority. 2001a. The Food Standards Code, Vol. 2, Standard 3.2.2; Food Safety Practices and General Requirements (Australia only). http://www.foodstandards.gov.au/foodsafetystandardsaustraliaonly/standard322.cfm. Accessed 2001 Oct 9.

[ANZFA] Australian New Zealand Food Authority. 2001b. Food safety: the priority classification system for food business. Lkd. http://www.anzfa.gov.au/mediareleasespublications/publications/index.cfm. Accessed 2001 Nov 20.

Baird-Parker TC. 1990. The staphylococci—an introduction. J Appl Bacteriol Symposium Suppl:1S-8S. (cited in ICMSF 1996a).

[CFIS] Canadian Food Inspection System, Canadian Food Inspection System Implementation Group. 2001a. Food retail and food services regulation. Lkd. Canadian Food Inspection System Model Regulations and Codes web page at http://www.cfis.agr.ca/english/regcode/frr_frme.htm. Accessed 2001 Oct 10.

[CFIS] Canadian Food Inspection System, Canadian Food Inspection System Imple-

mentation Group. 2001b. Food retail and food services code. Lkd. Canadian Food Inspection System Model Regulations and Codes web page at http://www.cfis.agr.ca/english/regcode/frfsrc-amendmts/frfsc_frme.htm Accessed 2001 Oct. 10.

[FDA] Food and Drug Administration. 1999. Food Code: 1999 recommendations of the United States Public Health Service, Food and Drug Administration. Springfield (VA): U.S. Dept. of Commerce, Technology Administration, National Technical Information Service. Report nr PB99-115925. Chapter 1, Part 1-201.10(B)(61). p 12. Available from: http://www.foodafety.gov/%7Edms/foodcode.html.

[HC] Health Canada, Health Protection Branch. 1992 Mar 1. Guidelines for production, distribution, retailing and use of refrigerated prepackaged foods with extended shelf life [Guideline No. 7].

Stewart CM, Cole MB, Legan JD, Slade L, Vandeven MH, Schaffner DW. 2001. Modeling the growth boundary of *Staphylococcus aureus* for risk assessment purposes. J Food Prot 64(1):51-7.

The Council of the European Communities. 1993. Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. Official Journal L 175, 19/07/1993. p 0001-11. Annex Chapter IX, paragraph 4. http://europa.eu.int/eur-lex/en/lif/dat/1993/en_393L0043.html. Accessed 2001 Nov 8.

[UK] United Kingdom, Ministry of Agriculture, Fisheries and Food. 1995. The Food Safety (Temperature Control) Regulations. HMSO London.

[USDA] U.S. Dept. of Agriculture, Food Safety and Inspection Service. 1996 [with change 98-01 (Sept 1996 thru Feb 1998)]. Food standards and labeling policy book. Available from: Superintendent of Documents, PO Box 371954, Pittsburgh, PA 15250-7954; (202)512-1800.

Chapter III
Factors that Influence Microbial Growth

1. Introduction

The factors discussed in this section constitute an inclusive, rather than exclusive, list of intrinsic, extrinsic, and other factors that may be considered when determining whether a food or category of foods requires time/temperature control during storage, distribution, sale and handling at retail and in food service to assure consumer protection.

Many factors must be evaluated for each specific food when making decisions on whether it needs time/temperature control for safety. These can be divided into intrinsic and extrinsic factors. Intrinsic factors are those that are characteristic of the food itself; extrinsic factors are those that refer to the environment surrounding the food. The need for time/temperature control is primarily determined by (1) the potential for contamination with pathogenic microorganisms of concern—including processing influences, and (2) the potential for subsequent growth and/or toxin production.

Most authorities are likely to divide foods among three categories based on an evaluation of the factors described below: those that do not need time/temperature control for protection of consumer safety; those that need time/temperature control; and those for which the exact status is questionable. In the case of questionable products, further scientific evidence—such as modeling of microbial growth or death, or actual microbiological challenge studies—may help to inform the decision.

2. Intrinsic factors

2.1. Moisture content

Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (a_w) of the food or environment. Water activity is defined as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (Jay 2000b, p 41):

$$a_w = p/p_o$$

where p = vapor pressure of the solution and p_o = vapor pressure of the solvent (usually water). The a_w of pure water is 1.00 and the a_w of a completely dehydrated food is 0.00. The a_w of a food on this scale from 0.00 to 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 to 100%. Thus, % Equilibrium Relative Humidity (ERH) = a_w x 100. The a_w of a food describes the degree to which water is "bound" in the food, its avail-

ability to participate in chemical/biochemical reactions, and its availability to facilitate growth of microorganisms.

Most fresh foods, such as fresh meat, vegetables, and fruits, have a_w values that are close to the optimum growth level of most microorganisms (0.97 to 0.99). Table 3–1 shows the approximate a_w levels of some common food categories. The a_w can be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food. Weight for weight, these food components will decrease a_w in the following order: ionic compounds > sugars, polyhydric alcohols, amino acids and other low-molecular-weight compounds > high-molecular-weight compounds such

Table 3–1—Approximate a_w values of selected food categories.

Animal Products	a_w
Fresh meat, poultry, fish	0.99 to 1.00
Natural cheeses	0.95 to 1.00
Pudding	0.97 to 0.99
Eggs	0.97
Cured meat	0.87 to 0.95
Sweetened condensed milk	0.83
Parmesan cheese	0.68 to 0.76
Honey	0.75
Dried whole egg	0.40
Dried whole milk	0.20

Plant Products	a_w
Fresh fruits, vegetables	0.97 to 1.00
Bread	~0.96
white	0.94 to 0.97
crust	0.30
Baked cake	0.90 to 0.94
Maple syrup	0.85
Jam	0.75 to 0.80
Jellies	0.82 to 0.94
Uncooked rice	0.80 to 0.87
Fruit juice concentrates	0.79 to 0.84
Fruit cake	0.73 to 0.83
Cake icing	0.76 to 0.84
Flour	0.67 to 0.87
Dried fruit	0.55 to 0.80
Cereal	0.10 to 0.20

Plant Products	a_w
Sugar	0.19
Crackers	0.10

Sources: Table 4.6 in Banwart 1979, p 115; Table 2 in FDA 1986; Table 18–3 in Jay 2000, p 367.

Table 3–2—Approximate a_w values for growth of selected pathogens in food

Organism	Minimum	Optimum	Maximum
Campylobacter spp.	0.98	0.99	
Clostridium botulinum type E*	0.97		
Shigella spp.	0.97		
Yersinia enterocolitica	0.97		
Vibrio vulnificus	0.96	0.98	0.99
Enterohemorrhagic			
Escherichia coli	0.95	0.99	
Salmonella spp.	0.94	0.99	>0.99
Vibrio parahaemolyticus	0.94	0.98	0.99
Bacillus cereus	0.93		
Clostridium botulinum types			
A & B**	0.93		
Clostridium perfringens	0.943	0.95 to 0.96	0.97
Listeria monocytogenes	0.92		
Staphylococcus aureus			
growth	0.83	0.98	0.99
toxin	0.88	0.98	0.99

ICMSF 1996.
**proteolytic
*nonproteolytic

Table 3-3—pH ranges of some common foods

Food	pH Range
Dairy Products	
Butter	6.1 to 6.4
Buttermilk	4.5
Milk	6.3 to 6.5
Cream	6.5
Cheese (American mild and cheddar)	4.9; 5.9
Yogurt	3.8 to 4.2
Meat and Poultry (and products)	
Beef (ground)	5.1 to 6.2
Ham	5.9 to 6.1
Veal	6.0
Chicken	6.2 to 6.4
Fish and Shellfish	
Fish (most species)	6.6 to 6.8
Clams	6.5
Crabs	7.0
Oysters	4.8 to 6.3
Tuna Fish	5.2 to 6.1
Shrimp	6.8 to 7.0
Salmon	6.1 to 6.3
White Fish	5.5
Fruits and Vegetables	
Apples	2.9 to 3.3
Apple Cider	3.6 to 3.8
Bananas	4.5 to 4.7
Figs	4.6
Grapefruit (juice)	3.0
Limes	1.8 to 2.0
Honeydew melons	6.3 to 6.7
Oranges (juice)	3.6 to 4.3
Plums	2.8 to 4.6
Watermelons	5.2 to 5.6
Grapes	3.4 to 4.5
Asparagus (buds and stalks)	5.7 to 6.1
Beans (string and lima)	4.6 to 6.5
Beets (sugar)	4.2 to 4.4
Broccoli	6.5
Brussels Sprouts	6.3
Cabbage (green)	5.4 to 6.0
Carrots	4.9 to 5.2; 6.0
Cauliflower	5.6
Celery	5.7 to 6.0
Corn (sweet)	7.3
Cucumbers	3.8
Eggplant	4.5
Lettuce	6.0
Olives (green)	3.6 to 3.8
Onions (red)	5.3 to 5.8
Parsley	5.7 to 6.0
Parsnip	5.3
Potatoes (tubers and sweet)	5.3 to 5.6
Pumpkin	4.8 to 5.2
Rhubarb	3.1 to 3.4
Spinach	5.5 to 6.0
Squash	5.0 to 5.4
Tomatoes (whole)	4.2 to 4.3
Turnips	5.2 to 5.5
Eggs yolks (white)	6.0 to 6.3 (7.6– 9.5)

Sources: Table 5.5 in ICMSF 1980, p 109–110; Table 3–2 in Jay 2000, p 39.

as cellulose, protein or starch (Mossel and others 1995, p 63–109).

Microorganisms respond differently to a_w depending on a number of factors. Microbial growth, and, in some cases, the production of microbial metabolites, may be particularly sensitive to alterations in a_w. Microorganisms generally have optimum and minimum levels of a_w for growth depending on other growth factors in their environments. One indicator of microbial response is their taxonomic classification. For example, Gram (–) bacteria are generally more sensitive to low a_w than Gram (+) bacteria. Table 3–2 lists the approximate minimum a_w values for the growth of selected microorganisms relevant to food. It should be noted that many bacterial pathogens are controlled at water activities well above 0.86 and only *S. aureus* can grow and produce toxin below a_w 0.90. It must be emphasized that these are approximate values because solutes can vary in their ability to inhibit microorganisms at the same a_w value. To illustrate, the lower a_w limit for the growth of *Clostridium botulinum* type A has been found to be 0.94 with NaCl as the solute versus 0.92 with glycerol as the solute (Mossel and others 1995, p 63–109). When formulating foods using a_w as the primary control mechanism for pathogens, it is useful to employ microbiological challenge testing to verify the effectiveness of the reduced a_w when target a_w is near the growth limit for the organism of concern.

Because a_w limits vary with different solutes or humectants, other measures may provide more precise moisture monitoring for certain products. For example, factors other than a_w are known to control the antibotulinal properties of pasteurized processed cheese spreads (Tanaka and others 1986). Also, a_w may be used in combination with other factors to control pathogens in certain food products (section 4.4). Care should be taken when analyzing multicomponent foods, because effective measurements of a_w may not reflect the actual value in a microenvironment or in the interface among the different components. In these cases, the a_w should be measured at the interface areas of the food, as well as in any potential microenvironment.

2.2. pH and acidity

Increasing the acidity of foods, either through fermentation or the addition of weak acids, has been used as a preservation method since ancient times. In their natural state, most foods such as meat, fish, and vegetables are slightly acidic while most fruits are moderately acidic. A few foods such as egg white are alkaline. Table 3–3 lists the pH ranges of some common foods. The pH is a function of the hydrogen ion concentration in the food:

$$pH = -\log_{10} [H+]$$

Another useful term relevant to the pH of foods is the pK_a. The term pK_a describes the state of dissociation of an acid. At equilibrium, pK_a is the pH at which the concentrations of dissociated

Table 3-4—Proportion of total acid undissociated at different pH values (expressed as percentages).

pH Values					
Organic Acids	**3**	**4**	**5**	**6**	**7**
Acetic acid	98.5	84.5	34.9	5.1	0.54
Benzoic acid	93.5	59.3	12.8	1.44	0.144
Citric acid	53.0	18.9	0.41	0.006	<0.001
Lactic acid	86.6	39.2	6.05	0.64	0.064
Methyl, ethyl, propyl parabens	>99.99	99.99	99.96	99.66	96.72
Propionic acid	98.5	87.6	41.7	6.67	0.71
Sorbic acid	97.4	82.0	30.0	4.1	0.48

Source: Table 7.3 in ICMSF 1980, p 133.

Table 3-5—Approximate pH values permitting the growth of selected pathogens in food

Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5 to 5.8	7.2	8.0 to 9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0 to 7.0	8.8
Campylobacter spp.	4.9	6.5 to 7.5	9.0
Shigella spp.	4.9		9.3
Vibrio parahaemolyticus	4.8	7.8 to 8.6	11.0
Clostridium botulinum			
toxin	4.6	8.5	
growth	4.6	8.5	
Staphylococcus aureus			
growth	4.0	6.0 to 7.0	10.0
toxin	4.5	7.0 to 8.0	9.6
Enterohemorrhagic			
Escherichia coli	4.4	6.0 to 7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2[1]	7.0 to 7.5	9.5
Yersinia enterocolitica	4.2	7.2	9.6

Sources: Table 5.3 in ICMSF 1980, p 101.
[1]pH minimum as low as 3.8 has been reported when acidulants other than acetic acid or equivalent are used.

and undissociated acid are equal. Strong acids have a very low pK_a, meaning that they are almost entirely dissociated in solution (ICMSF 1980, p 93). For example, the pH (at 25 °C [77 °F]) of a 0.1 M solution of HCl is 1.08 compared to the pH of 0.1 M solution of acetic acid, which is 2.6. This characteristic is extremely important when using acidity as a preservation method for foods. Organic acids are more effective as preservatives in the undissociated state. Lowering the pH of a food increases the effectiveness of an organic acid as a preservative. Table 3–4 lists the proportion of total acid undissociated at different pH values for selected organic acids. The type of organic acid employed can dramatically influence the microbiological keeping quality and safety of the food.

It is well known that groups of microorganisms have pH optimum, minimum, and maximum for growth in foods. Table 3–5 lists the approximate pH ranges for growth in laboratory media for selected organisms relevant to food. As with other factors, pH usually interacts with other parameters in the food to inhibit growth. The pH can interact with factors such as a_w, salt, temperature, redox potential, and preservatives to inhibit growth of pathogens and other organisms. The pH of the food also significantly impacts the lethality of heat treatment of the food. Less heat is needed to inactivate microbes as the pH is reduced (Mossel and others 1995).

Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins.

Titratable acidity (TA) is a better indicator of the microbiological stability of certain foods, such as salad dressings, than is pH. Titratable acidity is a measure of the quantity of standard alkali (usually 0.1 M NaOH) required to neutralize an acid solution (ICMSF 1980, p 94). It measures the amount of hydrogen ions released from undissociated acid during titration. Titratable acidity is a particularly useful measure for highly buffered or highly acidic foods. Weak acids (such as organic acids) are usually undissociated and, therefore, do not directly contribute to pH. Titratable acidity yields a measure of the total acid concentration, while pH does not, for these types of foods.

In general, pathogens do not grow, or grow very slowly, at pH levels below 4.6; but there are exceptions. Many pathogens can survive in foods at pH levels below their growth minima. It has been reported that *C. botulinum* was able to produce toxin as low as pH 4.2, but these experiments were conducted with high inoculum levels (10^3 to 10^4 CFU/g up to 10^6 CFU/g), in soy peptone, and with the presence of *Bacillus* spp. (Smelt and others 1982). The panel did not consider these results to be relevant to the foods under consideration in this report. It should also be noted

that changes in pH can transform a food into one that can support growth of pathogens (ICMSF 1980). For example, several botulism outbreaks have been traced to foods in which the pH increased due to mold growth. These are important considerations when determining the shelf life of a food formulation. Based on a comprehensive review of the literature, the panel concluded that a pH of 4.6 is appropriate to control spore-forming pathogens.

Among vegetative pathogens, *Salmonella* spp. are reported to grow at the lowest pH values; however, in a study by Chung and Goepfert (1970), the limiting pH was greatly influenced by the acidulant used. For example, when tryptone-yeast extract-glucose broth was inoculated with 10^4 CFU/ml of salmonellae, minimum pH values for growth ranged from 4.05 with hydrochloric and citric acids to 5.5 with propionic acid or acetic acid. Additionally, inoculum levels were unrealistically high (10^2 to 10^6 CFU/ml) for salmonellae in food systems. These investigators also noted that these results could not be extrapolated directly to food because the experiment was run in laboratory media under ideal temperature and a_w conditions and without the presence of competitive microorganisms. Similarly, Ferreira and Lund (1987) reported that six out of 13 strains of *Salmonella* spp. representing 12 serovars could grow at pH 3.8 at 30 °C (86 °F) within 1 to 3 d, and at 20 °C (68 °F) in 3 to 5 d, when using HCl as an acidulant. Other reports note that certain acids at pH 4.5 inactivate salmonellae. The panel therefore concluded that using a pH minimum of 4.0 for *Salmonella* spp. would not be scientifically substantiated for foods subject to Food Code requirements. Based on a comprehensive review of the literature data, the panel also concluded that it would be scientifically valid to use a pH minimum of 4.2 to control for *Salmonella* spp. and other vegetative pathogens.

As with other intrinsic properties, when analyzing multicomponent foods, the pH should be measured not only for each component of the food but also for the interface areas among components and for any potential microenvironment.

2.3. Nutrient content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Mossel and others 1995, p 47–8, 185–7; Ray 1996, p 62–65; Jay 2000, p 47–8).

Varying amounts of these nutrients are present in foods. Meats

have abundant protein, lipids, minerals, and vitamins. Most muscle foods have low levels of carbohydrates. Plant foods have high concentrations of different types of carbohydrates and varying levels of proteins, minerals, and vitamins. Foods such as milk and milk products and eggs are rich in nutrients. The role of water is discussed in section 2.1.

Foodborne microorganisms can derive energy from carbohydrates, alcohols, and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates, such as starch or cellulose found in plant foods, or glycogen found in muscle foods. Some microorganisms can use fats as an energy source.

Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals.

In general, the Gram (+) bacteria are more fastidious in their nutritional requirements and thus are not able to synthesize certain nutrients required for growth (Jay 2000, p 78). For example, the Gram (+) foodborne pathogen *S. aureus* requires amino acids, thiamine, and nicotinic acid for growth (Jay 2000, p 444). Fruits and vegetables that are deficient in B vitamins do not effectively support the growth of these microorganisms. The Gram (–) bacteria are generally able to derive their basic nutritional requirements from the existing carbohydrates, proteins, lipids, minerals, and vitamins that are found in a wide range of food (Jay 2000, p 47–8).

An example of a pathogen with specific nutrient requirements is *Salmonella* Enteritidis. Growth of *Salmonella* Enteritidis may be limited by the availability of iron. For example, the albumen portion of the egg, as opposed to the yolk, includes antimicrobial agents and limited free iron that prevent the growth of *Salmonella* Enteritidis to high levels. Clay and Board (1991) demonstrated that the addition of iron to an inoculum of *Salmonella* Enteritidis in egg albumen resulted in growth of the pathogen to higher levels compared to levels reached when a control inoculum (without iron) was used.

The microorganisms that usually predominate in foods are those that can most easily utilize the nutrients present. Generally, the simple carbohydrates and amino acids are utilized first, followed by the more complex forms of these nutrients. The complexity of foods in general is such that several microorganisms can be growing in a food at the same time. The rate of growth is limited by the availability of essential nutrients. The abundance of nutrients in most foods is sufficient to support the growth of a wide range of foodborne pathogens. Thus, it is very difficult and impractical to predict the pathogen growth or toxin production based on the nutrient composition of the food.

2.4. Biological structure

Plant- and animal-derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes.

Plant and animal foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms. Several factors may influence penetration of these barriers. The maturity of plant foods will influence the effectiveness of the protective barriers.

Physical damage due to handling during harvest, transport, or storage, as well as invasion of insects can allow the penetration of microorganisms (Mossel and others 1995, p 204; Jay 2000, p 49). During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers. Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food. For example, *Salmonella* spp. have been shown to grow on the interior of portions of cut cantaloupe, watermelon, honeydew melons (Golden and others 1993), and tomatoes (Lin and Wei 1997), given sufficient time and temperature.

Fruits are an example of the potential of pathogenic microorganisms to penetrate intact barriers. After harvest, pathogens will survive but usually not grow on the outer surface of fresh fruits and vegetables. Growth on intact surfaces is not common because foodborne pathogens do not produce the enzymes necessary to break down the protective outer barriers on most produce. This outer barrier restricts the availability of nutrients and moisture. One exception is the reported growth of *E. coli* O157:H7 on the surface of watermelon and cantaloupe rinds (del Rosario and Beuchat 1995). Survival of foodborne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens (bacteria or fungi). These conditions can also promote the multiplication of pathogens, especially at higher temperatures. Infiltration of fruit was predicted and described by Bartz and Showalter (1981) based on the general gas law, which states that any change in pressure of an ideal gas in a closed container of constant volume is directly proportional to a change in temperature of the gas. In their work, Bartz and Showalter describe a tomato; however, any fruit, such as an apple, can be considered a container that is not completely closed. As the container or fruit cools, the decrease in internal gas pressure results in a partial vacuum inside the fruit, which then results in an influx from the external environment. For example, an influx of pathogens from the fruit surface or cooling water could occur as a result of an increase in external pressure due to immersing warm fruit in cool water. Internalization of bacteria into fruits and vegetables could also occur due to breaks in the tissues or through morphological structures in the fruit itself, such as the calyx or stem scar. Although infiltration was considered a possible scenario, the panel concluded that there is insufficient epidemiological evidence to require refrigeration of intact fruit.

The egg is another good example of an effective biological structure that, when intact, will prevent external microbial contamination of the perishable yolk; contamination is possible, however, through transovarian infection. For the interior of an egg to become contaminated by microorganisms on the surface, there must be penetration of the shell and its membranes. In addition, the egg white contains antimicrobial factors. When there are cracks through the inner membrane of the egg, microorganisms penetrate into the egg. Factors such as temperature of storage, relative humidity, age of eggs, and level of surface contamination will influence internalization. For example, conditions such as high humidity and wet and dirty shells, along with a drop in the storage temperature will increase the likelihood for entry of bacteria. If eggs are washed, the wash water should be 12 °C (22 °F) higher than the temperature of the eggs to prevent microbial penetration. After washing, the eggs should be dried and then cooled. The Food and Drug Administration (FDA) published a final rule that applies to shell eggs that have not be processed to destroy all live Salmonella before distribution to the consumer. The rule mandates that eggs should be kept dry and chilled below 7.2 °C (45 °F) to prevent growth of *Salmonella* Enteritidis (Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution, 65 FR 76092 [Dec. 5, 2000] [to

be codified at 21 C.F.R. parts 16, 101, and 115]).

Heating of food as well as other types of processing will break down protective biological structures and alter such factors as pH and a_w. These changes could potentially allow the growth of microbial pathogens.

2.5. Redox potential

The oxidation-reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential is a measurement of the ease by which a substance gains or loses electrons. The redox potential (Eh) is measured in terms of millivolts. A fully oxidized standard oxygen electrode will have an Eh of +810 mV at pH 7.0, 30 °C (86 °F), and under the same conditions, a completely reduced standard hydrogen electrode will have an Eh of –420 mV. The Eh is dependent on the pH of the substrate; normally the Eh is taken at pH 7.0 (Jay 2000, p 45–7).

The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes, and microaerophiles. Examples of foodborne pathogens for each of these classifications include *Aeromonas hydrophila, Clostridium botulinum, Escherichia coli* O157:H7, and *Campylobacter jejuni,* respectively. Generally, the range at which different microorganisms can grow are as follows: aerobes +500 to +300 mV; facultative anaerobes +300 to –100 mV; and anaerobes +100 to less than –250 mV (Ray 1996, p 69–70). For example, *C. botulinum* is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values. The relationship of Eh to growth can be significantly affected by the presence of salt and other food constituents. For example, in one study with smoked herring, toxin was produced in inoculated product stored at 15 °C (59 °F) within three days at an Eh of +200 to +250 mV (Huss and others 1979). In this case, the major oxidant would be trimethylamine oxide, which becomes the electron acceptor for *C. botulinum.* The anaerobe *Clostridium perfringens* can initiate growth at an Eh close to +200 mV; however, in the presence of increasing concentrations of certain substances, such as salt, the limiting Eh increases (Morris 2000).

The measured Eh values of various foods are given in Table 3–6. These values can be highly variable depending on changes in the pH of the food, microbial growth, packaging, the partial pressure of oxygen in the storage environment, and ingredients and composition (protein, ascorbic acid, reducing sugars, oxidation level of cations, and so on). Another important factor is the poising capacity of the food. Poising capacity, which is analogous to buffering capacity, relates to the extent to which a food resists external affected changes in Eh. The poising capacity of the food will be affected by oxidizing and reducing constituents in the food as well as by the presence of active respiratory enzyme systems. Fresh fruits and vegetables and muscle foods will continue to respire; thus low Eh values can result (Morris 2000).

The measurement of redox potential of food is done rather easily, either for single or multicomponent foods. For multicomponent foods, in addition to measurement of each component, the redox potential of the interface areas and microenvironments should be considered. However, difficulties arise in taking accurate measurements and in accounting for the differences throughout the food and the equilibrium at the point of measurement. According to Morris (2000): "This imposes the further requirements (1) that the measuring electrode be so prepared and calibrated that it gives stable and reproducible readings, and (2) that a foodstuff is tested in a manner that does not cause any change in the potential that is to be measured. … it would be unwise to use redox potential information in isolation to predict food safety, or to rely exclusively on control of redox potential as the means of preventing growth

Table 3–6—Redox potentials on some foods.

Food	Presence of air	Eh (mV)	pH
Milk	+	+300 to +340	NR
Cheese			
Cheddar	+	+300 to −100	NR
Dutch	+	−20 to −310	4.9 to 5.2
Emmenthal	+	−50 to −200	NR
Butter serum	−	+290 to +350	6.5
Egg (infertile after 14 d)	+	+500	NR
Meats			
Liver, raw minced	−	−200	~7
Muscle			
Raw, postrigor	−	−60 to −150	5.7
Raw, minced	+	+225	5.9
Minced, cooked	+	+300	7.5
Cooked sausages			
and canned meat	−	−20 to −150	~6.5
Cereals			
Wheat (whole grain)	−	−320 to −360	6.0
Wheat (germ)	−	−470	NR
Barley (ground)	+	+225	7
Potato tuber	−	~ −150	~6
Plant juices			
Grape	−	+409	3.9
Lemon	−	+383	2.2
Pear	−	+436	4.2
Spinach	−	+74	6.2
Canned foods			
"Neutral"	−	−130 to −550	> 4.4
"Acid"	−	−410 to −550	< 4.4

NR = Not reported
Reproduced from Mossel and others 1995, p 185 by permission of D.A.A. Mossel.

of specific microorganisms." Redox measurements could possibly be used in combination with other factors to evaluate the potential for pathogen growth. However, the limitations discussed above make it a rather difficult and variable factor that could result in erroneous conclusions in the absence of other comprehensive information.

2.6. Naturally occurring and added antimicrobials

Some foods intrinsically contain naturally occurring antimicrobial compounds that convey some level of microbiological stability to them. There are a number of plant-based antimicrobial constituents, including many essential oils, tannins, glycosides, and resins, which can be found in certain foods. Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano (Jay 2000, p 266–7). Other plant-derived antimicrobial constituents include the phytoalexins and the lectins. Lectins are proteins that can specifically bind to a variety of polysaccharides, including the glycoproteins of cell surfaces (Mossel and others 1995, p 175–214). Through this binding, lectins can exert a slight antimicrobial effect. The usual concentration of these compounds in formulated foods is relatively low, so that the antimicrobial effect alone is slight. However, these compounds may produce greater stability in combination with other factors in the formulation.

Some animal-based foods also contain antimicrobial constituents. Examples include lactoferrin, conglutinin and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk, and other factors in fresh meat, poultry, and seafood (Mossel and others 1995, p 175–214). Lysozyme is a small protein that can hydrolyze the cell wall of bacteria. The lactoperoxidase system in bovine milk consists of three distinct components that are required for its antimicrobial action: lactoperoxidase, thiocyanate, and hydrogen peroxide. Gram (–) psychotrophs such as the

Table 3–7—Preservatives frequently used in conjunction with main groups of foods in the U.S.

Foodstuff	Nitrate, Nitrite	Sulfur Dioxide	Acetic Acid	Propionic Acid	Sorbic Acid	Benzoic Acid	BHA and BHT	Smoke	Nisin	Parabens
Fat Emulsions	−	−	+	−	++	+	+	−	−	+
Cheese	−	−	−	+	++	(+)	−	−		
Meat products	++	−	−	−	+	−	−	++	−	−
Seafood products	+	+	++	−	+	+	−	++	−	(+)
Vegetable products	−	+	++	−	++	++	+	−	−	−
Fruit products	−	++	+	−	++	++	(+)	−	−	+
Beverages	−	(+)	−	−	++	++	+	−	−	+
Wine	−	++	−	−	++	−	−	−	−	−
Baked goods	−	−	+	++	++	−	−	−	−	(+)
Confectionery	−	−	−	−	++	(+)	(+)	−	−	−

Source: Adapted from Davidson and Branen 1993; Table 11 in Lück and Jager 1997, p 61;
++ used frequently
+ used occasionally
(+) used in exceptional cases only
−not used

pseudomonads have been shown to be very sensitive to the lactoperoxidase system. Consequently, this system, in an enhanced form, has been suggested to improve the keeping quality of raw milk in developing countries where adequate refrigeration is scarce (Mossel and others 1995, p 188). Similar to the plant-derived antimicrobial compounds, the animal-derived compounds have a limited effect on ambient shelf life of foods.

It is also known that some types of food processing result in the formation of antimicrobial compounds in the food. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. Maillard compounds resulting from condensation reactions between sugars and amino acids or peptides upon heating of certain foods can impart some antimicrobial activity (Mossel and others 1995, p 195–6). Smoke condensate includes phenol, which is not only an antimicrobial, but also lowers the surface pH. Some processors also lower the surface pH with liquid smoke to achieve an unsliced shelf-stable product.

Some types of fermentations can result in the natural production of antimicrobial substances, including bacteriocins, antibiotics, and other related inhibitors. Bacteriocins are proteins or peptides that are produced by certain strains of bacteria that inactivate other, usually closely related, bacteria (Lück and Jager 1997, p 251). The most commonly characterized bacteriocins are those produced by the lactic acid bacteria. The antibiotic nisin produced by certain strains of *Lactococcus lactis* is one of the best characterized of the bacteriocins. Nisin is approved for food applications in over 50 countries around the world (Jay 2000, p 269–72). Nisin's first food application was to prevent late blowing in Swiss cheese by *Clostridium butyricum.* Nisin is a polypeptide that is effective against most Gram (+) bacteria but is ineffective against Gram (−) organisms and fungi. Nisin can be produced in the food by starter cultures or, more commonly, it can be used as an additive in the form of a standardized preparation (Lück and Jager 1997). Nisin has been used to effectively control spore-forming organisms in processed cheese formulations, and has been shown to have an interactive effect with heat. For example, an F_O process for conventional low acid canned foods may be in the 6 to 8 range, but with the addition of nisin, can be reduced to a F_O of 3 for inactivating thermophilic spores.

There are a number of other bacteriocins and natural antimicrobials that have been described, however, these have found very limited application in commercial use as food preservatives because of their restricted range of activity, limited compatibility with the food formulation or their regulatory status.

In addition to naturally occurring antimicrobial compounds in foods, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination. Table 3–7 lists some of the most frequently used preservatives in the United States by food category (Lück and Jager 1997). The selection and use of these preservatives is typically governed by food law regulation of a country or region of the world. A number of criteria should be followed when selecting a preservative for a specific food application. Ideally, the preservative should have a wide spectrum of activity against the target spoilage organisms and pathogens expected to be encountered in the food. The preservative must be active for the desired shelf life of the food and under the expected formulation conditions in the food. It should cause minimal organoleptic impact on the food and should not interfere with desirable microbiological processes expected to occur in the food, such as the ripening of cheese or leavening of baked goods.

Added antimicrobial compounds can have an interactive or synergistic effect with other parameters of the formulation. One example is the interaction with pH. Many preservatives have an optimum pH range for effectiveness. Other factors include a_w, presence of other preservatives, types of food constituents, presence of certain enzymes, processing temperature, storage atmosphere, and partition coefficients. The effective use of combinations of preservatives with other physicochemical parameters of a food formulation can stabilize that food against spoilage organisms or pathogens. Leistner systematically developed the "hurdle concept" to describe these effects (Leistner 1995). The hurdle concept states that several inhibitory factors (hurdles), while individually unable to inhibit microorganisms, will, nevertheless, be effective in combination. A classic example of applying the hurdle concept is the antibotulinal stability of certain shelf-stable processed cheese formulations. Combinations of moisture, total salt, and pH have been shown to allow for the safe storage of these products at room temperature for extended time even though the individual factors, taken singly, would not support that practice (Tanaka and others 1986). In combination products, the effectiveness of an antimicrobial may be altered by other factors including the potential for migration of the antimicrobial to other components of the food and the different food parameters at the interface areas.

There are a number of food formulations that, either by addition of preservatives or through the application of the hurdle concept do not require refrigeration for microbiological stability or safety. However, in the absence of a well defined and validated microbiological model, it is usually difficult to evaluate the microbiological safety of these products. In the majority of these cases, the application of appropriate microbiological challenge testing is the most effective tool for judging the suitability of these formulations for nonrefrigerated storage.

2.7. Competitive microflora

The potential for microbial growth of pathogens in temperature-

sensitive foods depends on the combination of the intrinsic and extrinsic factors, and the processing technologies that have been applied. Within the microbial flora in a food, there are many important biological attributes of individual organisms that influence the species that predominates. These include the individual growth rates of the microbial strains and the mutual interactions or influences among species in mixed populations (ICMSF 1980, p 221–31)

2.7.1. Growth. In a food environment, an organism grows in a characteristic manner and at a characteristic rate. The length of the lag phase, generation time, and total cell yield are determined by genetic factors. Accumulation of metabolic products may limit the growth of particular species. If the limiting metabolic product can be used as a substrate by other species, these may take over (partly or wholly), creating an association or succession (ICMSF 1980, p 222). Due to the complex of continuing interactions between environmental factors and microorganisms, a food at any one point in time has a characteristic flora, known as its association. The microbial profile changes continuously and one association succeeds another in what is called succession. Many examples of this phenomenon have been observed in the microbial deterioration and spoilage of foods (ICMSF 1980, p 226).

As long as metabolically active organisms remain, they continue to interact, so that dominance in the flora occurs as a dynamic process. Based on their growth-enhancing or inhibiting nature, these interactions are either antagonistic or synergistic.

2.7.2. Competition. In food systems, antagonistic processes usually include competition for nutrients, competition for attachment/adhesion sites (space), unfavorable alterations of the environment, and a combination of these factors. Early studies demonstrated that the natural biota of frozen pot pies inhibited inoculated cells of *S. aureus*, *E. coli* and *Salmonella* Typhimurium (Jay 2000, p 52). Another example of this phenomenon is raw ground beef. Even though *S. aureus* is often found in low numbers in this product, staphylococcal enterotoxin is not produced. The reason is that the *Pseudomonas-Acinetobacter-Moraxella* association that is always present in this food grows at a higher rate, outgrowing the staphylococci (ICMSF 1980, p 222).

Organisms of high metabolic activity may consume required nutrients, selectively reducing these substances, and inhibiting the growth of other organisms. Depletion of oxygen or accumulation of carbon dioxide favors facultative obligate anaerobes, which occur in vacuum-packaged fresh meats, held under refrigeration (ICMSF 1980, p 222).

Staphylococci are particularly sensitive to nutrient depletion. Coliforms and *Pseudomonas* spp. may utilize amino acids necessary for staphylococcal growth and make them unavailable. Other genera of *Micrococcaceae* can utilize nutrients more rapidly than staphylococci. Streptococci inhibit staphylocci by exhausting the supply of nicotinamide or niacin and biotin (ICMSF 1980, p 222). *Staphylococcus aureus* is a poor competitor in both fresh and frozen foods. At temperatures that favor staphylococcal growth, the normal food saprophytic biota offers protection against staphylococcal growth through antagonism, competition for nutrients, and modification of the environment to conditions less favorable to *S. aureus* (Jay 2000, p 455). Changes in the composition of the food, as well as changes in intrinsic or extrinsic factors may either stimulate or decrease competitive effects.

2.7.3. Effects on growth inhibition. Changes in growth stimulation have been reported among several foodborne organisms, including yeasts, micrococci, streptococci, lactobacilli and *Enterobacteriaceae* (ICMSF 1980, p 224). Growth stimulating mechanisms can have a significant influence on the buildup of a typical flora. There are several of these mechanisms, a few of which are listed below (ICMSF 1980, p 224):

- Metabolic products from one organism can be absorbed and utilized by other organisms.
- Changes in pH may promote the growth of certain microorganisms. An example is natural fermentations, in which acid production establishes the dominance of acid tolerant organisms such as the lactic acid bacteria. Growth of molds on high acid foods has been found to raise the pH, thus stimulating the growth of *C. botulinum*.
- Changes in Eh or a_w in the food can influence symbiosis. At warm temperatures, *C. perfringens* can lower the redox potential in the tissues of freshly slaughtered animals so that even more obligately anaerobic organisms can grow.
- There are some associations where maximum growth and normal metabolic activity are not developed unless both organisms are present.

This information can be used in the hurdle concept to control microorganisms in temperature-sensitive foods.

3. Extrinsic factors

3.1. Types of packaging/atmospheres

Many scientific studies have demonstrated the antimicrobial activity of gases at ambient and subambient pressures on microorganisms important in foods (Loss and Hotchkiss 2002, p 245).

Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that are directly toxic to certain microorganisms. This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. Oxidizing radicals generated by O_3 and O_2 are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms. A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. This effect may have positive or negative consequences depending upon the native pathogenic microflora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity (Loss and Hotchkiss 2002, p 245).

A variety of common technologies are used to inhibit the growth of microorganisms, and a majority of these methods rely upon temperature to augment the inhibitory effects. Technologies include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage (Loss and Hotchkiss 2002, p 246).

Controlled atmosphere and modified atmosphere packaging of certain foods can dramatically extend their shelf life. The use of CO_2, N_2, and ethanol are examples of MAP applications. In general, the inhibitory effects of CO_2 increase with decreasing temperature due to the increased solubility of CO_2 at lower temperatures (Jay 2000, p 286). Carbon dioxide dissolves in the food and lowers the pH of the food. Nitrogen, being an inert gas, has no direct antimicrobial properties. It is typically used to displace oxygen in the food package either alone or in combination with CO_2, thus having an indirect inhibitory effect on aerobic microorganisms (Loss and Hotchkiss 2002, p 246). Table 3–8 shows some examples of combinations of gases for MAP applications in meat, poultry, seafood, hard cheeses, and baked goods (Farber 1991, p 67).

The preservation principle of antimicrobial atmospheres has

been applied to fruits and vegetables, raw beef, chicken and fish, dairy foods including milk and cottage cheese, eggs, and a variety of prepared, ready-to-eat foods.

There are several intrinsic and extrinsic factors that influence the efficacy of antimicrobial atmospheres. These factors—including product temperature, product-to-headspace gas volume ratio, initial microbial loads and type of flora, package barrier properties, and biochemical composition of the food—all interact to determine the degree to which the microbial quality and safety are enhanced (Loss and Hotchkiss 2002, p 255).

Temperature, the most important factor affecting the efficacy of antimicrobial atmospheres, directly affects growth rate, but also indirectly affects growth by affecting gas solubility. At practical food storage temperatures, packaging configurations, especially the product-to-headspace volume ratio, play a major role in determining the magnitude of microbial inhibition.

In MAP, package barrier properties have a major effect on the microbial growth by influencing the time in which the selected modified atmosphere gases remain in contact with the product and the rate at which oxygen enters the package.

Water activity, salt content of the aqueous phase, pH, and fat content of foods also play a role in overall inhibitory effects of antimicrobial gases. As with temperature, the physical and chemical characteristics of the food have an effect on the solubility of the inhibitory gas. For example, increasing salt concentrations decreases CO_2 solubility.

The major safety consideration in extending shelf life of foods by MAP or related technologies is the loss of sensory cues to spoilage provided by bacterial growth. Without spoilage bacteria indicators, it is conceivable that a food could have acceptable organoleptic quality, but be unsafe. The effect of loss of competitive inhibition by spoilage bacteria is most pronounced on the facultative anaerobic pathogenic bacterial populations in foods under altered atmospheres (Loss and Hotchkiss 2002, p 261).

By combining antimicrobial atmospheres with other techniques, hurdle technology strategies may be generated that can further enhance food quality and safety.

3.2. Effect of time/temperature conditions on microbial growth

3.2.1. Impact of time. When considering growth rates of microbial pathogens, in addition to temperature, time is a critical consideration. Food producers or manufacturers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from when the product is produced until the time it is intended to be consumed or used. Several factors are used to determine a product's shelf life, ranging from organoleptic qualities to microbiological safety. For the purpose of this report, the key consideration is the microbiological safety of the product. The Uniform Open Dating Regulation requires the shelf life of a perishable food product to be expressed in terms of a "sell by" date (NIST 2000). The "sell by" date must incorporate the shelf life of the product plus a reasonable period for consumption that consists of at least one-third of the approximate total shelf life of the perishable food product.

At retail or foodservice, an additional period of time referred to herein as "use-period" should also be considered. As an example, fast food locations may find it operationally desirable to hold processed cheese slices at ambient temperatures for a complete shift or meal period, which may be in excess of 4 h. This practice provides operational efficiency by allowing the cheese to melt faster on a hot sandwich as well as providing a better quality sandwich. Although refrigeration may be required for safety under long-term storage conditions, for use-periods measured in hours, storage at ambient temperatures may be acceptable.

Under certain circumstances, time alone at ambient tempera-

Table 3-8—Examples of gas mixtures used for various MAP products.

Product	% CO_2	% O_2	%N_2
Fresh meat	30	30	40
	15 to 40	60 to 85	0
Cured meat	20 to 50	0	50 to 80
Sliced cooked roast beef	75	10	15
Eggs	20	0	80
	0	0	100
Poultry	25 to 30	0	70 to 75
	60 to 75	5 to 10	$20
	100	0	0
	20 to 40	60 to 80	0
Pork	20	80	0
Processed Meats	0	0	100
Fish (White)	40	30	30
Fish (Oily)	40	0	60
	60	0	40
Hard cheese	0 to 70		30 to 100
Cheese	0	0	100
Cheese; grated/sliced	30	0	70
Sandwiches	20 to 100	0 to 10	0 to 100
Pasta	0	0	100
	70 to 80	0	20 to 30
Baked goods	20 to 70	0	20 to 80
	0	0	100
	100	0	0

Source: Table 9 in Farber 1991

tures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question. For refrigerated food products, the shelf life or use-period required for safety may vary depending on the temperature at which the product is stored. For example, Mossel and Thomas (1988) report that the lag time for growth of *L. monocytogenes* at 10 °C (50 °F) is 1.5 d, while at 1 °C (34 °F) lag time is ~3.3 d. Likewise, they report that at 10 °C (50 °F) the generation time for the same organism is 5 to 8 h, while at 1 °C (34 °F), the generation time is between 62 and 131 h. Figure 1 shows the effect of temperature and pH on lag times of L. monocytogenes. The data were obtained by using the USDA Pathogen Micromodel Program (version 5.1) at a NaCl concentration of 2% and a_w of 0.989. It should be noted that this model was developed in broth under various salt and pH combinations, and that growth of bacteria in food systems will likely differ. According to the model results, a temperature shift from 10 (50) to 25 °C (77 °F) decreases the lag time of *L. monocytogenes* from 60 to 10 h. In a similar manner, a pH increase from 4.5 to 6.5 decreases the lag time from 60 to 5 h. In conclusion, the safety of a product during its shelf life may differ, depending upon other conditions such as temperature of storage, pH of the product, and so on. This study by Mossel and Thomas (1988), along with numerous others, illustrates that various time/temperature combinations can be used to control product safety depending on the product's intended use.

As stated earlier, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question.

3.2.2. Impact of temperature

All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. An understanding of the interplay between time, temperature, and other intrinsic and extrinsic factors is crucial to selecting the proper storage conditions for a food product. Temperature has dramatic impact on both the generation time of an organism and its lag period. Over a defined temperature range, the growth rate of an organism is classically defined as an Arrhenius relationship (Mossel and others 1995, p 79–80). The log growth rate constant is found to be proportional to the reciprocal of the absolute temperature:

$$G = -m / 2.303 \, RT \text{ where,}$$
G = log growth rate constant
m = temperature characteristic (constant for a particular microbe)
R = gas constant
T = temperature (°K)

The above relationship holds over the linear portion of the Arrhenius plot. However, when temperatures approach the maxima for a specific microorganism, the growth rate declines more rapidly than when temperatures approach the minima for that same microorganism. A relationship that more accurately predicts growth rates of microorganisms at low temperatures follows (Jay 2000, p 51):

$$\sqrt{r} = b(T - To) \text{ where,}$$
r = growth rate
b = slope of the regression line
T = temperature (°K)
To = conceptual temperature of no metabolic significance

At low temperatures, two factors govern the point at which growth stops: (1) reaction rates for the individual enzymes in the organism become much slower, and (2) low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms (Mossel and others 1995). At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. While the growth rate increases with increasing temperature, the rate tends to decline rapidly thereafter, until the temperature maximum is reached.

The relationship between temperature and growth rate constant varies significantly across groups of microorganisms. Four major groups of microorganisms have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles, and psychrotrophs. Tables 9 and 10 list the tempera-
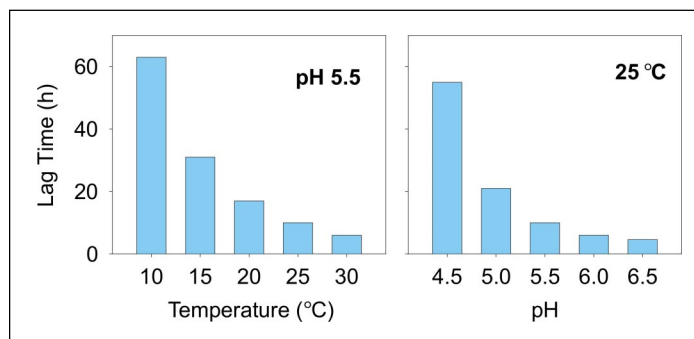
Table 3-9—Temperature ranges for prokaryotic microorganisms.

Group	Temperature °C (°F)		
	Minimum	Optimum	Maximum
Thermophiles	40 to 45 (104 to 113)	55 to 75 (131 to 167)	60 to 90 (140 to 194)
Mesophiles	5 to 15 (41 to 59)	30 to 45 (86 to 113)	35 to 47 (95 to 117)
Psychrophiles	−5 to +5 (23 to 41)	12 to 15 (54 to 59)	15 to 20 (59 to 68)
Psychrotrophs	−5 to +5 (23 to 41)	25 to 30 (77 to 86)	30 to 35 (86 to 95)

Source: Table 1.1 in ICMSF 1980, p 4.

Table 3–10—Approximate minimum, maximum and optimum temperature values in °C (°F) permitting growth of selected pathogens relevant to food.

Organism	Minimum	Optimum	Maximum
Bacillus cereus	5 (41)	28 to 40 (82 to 104)	55 (131)
Campylobacter spp.	32 (90)	42 to 45 (108 to 113)	45 (113)
Clostridium botulinum types A & B*	10 to 12 (50 to 54)	30 to 40 (86 to 104)	50 (122)
Clostridium botulinum type E**	3 to 3.3 (37 to 38)	25 to 37 (77 to 99)	45 (113)
Clostridium perfringens	12 (54)	43 to 47 (109 to 117)	50 (122)
Enterotoxigenic *Escherichia coli*	7 (45)	35 to 40 (95 to 104)	46 (115)
Listeria monocytogenes	0 (32)	30 to 37 (86 to 99)	45 (113)
Salmonella spp.	5 (41)	35 to 37 (95 to 99)	45 to 47 (113 to 117)
Staphylococcus aureus growth	7 (45)	35 to 40 (95 to 104)	48 (118)
toxin	10 (50)	40 to 45 (104 to 113)	46 (115)
Shigella spp.	7 (45)	37 (99)	45 to 47 (113 to 117)
Vibrio cholerae	10 (50)	37 (99)	43 (109)
Vibrio parahaemolyticus	5 (41)	37 (99)	43 (109)
Vibrio vulnificus	8 (46)	37 (99)	43 (109)
Yersinia enterocolitica	−1 (30)	28 to 30 (82 to 86)	42 (108)

ICMSF 1996; Lund and others 2000; Doyle and others 2001
*proteolytic
**nonproteolytic

ture ranges for these four groups (ICMSF 1980) and for pathogens of concern (ICMSF 1996; Doyle and others 2001; Lund and others 2000). The optimum temperature for growth of thermophiles is between 55 to 65 °C (131 to 149 °F) with the maximum as high as 90 °C (194 °F) and a minimum of around 40 °C (104 °F). Mesophiles, which include virtually all human pathogens, have an optimum growth range of between 30 °C (86 °F) and 45 °C (113 °F), and a minimum growth temperature ranging from 5 to

Figure 1—Effect of temperature or pH on lag times of *Listeria monocytogenes* from USDA PMP ver 5.1 (2% NaCl, a_w 0.989)

10 °C (41 to 50 °F). Psychrophilic organisms have an optimum growth range of 12 °C (54 °F) to 15 °C (59 °F) with a maximum range of 15 °C (59 °F) to 20 °C (68 °F). There are very few true psychrophilic organisms of consequence to foods. Psychrotrophs such as *L. monocytogenes* and *C. botulinum* type E are capable of growing at low temperatures (minimum of –0.4 °C [31 °F] and 3.3 °C [38 °F], respectively, to 5 °C [41 °F]), but have a higher growth optimum range (37 °C [99 °F] and 30 °C [86 °F], respectively) than true psychrophiles. Psychrotrophic organisms are much more relevant to food and include spoilage bacteria, spoilage yeast and molds, as well as certain foodborne pathogens.

Growth temperature is known to regulate the expression of virulence genes in certain foodborne pathogens (Montville and Matthews 2001). For example, the expression of proteins governed by the *Yersinia enterocolitica* virulence plasmid is high at 37 °C (99 °F), low at 22 °C (72 °F), and not detectable at 4 °C (39 °F). Growth temperature also impacts an organism's thermal sensitivity. *Listeria monocytogenes,* when held at 48 °C (118 °F) in inoculated sausages, has an increase of 2.4-fold in its D value at 64 °C (147 °F).

It must be emphasized that the lag period and growth rate of a microorganism are influenced not only by temperature but by other intrinsic and extrinsic factors as well. For example, as shown in Table 3–11, the growth rate of *Clostridium perfringens* is significantly lower at pH 5.8 versus pH 7.2 across a wide range of temperatures (ICMSF 1980, p 10). Salmonellae do not grow at temperatures below 5.2 °C (41 °F). The intrinsic factors of the food product, however, have been shown to impact the ability of salmonellae to grow at low temperatures. *Salmonella* Senftenberg, *S.* Enteritidis, and *S.* Manhattan were not able to grow in ham salad or custard held at 10 °C (50 °F), but were able to grow in chicken à la king held at 7 °C (45 °F) (ICMSF 1980, p 9).

Staphylococcus aureus has been shown to grow at temperatures as low as 7 °C (45 °F), but the lower limit for enterotoxin production has been shown to be 10 °C (50 °F). In general, toxin production below about 20 °C (68 °F) is slow. For example, in laboratory media at pH 7, the time to produce detectable levels of enterotoxin ranged from 78 to 98 h at 19 °C (66 °F) to 14 to 16 h at 26 °C (79 °F) (ICMSF 1980, p 10). Less favorable conditions, such as reduced pH, slowed enterotoxin production even further.

Table 3–12 illustrates the combined impact of temperature, pH, and a_w on the growth of proteolytic *C. botulinum* type B. This table clearly shows that an interactive effect occurs between these three factors. When measuring the suitability of holding a refrigerated food at room temperature for a period of time, consideration may be given to each factor independently. Doing so, however, ignores the potential to safely hold products for a period of time out of refrigeration based on interaction effects. Consideration of each relevant factor independently may lead to the conclusion that it is not a safe practice to do so, while, in reality, it is actually safe based on the interactive effects. The most appropriate method for evaluating such interactive effects is through a properly designed microbiological challenge study using relevant target microorganisms. Appropriate, validated predictive microbiological models may also be employed for this purpose. The use of challenge studies and/or predictive models can yield scientific data that supports holding a product with a certain formulation for a given time and temperature. It is incumbent upon the producer to have specific knowledge of the food formulation to generate valid scientific data.

3.3. Storage/holding conditions

This discussion of storage conditions will be limited to the storage/holding temperature, and the time/temperature involved in cooling of cooked items, and the relative humidity to which the food or packaging material may be exposed. Other factors that

Table 3-11—The relationship of pH and temperature to growth rate of *Clostridium perfringens* (welchii) F2985/50.

Incubation temperature	Hours to visible turbidity in RCM broth at pH	
	5.8	7.2
15 °C (59 °F)	>700	>700
20 °C (68 °F)	74	48
25 °C (77 °F)	30	24
30 °C (86 °F)	24	8
37 °C (99 °F)	5	5

Source: Table 1.3 in ICMSF 1980, p 10.

Table 3-12—Incubation period, in days, before growth of proteolytic *Clostridium botulinum* type B was observed at various levels of temperature, pH, and a_w.

Temperature	pH	a_w						
		0.997	0.99	0.98	0.97	0.96	0.95	0.94
20 °C (68 °F)	5	—	—	—	—	—	—	—
	6	49	9	—	—	—	—	—
	7	2	2	4	9	—	—	—
	8	2	2	4	14	—	—	—
	9	—	—	—	—	—	—	—
30 °C (86 °F)	5	—	—	—	—	—	—	—
	6	2	2	3	9	—	—	—
	7	1	1	2	3	9	14	—
	8	1	1	2	4	14	—	—
	9	—	—	—	—	—	—	—
40 °C (104 °F)	5	—	—	—	—	—	—	—
	6	1	2	2	3	14	—	—
	7	1	1	1	2	3	9	17
	8	1	1	1	2	9	14	—
	9	—	—	—	—	—	—	—

No growth observed at any pH or a_w level at 10 °C (50 °F).
Source: Table 6 in FDA 1986

may be included as important considerations for storage, such as the effectiveness of the packaging material at conserving certain characteristics, are discussed in other sections of this chapter.

When considering growth rate of microbial pathogens, time and temperature are integral and must be considered together. As has been stated previously in this chapter, increases in storage and/or display temperature will decrease the shelf life of refrigerated foods since the higher the temperature, the more permissive conditions are for growth. At the same time, those foods that have been cooked or reheated and are served or held hot may require appropriate time/temperature control for safety. For example, the primary organism of concern for cooked meat and meat-containing products is *C. perfringens.* Illness symptoms are caused by ingestion of large numbers (greater than 10^8) of vegetative cells. The organism has an optimal growth range of 43 to 47 °C (109–116 °F) and a growth range of 12 to 50 °C (54 to 122 °F). Generation times as short as 8 min have been reported in certain foods under optimal conditions (ICMSF 1996). Thus time/temperature management is essential for product safety.

The literature is replete with examples of outbreaks of foodborne illness that have resulted from cooling food too slowly, a practice that may permit growth of pathogenic bacteria. Of primary concern in this regard are the spore-forming pathogens that have relatively short lag times and the ability to grow rapidly and/or that may normally be present in large numbers. Organisms that possess such characteristics include *C. perfringens*, and *Bacillus cereus*. As with *C. perfringens,* foodborne illness caused by *B. cereus* is typically associated with consumption of food that has supported growth of the organism to relatively high numbers. The

FDA "Bad Bug Book" notes that "The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health" (FDA 2001). In this case, the time and temperature (cooling rate) of certain foods must be addressed to assure rapid cooling for safety.

The effect of the relative humidity of the storage environment on the safety of foods is somewhat more nebulous. The effect may or may not alter the a_w of the food. Such changes are product dependent. The earlier discussion on a_w and its effect on microorganisms in foods provides some background information. In addition, the possibility of surface evaporation or condensation of moisture on a surface should be considered.

Generally, foods that depend on a certain a_w for safety or shelf life considerations will need to be stored such that the environment does not markedly change this characteristic. Foods will eventually come to moisture equilibrium with their surroundings. Thus, processors and distributors need to provide for appropriate storage conditions to account for this fact.

Packaging, as discussed previously in this chapter, will play a major role in the vulnerability of the food to the influence of relative humidity. But even within a sealed container, moisture migration and the phenomenon of environmental temperature fluctuation may play a role. It has been observed that certain foods with low a_w can be subject to moisture condensing on the surface due to wide environmental temperature shifts. This surface water will result in microenvironments favorable to growth of spoilage, and possibly pathogenic, microorganisms. As a general guideline, the product should be held such that environmental moisture, including that within the package, does not have an opportunity to alter the a_w of the product in an unfavorable way.

3.4. Processing steps

The current definition of "potentially hazardous foods" considers the effect of processing in much the same way that it considers pH and a_w: it divides foods into two categories. Low-acid canned foods in a hermetically sealed container do not require temperature control for safety. This rigid definition fails to address less processed foods, in less robust packaging, which still would not require temperature control for safety. Consider a baked product, such as a pie, with a pH of 5.5 and a_w of 0.96. Since this product is baked to an internal temperature >180 °F (82 °C) to set the product structure of the pie, it will not contain any viable vegetative pathogens. Any pathogenic spores that survive the baking process will be inhibited by the pH and a_w values listed above (ICMSF 1996; see Tables 2 and 5). If the product is cooled and packaged under conditions that do not allow recontamination with vegetative pathogens, the product is safe and stable at room temperature until consumed, or until quality considerations (that is, staling) make it unpalatable.

Scientifically sound criteria for determining whether foods require time/temperature control for safety should consider (1) processes that destroy vegetative cells but not spores (when product formulation is capable of inhibiting spore germination); (2) postprocess handling and packaging conditions that prevent reintroduction of vegetative pathogens onto or into the product before packaging; and (3) the use of packaging materials that while they do not provide a hermetic seal, do prevent reintroduction of vegetative pathogens into the product.

4. Other factors

4.1. Intended end-use of product

In addition to carefully assessing how the product is produced and distributed, it is important to consider how the food will ultimately be prepared, handled, and/or stored by the end user. A food product that does not require time/temperature control for safety at one point in the food production or distribution chain may require time/temperature control at another point, depending on its intended use. For example, a thermally processed food that is hot-filled into its final packaging may not require refrigeration if spore-forming pathogens are not capable of outgrowth. However, once the food item is taken out of its original packaging, it may require time/temperature control for safety if the product is likely to be recontaminated during its intended use.

4.2. Product history and traditional use

The panel struggled with the concept of product history and traditional use as a means to determine the need for time/temperature control for safety. For example, there are foods which have a long history of safe storage use at ambient temperatures, yet have formulations, pH, and a_w that would designate them as "temperature controlled for safety" (TCS) foods. Paramount among them is white bread, but products such as intact fruits and vegetables, other breads, bottled waters, and some processed cheeses have a history of being stored and used at ambient temperatures with no public health impact. In addition, moisture protein ratios (MPR) for shelf-stable fermented sausages were developed to ensure process control values for these sausages that also have a traditional history of safety as a non-TCS food. Moreover, an evaluation of the food characteristics provides a scientific explanation for the products to be safely stored at ambient temperatures. For example, baking of bread controls the growth of pathogens in the interior, and the low a_w precludes the growth of pathogens on the outer surface, so that it can be stored safely at ambient temperatures. Clearly these products' traditional uses and histories provide a valid justification for a decision to be made based on history. Care must be observed, however, as this traditional history can be influenced by the intrinsic and extrinsic factors and any changes in product end-use, processes, formulation, physical structure, processing, distribution, and/or storage. Changes in any of these parameters may invalidate the sole use of history as a basis for decisions on whether a food needs temperature control for safety.

The panel recognizes that the use of history as a factor to decide whether a product needs time/temperature control for safety can be subjective. As a guidance, one should determine whether the food in question or any of its ingredients have been previously implicated as a common vehicle of foodborne disease as a result of abuse or storage at ambient temperature. Of particular importance are the microbiological agents that may be of concern based on food formulation, or that may be responsible for illnesses associated with the food and the reported contributing factors that have led to documented illnesses. Has adequate temperature control been clearly documented as a factor that can prevent or reduce the risk of illness associated with the food? As intrinsic or extrinsic factors change (for example, MAP or greatly extended shelf life), historical evidence alone may not be appropriate in determining potential risk. Therefore, for a product to be identified as non-TCS based on history and traditional use, the intrinsic and extrinsic factors affecting microbial growth need to have remained constant. Lastly, product history alone should not be used as the sole factor in determining whether a food needs time/temperature control for safety. This decision requires a valid scientific rationale such as that provided above for white bread.

4.3. Interactions of factors

Traditional food preservation techniques have used combinations of pH, a_w, atmosphere, numerous preservatives, and other inhibitory factors. Microbiologists have often referred to this phenomenon as the "hurdle effect". For example, certain processed meat products and pickles may use the salt-to-moisture ratio

Table 3-13—USDA pathogen modeling program predictions for time in hours needed for a 3 log increase in *Staphylococcus aureus* concentration as a function of the pH and water activity at 25 °C (77 °F)[1]

Critical a_w values	Critical pH values			
	4.2	4.6	5.0	5.5
0.85	Outside	Outside	Outside	Outside
0.90	Outside	Outside	Outside	Outside
0.92	Outside	171.3	113.1	80.7
0.93	Outside	143.0	93.0	65.5
0.94	Outside	120.6	77.3	53.6
0.95	Outside	101.4	63.9	43.6
0.96	Outside	86.3	53.4	35.9

[1]Conditions labeled "outside" are outside the range of the current model

(brine ratio) to control pathogens. USDA recognizes this strategy in designating as shelf-stable semi-dry sausages with a moisture-protein ratio of less than or equal to 3.1:1 and pH less than or equal to 5.0.

In salad dressings and mayonnaise-type products, the acid-to-moisture ratio along with pH is the governing factor for pathogen control. An acid:moisture ratio > 0.70 in combination with a pH < 4.1 is often used as the pathogen-control target level for these products. Usually, these ratios are combined with other factors such as pH or added antimicrobials to effect pathogen control (Mossel and others 1995). It is the interaction of these factors that controls the ability of pathogens to proliferate in foods.

Despite this long-standing recognition of the concept of hurdle technology (the possible synergistic effect of combining different inhibitory factors), the current definition of potentially hazardous foods only considers pH and a_w independently, and does not address their interaction. The panel believes that these interactions have to be taken into consideration.

Scientific advances in predictive food microbiology over the last two decades have repeatedly shown that different inhibitory factors that might not prevent pathogen growth when considered singly will prevent pathogen growth when used in concert. Table 3-13 summarizes a series of predictions from the USDA Pathogen Modeling Program ver. 5.1. It should be noted that this model was developed in broth with salt and pH combinations and that growth of bacteria in food systems will likely differ. Also, the salt used to control the a_w results in additional microbial inhibitory effects that may be lacking if other compounds are used. The values are the time in hours needed for a 3 log increase in *S. aureus* (see Chapter 6, section 9) concentration as a function of the pH and a_w values shown.

It is clear from the numerical values shown that even though a food might have a pH of 5.0 and an a_w of 0.92 (for example), after 72 h at room temperature, it may show a minimal increase in *S. aureus* concentration, and thus not constitute a significant risk to public health.

Models that address the interaction of other factors (for example, atmosphere, preservatives) have been published, but are not nearly as numerous as models using pH and a_w. Individual companies have shown, however, that in-house models incorporating preservative effects can be useful tools in reducing the need for extensive challenge testing and assessing risk. However, a general model for foods to cover all interactions of atmospheric gases and/or preservative combinations with pH and a_w does not currently exist.

Scientifically sound criteria for determining whether foods require time/temperature control for safety could consider the interaction of only pH and a_w factors using data from microbial growth models such as those shown in the table above. In order to design effective combinations of factors, an understanding of the pathogen (vegetative or spore-forming) and of the mechanisms by which individual factors exert their impact are necessary.

References

Banwart GJ. 1979. Basic Food Microbiology. Westport, Conn.: AVI. Chapter 4, Factors that affect microbial growth in food; p 115 (table 4.6).

Bartz JA, Showalter RK. 1981. Infiltration of tomatoes by aqueous bacterial suspensions. Phytopathology 71(5):515-8.

Chung KC, Goepfert JM. 1970. Growth of *Salmonella* at low pH. J Food Sci 35:326-8.

Clay CE, Board RG. 1991. Growth of *Salmonella enteritidis* in artificially contaminated hens' shell eggs. Epidemiol Infect 106:271-81.

Davidson PM, Branen AL, editors. 1993. Antimicrobials in foods. 2nd ed. New York: Marcel Dekker. 647 p. (Food Science, 10).

Del Rosario BA, Beuchat LR. 1995. Survival and growth of enterohemorrhagic *Escherichia coli* 0157:H7 in cantaloupe and watermelon. J Food Prot 58:105-7.

Doyle MP, Beuchat LR, Montville TJ, editors. 2001. Food microbiology: fundamentals and frontiers. 2nd ed. Washington DC: ASM Press.

Farber JM. 1991. Microbiological aspects of modified atmosphere packaging technology—a review. J Food Prot 54:58-70.

[FDA] U.S. Food and Drug Administration. 1986 May 9. Retail food protection program information manual, part 6—Inspection, chapter 01—code interpretations, section 04—interpretations by code section. Washington DC: FDA, Center for Food Safety and Applied Nutrition, Retail Food Protection Branch. Table 6, p 11-12.

[FDA] Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. The "Bad Bug Book" [Foodborne pathogenic microorganisms and natural toxins handbook]. http://www.cfsan.fda.gov/~mow/intro.html. Accessed 2001 Dec 10.

Ferreira MASS, Lund BM. 1987. The influence of pH and temperature on initiation of growth of *Salmonella* spp. Lett Appl Microbiol 5:67-70.

Golden DA, Rhodehamel EJ, Kautter DA. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J Food Prot 56:194-6.

Huss HH, Schaeffer I, Rye Peterson E, Cann DC. 1979. Toxin production by *Clostridium botulinum* type E in fresh herring in relation to the measured oxidation-reduction potential (Eh). Nord Veterinaermed 31:81-6.

[ICMSF] International Commission on Microbiological Specification for Foods. 1980. Microbial ecology of foods. Volume 1, Factors affecting life and death of microorganisms. Orlando: Academic Pr. p 311.

[ICMSF] International Commission on Microbiological Specification for Foods. 1996. Microorganisms in foods. Roberts TA, Baird-Parker AC, Tompkin RB, editors. Volume 5, Characteristics of microbial pathogens. London: Blackie Academic & Professional. p 513.

Jay JM. 2000. Modern food microbiology. 6th ed. Gaithersburg MD: Aspen. p 679.

Leistner L. 1995. Principles and applications of hurdle technology. In: Gould GW, editor. New methods of food preservation. London: Blackie Academic & Professional. p 1-21.

Lin CM, Wei CI. 1997. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. J Food Prot 60(7):858-63.

Loss CR, Hotchkiss JH. 2002. Inhibition of microbial growth by low-pressure and ambient pressure gasses. In: Juneja VK, Sofos JN, editors. Control of foodborne microorganisms. New York: Marcel Dekker. p 245-79. Forthcoming.

Luck E, Jager M. 1997. Antimicrobial food additives: characteristics, uses, effects. Springer: Berlin. 260 p.

Lund BM, Baird-Parker TC, Gould GW, editors. 2000. The microbiological safety and quality of foods. Volume 1 & 2. Gaithersburg MD: Aspen.

Montville TJ, Matthews KR. 2001. Chapter 2: Principles which influence microbial growth, survival, and death in foods. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington DC: ASM Pr. p 13-32.

Morris JG. 2000. The effect of redox potential. In: Lund BL, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Volume 1. Gaithersburg MD: Aspen. p 235-50.

Mossel DAA, Thomas G. 1988. Securite microbioligique des plats prepares refrigeres: recommendations en matiere d'analyse des risques, conception et surveillance du processus de fabrication. Microbiologie—Aliements—Nutrition 6:289-309.

Mossel DAA, Corry JEL, Struijk CB, Baird RM. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester England: John Wiley and Sons. 699 p.

[NIST] National Institute of Standards and Technology. 2000. Uniform laws and regulations in the areas of legal metrology and engine fuel quality [as adopted by the 84th National Conference on Weights and Measures 1999]. 2000 ed. Gaithersburg MD: U.S. Dept. of Commerce, Technology Administration, National Institute of Standards and Technology. Uniform open dating regulation; p 117-22. (NIST Handbook 130).

Ray B. 1996. Fundamental food microbiology. Boca Raton FL: CRC Press. 516 p.

Smelt JPPM, Raatjes JGM, Crowther JC, Verrips CT. 1982. Growth and toxin formation by *Clostridium botulinum* at low pH values. J Appl Bacteriol 52:75-82.

Tanaka N, Traisman E, Plantong P, Finn L, Flom W, Meskey L, Guggisberg J. 1986. Evaluation of factors involved in antibotulinal properties of pasteurized process cheese spreads. J Food Prot 49(7):526-31.

[USDA] U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Laboratory. USDA Pathogen Modeling Program Version 5.1.

Analysis of Microbial Hazards Related to Time/ Temperature Control of Foods for Safety

1. Introduction

To make decisions on whether a food requires time/temperature control for safety, the properties of the food itself must be considered. This chapter describes properties of common food commodities, including added preservatives and processing steps, and the environmental circumstances that may affect their microbial ecology. The microbiological hazards that may occur from consuming particular food commodities or their derived products are also discussed. The chapter emphasizes microbial concerns that would be associated with temperature abuse of the products, and discusses foods for which time/temperature control may be necessary for safety and those that might be safely stored at room temperature. Consideration is also given to processing technologies or other methods that may be useful in minimizing hazards. Special considerations unique to each food category are also provided. Pathogens of concern and control methods for the various product categories evaluated in this chapter was summarized by the panel and are listed in Table 4-1.

2. Meat and poultry products

2.1. Types of products

Raw meat and poultry products consist of raw products; shelf-stable, raw-salted and salted-cured products (salt pork, dry-cured bacon, country ham); perishable raw-salted and salted-cured products (fresh sausage, chorizo, bratwurst, Polish and Italian sausage); marinated products; and raw breaded products. Ready-to-eat products include perishable cooked uncured products (cooked roast beef, cooked pork, cooked turkey); perishable cooked cured products (franks, bologna, ham, and a variety of luncheon meats); canned shelf-stable cured products (Vienna sausages, corned beef, meat spreads, small canned hams, canned sausages with oil and water activity [a_w] <0.92, dried beef, and prefried bacon); perishable canned cured products (ham and other cured meats); shelf-stable, canned uncured products (roast beef with gravy, meat stew, chili, chicken and spaghetti sauce with meat); fermented and acidulated sausages (German and Italian style salamis, pepperoni, Lebanon bologna, and summer sausage); and dried meat products (jerky, beef sticks, basturma, and other dried meats). Because of the complexity of the product/processing matrices, product parameters (moisture protein ratio, a_w, and pH) and processing schedules are needed to ascertain whether ready-to-eat products require time/temperature control for safety or are shelf stable.

2.2. Microbial concerns

Red meats and poultry come from warm-blooded animals and,

as such, their microbial flora is heterogeneous, consisting of mesophilic and psychrotrophic bacteria. These bacteria include pathogenic species from the animal itself and from the environment, and bacterial species introduced during slaughter and processing of raw products. Raw meat and poultry have an $a_w > 0.99$ and a pH range of 5 to 7, which is an optimal combination for microbial growth. When red meats and poultry are cooked or processed and subsequently refrigerated, the bacterial load from the raw tissue is greatly reduced, leaving only spore-formers, enterococci, micrococci, and some lactobacilli. In addition, environmental post-processing pathogen contamination can occur and the reduction in competitive bacterial flora may allow for pathogen growth. Some products are shelf stable because they received either a botulinum cook or a lesser cook in combination with other controls, such as acidity or other additives (for example, spaghetti meat sauce and Sloppy Joe mix).

2.3. Pathogens of concern

The principal pathogens of concern are *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli* (ruminants), *Salmonella* spp. (all meats), *Listeria monocytogenes* (all meats), *Campylobacter jejuni/coli* (poultry), *Yersinia enterocolitica* (pork), and *Clostridium perfringens and Clostridium botulinum* (mainly processed products). There is a particular concern when these species are present and/or can grow in cooked products without competition.

2.4. Effects of processing

Meat and poultry products require a wide array of control measures in their processing. Cured meats and some sausage products utilize additives such as salt, nitrate, nitrite, and sugars with processing procedures such as cooking and smoking. Salt, for example, may restrict bacterial flora to salt-tolerant species. Smoking and/or cooking will destroy many vegetative cells. However, the processing environment and product handling and packaging may introduce microorganisms, including pathogens, into the packaged product that also must be considered.

While some canned products may be processed as "commercially sterile," others are canned "semi-preserved" and must be stored under refrigeration. Some products utilize a secondary control such as acidity and are shelf stable though not necessarily "commercially sterile." Specific labeling for refrigeration is required on the semi-preserved products that require refrigeration as a control. Pickled products depend on a low pH, absence of oxygen, and the lack of a fermentable sugar to inhibit the growth of most bacteria. Acid-tolerant species may develop, such as certain lactobacilli, and if air is available, certain yeast and molds may grow. The activity of lactic acid bacteria in fermented sausag-

Table 4-1—Pathogens of concern and control methods for various product categories (including examples of foods that may need to be evaluated for time/temperature control needs for safety).

Product Category (examples of possible foods for evaluation)	Pathogens of Concern	Types of Process Control[1] (alone and in combination)
Meats and poultry (fermented sausage)	*Clostridium botulinum*[5] and *Clostridium perfringens*, *Salmonella* spp., enterohemorrhagic *Escherichia coli*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes*	Time/temperature, pH, a_w, preservatives, moisture protein ratio, fermentation, heat processing
Fish and seafood (smoked fish)	*Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *C. botulinum*[5], *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *S. aureus*	Time/temperature, harvest site control, fermentation, pH, a_w, water-phase salt, preservatives, drying, salting
Fruits and vegetables (peeled carrots)	*Salmonella* spp., *Shigella* spp., enterohemorrhagic *E. coli*, *L. monocytogenes*, *Bacillus cereus*, *C. botulinum*[5], *Y. enterocolitica*	Production control (Good Agriculture Practices), time/temperature, cooking, preservation techniques
Cereal grains and related products (fresh pasta, focaccia bread)	*Salmonella* spp., *S. aureus*, *B. cereus*, *C. botulinum*[5]	Cooking, a_w, pH, preservatives, time/temperature
Fats, oils & salad dressings (garlic-in-oil)	*S. aureus*[2], *Salmonella* spp.[2], *B. cereus*[2], *C. botulinum*[2]	pH, a_w, salt
Butter and margarine (light salted butter)	*S. aureus*, *L. monocytogenes*, *Y. enterocolitica*	Production/raw ingredient quality control, moisture droplet size in the water-in-oil emulsion, water phase salt, a_w
Sugars and syrups (light maple syrup)	*C. botulinum*[3]	a_w, acidification (light syrups)
Eggs and egg products (meringue)	*Salmonella* spp.[4], *L. monocytogenes*[4]	Production control, cooking/pasteurization, time/temperature
Milk and milk products (yogurt)	*Salmonella* spp.[4], *L. monocytogenes*[4], enterohemorrhagic *E. coli*[4], *S. aureus*[4], *B. cereus* (cells[4] and spores[5]), *C. botulinum* (cells[4] and spores[5]), *Campylobacter jejuni*[4]	Production control, time/temperature, cooking/pasteurization, a_w, preservatives
Cheese and cheese products (Natural Swiss cheese)	*Salmonella* spp.[4], *L. monocytogenes*[4], enterohemorrhagic *E. coli*[4], *S. aureus*[4], *Shigella* spp.[4], *C. botulinum* (cells[4] and spores[5])	Production control, moisture content, a_w, pasteurization, preservatives, pH
Combination products (cheese with veg. pieces, pumpkin pie, stuffed pastry)	Variable, based on raw materials and processing	Variable, based on raw materials and product

[1]Good Manufacturing Practices would help in reducing the hazards. For meats, poultry, fish, and seafood products the Hazard Analysis Critical Control Point principles should be implemented as a control system.
[2]A pH > 4.0 and a_w ~ 0.92 in salad dressings and mayonnaise would preclude the growth of pathogens of concern.
[3]Only a concern in light syrups and can be controlled by acidification.
[4]In pasteurized products, all pre-processing vegetative pathogens would be controlled.
[5]Only a concern in anoxic environments.

es is desirable and is an integral part of the process control for achieving the desired pH for these products.

Because of the complexities of products and processing, the USDA Food Safety and Inspection Service (FSIS) has provided guidelines for product parameters in its "*Food Standards and Labeling Policy Book*" (USDA 1996, with change 98-01). The FSIS guidelines include product specifications such as "meat sticks and cheese," along with general topic categories such as for example "Sausage – Shelf Stable"; "Moisture Protein Ratio – MPR;" and "Moisture Protein Ratio – pH." These policies must always be considered in conjunction with process controls under the HACCP Rule, 9 C.F.R. 417. A product processed in the retail environment is not covered by this rule; however, the variance requirements of the Food Code should require that meat and poultry products have equivalent product specifications for shelf stability and process records documenting control of hazards.

There is substantial history of safety of meat and poultry products that meet these criteria. In addition to the above criteria, certain combinations of pH, a_w, and/or other factors can be used to prevent pathogen level increase when meat products are held at ambient temperatures. Products processed in the retail environment and exempt from the HACCP Rule should also follow these guidelines and maintain records documenting control of hazards.

2.5. Time/temperature control

Unless the specific product parameters referenced in the previous section are met, meat and poultry products must be considered as requiring time/temperature control. Raw meat and poultry products currently require safe-handling instruction labeling that includes a time/temperature control provision. For ready-to-eat foods, product parameters and processing schedules are needed to ascertain whether temperature control for safety is required. Post-processing contamination is also an important consideration and should not be overlooked. Because meat offers a rich nutrient media for microbial growth, products that incorporate meat and poultry as ingredients, such as meat salads and meat pastries, also must be considered as requiring time/temperature control.

3. Fish and seafood products

3.1. Types of products

Fish and seafood products include fresh and frozen fish and crustaceans; cooked crustacean products; breaded and prepared seafood products; salted and smoked seafood products; sushi and seafood products such as minced fish flesh, surimi, pickled fish products, fermented fish, and seafood analogs; and mollus-

can shellfish (oysters, mussels, and clams).

3.2. Microbial concerns

Seafood is more perishable than other high-protein products due to the high level of soluble nitrogen compounds in the tissue. Microbial activity is responsible for changes in flavor, odor, texture, and color that reflect the extent of decomposition. Seafood is largely harvested from the wild and is subject to environmental contaminants, including pathogens, from the harvest site and on-board-ship handling practices. The numbers and types of indigenous microorganisms on freshly harvested fish, crustaceans, and mollusks depend on the geographical location of the harvest site, the season, and the method of harvest. While microbial concerns center mainly on foodborne illness, poor quality (spoiled or decomposed) products rarely cause illness because they usually are discarded before consumption. With the exception of scombroid poisoning in other foods, problems generally arise from contaminated harvest sites or from mishandling during or after processing.

3.3. Pathogens of concern

Inshore water sites increase the likelihood of enteric pathogen contaminants. Indigenous pathogens including *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae,* and *C. botulinum* Type E, and enteric microorganisms such as *Salmonella* spp. and *Shigella* spp. have been isolated from freshly caught fish, crustaceans, and mollusks due to contaminated harvest waters, but they are not present in deep sea waters. Other nonindigenous pathogens such as *L. monocytogenes* and *S. aureus* can be present in cooked products as a result of processing, handling, or post-processing environmental contamination.

Sushi products that incorporate raw fish as an ingredient must meet the additional requirements of a process for destruction of parasites. Sushi is also made from acidified rice and other ingredients that are subject to the environmental/processing contamination already discussed. Rice, without proper acidification control, introduces a risk of toxin formation from *Bacillus cereus.*

Cooked seafood, especially crustaceans that are heavily handled during processing, is subject to contamination by *S. aureus*, *Salmonella* spp., *L. monocytogenes*, *Shigella* spp.*,* and other enteric microorganisms. In addition, poor manufacturing practices may result in cross contamination by indigenous pathogens, especially *V. parahaemolyticus. Clostridium botulinum* spores may survive depending on the nature of the heating process.

3.4. Effects of processing

Since 1997, all seafood processors must comply with the HACCP rule, mandated by FDA in an attempt to minimize the microbiological hazards in the final products. Seafood can be sold raw, frozen, canned, cured, smoked, or fermented. Much seafood is frozen, a factor that does not affect the level of pathogens, except in the case of *Vibrio* spp., which are sensitive to freezing temperatures. *Vibrio parahaemolyticus*, for instance, has been shown to survive freezing at sufficient levels to cause illness.

The cooking process usually eliminates vegetative pathogens. However, to maintain quality, the duration of these cooks may be shortened and may not fully destroy all pathogens. In addition, meat from cooked crabs and lobsters is picked by hand, a practice that can cause contamination by *S. aureus* and by *Salmonella* spp.*, Shigella* spp., and other enteric pathogens. *Listeria monocytogenes* is also a significant contaminant in cooked/processed seafood because the cool, wet processing environment is conducive to its presence and subsequent product contamination. Cooked seafood products should be cooled and refrigerated immediately.

Canned seafood given a full retort process is shelf stable. Of concern with canned fish are those species with high histidine levels, such as tuna, mackerel, and sardines, that have been mishandled when fresh, and that may develop significant levels of heat-stable histamine and cause food illness.

Large amounts of fish are cured and/or smoked as a preservation technique. These products are subject to contamination by environmental species, especially *L. monocytogenes.* The curing process uses salt to lower a_w and sometimes uses smoke to provide flavor. A wide variety both hot- and cold- smoked products are available. The safety of these products relies on the amount of water phase salt, preservatives, and the type and amount of heat treatment. Except for a fully salted and dried product (> 20% salt), these products cannot be considered shelf stable without full validation and process control. Cold-smoked fish that are vacuum packaged have been implicated in outbreaks from *C. botulinum* toxin. In addition, the high frequency of isolations of *L. monocytogenes,* especially in cold-smoked fish, has resulted in numerous product recalls. Several states have specific requirements based on the Association of Food and Drug Officials' Model Code: "Cured, Salted and Smoked Fish Establishment GMPs," for the amount of water-phase salt, heat treatment, and storage temperature for salt-cured, smoked fish (AFDO 1991). Curing and smoking of uneviserated fish is prohibited.

Fish is also preserved by fermentation. Fermented seafood uses salt and acids, such as vinegar, to produce acidic products with high salt contents that preclude pathogen survival.

3.5. Time/temperature control

Most seafood, including cooked seafood and sushi, requires time/temperature control. Only fully retorted or fully dried and salted products are considered shelf stable. Most smoked seafood products require time/temperature control because of the concern with *C. botulinum* growth and toxin production, in addition to their being highly perishable. Heavily smoked products with low water activities are spoiled primarily by molds.

4. Fruits and vegetables

4.1. Types of products

Fruits are the portions of plants that bear seeds, while vegetables are the edible components of a plant, including the leaves, stalks, roots, tubers, bulbs, flowers, and seeds (ICMSF 1998, p 253). A wide variety of products, including citrus fruits, apples, pears, bananas, tropical fruits, compound fruits (for example, berries), tomatoes, olives, cucumbers, and melons, as well as vegetables ranging from asparagus to zucchini, are available in the market place (ICMSF 1998, p 215-273).

Fruits and vegetables and related products include foods that are sold fresh, minimally processed (for example, cut, sliced, chopped, shredded, or peeled), canned, frozen, juiced, or dried. Some commodities are retained in storage under controlled or modified atmospheres before packaging, while others are packaged by using modified atmospheres in films that control the permeability of gases. In addition to being sold fresh, fruits are also sold dried and packaged with preservatives. Dried fruits are also used in a variety of products such as confectionary bars, cookies, chocolates, breads, and many cereal based products. Minimally processed fruit can be sold as fruit salads or incorporated into dairy products such as yogurt, cottage cheese, or ice cream (ICMSF 1998, p 253).

Fresh-cut vegetables include ready-to-eat washed, sliced, chopped, or shredded vegetables, dry coleslaw mixes (without dressing), and complex mixed salads, as well as stir-fry products. Raw or cooked vegetables (with or without fruit and meat or poultry) are used as ingredients in prepared (deli) salads with mayonnaise or other types of dressings. Due to their highly perishable

nature, most fresh fruits and vegetables need temperature control to extend their shelf life. Preservation of fruits and vegetables is achieved by drying, salting, freezing, refrigeration, canning, fermentation, irradiation, and packaging under vacuum or modified atmospheres (ICMSF 1998, p 215).

Over the past several years, seeds, either fresh or cooked, have become a commonly consumed produce item. Seed sprouts may harbor very low levels of pathogens (*Salmonella* serotypes, *B. cereus*, *E. coli* O157:H7, and *Y. enterocolitica)* that can multiply to very high levels during the 3 to 10 d sprouting process and survive through the typical refrigerated shelf life of the products (IFT 2001). Whereas mung bean sprouts are often stir-fried or otherwise heated prior to consumption, which would reduce the risk of disease, other seed sprouts are often consumed raw and have been associated with foodborne illness (IFT 2001). For these products, time/temperature control would not prevent microbial hazards and, therefore, sanitation procedures that would reduce the contamination and growth of pathogens growth should be in place.

4.2. Microbial concerns

The initial bacteria of fresh produce derive from contamination from air, soil, water, insects, animals, workers, and harvesting and transportation equipment. In fruits, bacteria are usually present in low numbers, but contamination by yeasts and molds is more prevalent due to the lower pH of fruits and the lack of competition from other microorganisms (ICMSF 1998, p 253). Microorganisms also found in vegetables include *Pseudomonas* and *Erwinia* as well as coryneforms, lactic acid bacteria, spore formers, coliforms, and micrococci. Yeasts and molds are often present but in lower numbers than bacteria (ICMSF 1998, p 216). Sufficient moisture, abusive temperature, and adequate time will ensure a continuing increase in the bacterial population on fruits and vegetables, particularly in fresh-cut products.

4.3. Pathogens of concern

Since 1973, the number of reported outbreaks of foodborne illness associated with produce has more than doubled. As a result, pathogens on fresh fruits and vegetables have become a major concern. Pathogenic bacteria are not usually associated with fruit, but pathogens can be present due to fecal contamination. There have been a number of outbreaks of salmonellosis and *E. coli* O157:H7 infection associated with the consumption of a variety of fruits, including raw tomatoes, sliced watermelons, cantaloupes, and unpasteurized apple and orange juice. Human pathogens have been isolated from more than thirty kinds of vegetables and include *Salmonella* spp., *Shigella* spp, *Y. enterocolitica*, *E. coli* O157:H7, *L. monocytogenes*, *C. botulinum,* and *B. cereus* (ICMSF 1998, p 221). Fresh-cut produce presents a special concern because of the disruption of natural protective barriers that may result in increased pathogen multiplication.

4.4. Effects of processing

Fruits and vegetables are frequently consumed raw without being exposed to a process that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens. Traditional processing methods such as freezing, canning, dehydration, fermentation, and acidification are used to improve the stability of fruits and vegetables.

4.5. Time/temperature control

Outbreaks of salmonellosis and E. *coli* O157:H7 infection linked with a variety of fruits and vegetables have increased the concerns as to the safety of these foods. Strategies to reduce microbial hazards in produce include the implementation of Good

Agricultural Practices on farms, and Good Manufacturing Practices in packing, handling, and storage. Due to their highly perishable nature, most fresh fruits and vegetables need time/temperature control to extend their shelf life. In any case, attention should be paid to storage times and temperatures since pathogens, if present, are able to grow—particularly in the case of fresh-cut produce or where internalization is possible. Storage temperature and time management are important in reducing the risks of foodborne illness, and become critical parameters for any fresh-cut produce. However, as mentioned above, the time/temperature for seed sprouts will not reduce the risk of presence of high levels of pathogens. While, traditional processing methods such as freezing, canning, dehydration, fermentation, and acidification are used to improve the stability of fruits and vegetables, and time/temperature control may not be a requirement for these processed products.

5. Cereal grains and related products

5.1. Types of products

Cereal grains and related products include baked goods (breads, muffins, cakes, pastries, cookies, biscuits, bagels, and so on), frozen and refrigerated dough, breakfast cereals (cold cereal, oatmeal, grits, and so on), refrigerated or dry pasta and noodles, and cooked grains (for example, rice). Some products, such as baked goods, have a long history of safe storage at room temperature; others, such as rice, require time/temperature control after preparation.

5.2. Pathogens of concern

Grains and milled products are raw agricultural commodities; therefore, a variety of microorganisms, including mold, yeast, coliforms and other bacteria, occur naturally. Grains and milled products are dried to inhibit mold growth during storage, a process that easily controls growth of bacterial pathogens. Therefore, while organisms such as *Salmonella* spp. may be present, the prevalence and levels are low (usually < 1%). Raw ingredients used to prepare dough products (for example, eggs, dairy products, meats) may introduce *Salmonella* spp., and need to be considered when analyzing potential hazards. *Staphylococcus aureus* may present a potential hazard for certain raw dough, such as pasta dough processed at warm temperatures for extended periods of time (days); however, yeast leavened dough and cookie dough control the organism through competitive inhibition and low a_w, respectively. *Bacillus cereus* presents a concern in cooked rice.

5.3. Effects of processing

Baking, boiling, steaming, or frying are the methods used to cook the cereal-grain products. The temperatures required to achieve product quality easily destroy vegetative pathogens that may be present. These temperatures are needed to properly set the starch structure and/or to rehydrate dry products. Baking and frying not only destroy vegetative pathogens such as *S. aureus* and *Salmonella* spp., but they also remove moisture from the product—especially at the exterior surface. This dehydrated surface inhibits the growth of most bacteria; thus, mold is the primary microbial mode of failure for baked goods. When stored at room temperature, baked and fried products typically continue to lose moisture to the atmosphere, further reducing the potential for pathogen growth. Thus, baked and fried cereal-grain products such as cakes, breads, muffins, and biscuits have a long history of safe storage at room temperature despite having an internal a_w of approximately 0.94 to 0.95 (but may be as high as 0.98).

While boiled or steamed cereal products achieve temperatures

lethal to vegetative pathogens during the cooking process, these products increase in a_w to levels that support the growth of many microbial pathogens. Thus, time/temperature control is required to assure the safety of these products. For example, numerous *B. cereus* outbreaks have been associated with fried rice prepared using boiled rice that was held for hours at room temperature.

5.4. Time/temperature control

Although baked and fried cereal-grain products (for example, cakes, breads, muffins, and biscuits) have a high a_w, a number of reasons may justify their shelf stability: they have a long history of safe storage at ambient temperature; processing temperatures and moisture reduction, especially on the surface, preclude the growth of pathogens; and they are often formulated to include ingredients that enhance product safety and stability so as to permit distribution without temperature control for limited periods of time. Ingredients that are used to enhance safety and stability include humectants to reduce a_w (sugars and glycerine), preservatives (calcium propionate, potassium sorbate, sorbic acid), acids to reduce pH (vinegar, citric acid, phosphoric acid, malic acid, fumaric acid), spices with antimicrobial properties (cinnamon, nutmeg, garlic), and water-binding agents to control free water (gums, starches). The primary mode of spoilage of baked goods is mold growth, which is visible and alerts the consumer to avoid consumption, further reducing the risk of illness due to spoiled product. These characteristics plus their long history of safe storage at room temperature would allow these products to be stored at ambient temperature. Boiled or steamed cereal products, such as rice, require time/temperature control after preparation due to the increase in a_w.

Dough is frequently used to enrobe other food ingredients. Careful consideration must be given to these combination products to accurately assess the need for time/temperature control. For example, egg and dairy ingredients baked inside a pastry, such as cream-cheese croissant, will receive sufficient heat treatments to destroy vegetative pathogens and may therefore be stable at room temperature with water activities above 0.86. However, if the filling is injected after the baking process, as in the case of a cream-filled éclair, the potential for contamination must be assessed. Meat and vegetable-filled cereal products with high water activities (> 0.94) and neutral pH generally require time/temperature control because the baking process can activate spore formers such as *C. botulinum* that are present in these ingredients.

6. Fats, oils, and salad dressings

6.1. Types of products

Fats and oils are primary components of many foods that are emulsions comprised of oil as the continuous phase and water as the discontinuous phase. Mayonnaise, salad dressings, and related products are examples where water is the continuous phase and oil (fat) the discontinuous phase. Product types have grown to also include pourable dressings and starch-based dressings that resemble mayonnaise. In addition, in recent years products such as garlic-in-oil, various herb/spices-in-oil, and flavored oils have proliferated.

6.2. Microbial/pathogen concerns

The form of the water-in-oil emulsion in mayonnaise and salad dressings, particularly the chemical composition of the water phase, plays a key role in their microbiological stability. The pH range is 3.2 to 4.0 due to acetic acid; the oil content, 65 to 80%; the aqueous phase salt content, 9 to 11%; and the sugar content is 7 to 10%. This composition provides an a_w of ~0.925. Pourable dressings have a pH in the range of 3.5 to 3.9. Microbial stability

is largely related to the maximum preservative effect of acetic acid, mostly undissociated at those low pH levels. Although the a_w of mayonnaise and salad dressings is not sufficiently low to preclude growth of *S. aureus,* at pH 4.1 and below, *S. aureus* does not survive. Additionally, mayonnaise and salad dressings do not support the growth of *C. botulinum* because of the low pH and a_w. The low a_w also precludes the growth of *B. cereus.* The few documented cases of *Salmonella*-related foodborne illnesses have been related to deviations in pH and in the proportion of egg yolk and vinegar. These deviations typically occurred with noncommercially prepared products that lack the proper control of pH and the hold time to allow pathogen die-off.

Oil products that can create anaerobic sites of sufficient a_w favorable for *C. botulinum* growth and toxin production are problematic; for example, the addition of fresh garlic to oil. The moisture surrounding the garlic fragments coupled with no acidulant creates the conditions necessary for *C. botulinum* growth and toxin production. To maintain a pH that precludes growth and toxin production, an acidulant is required in these products.

6.3. Effects of processing

Following Good Manufacturing Practices can protect these products from contamination. Formulating with appropriate levels of acetic acid is essential to protect fats and oils against pathogenic bacteria; salad dressings with a pH less than 4.0 are very safe. Refrigeration after opening is recommended to prevent oxidation of the oils and product separation, but not for safety. A recent review of the microbiological safety of mayonnaise, salad dressings, and other sauces revealed that *Salmonella, E. coli* O157:H7, *L. monocytogenes, S. aureus*, and *Y. enterocolitica* die when inoculated into mayonnaise and dressings (Smittle 2000).

6.4. Time/temperature control

Products with formulations that do not meet a_w, pH, and acidity requirements as outlined above may require time/temperature control. Addition of flavoring components to traditional oils must be done in conjunction with added acidifying agents. Addition of other ingredients, such as garlic or herbs, would require an assessment or challenge testing before the product is designated shelf-stable.

7. Butter and margarine

7.1. Types of products

Butter, one of the few foods defined by law, must be at least 80% milk fat. It is a water-in-oil emulsion that can be salted or unsalted and may contain starter cultures for additional flavor. The composition and manufacturing process of butter are critical to its stability because uneven churning of butter may result in pockets of high moisture that would permit microbial growth if contamination is present. Additional stability is provided by salt, which normally results in a water-phase salt level around 16%.

Regular margarine, as defined in CFR 21.166.110, includes any plastic fat composition emulsified to at least 80% fat and with moisture in excess of 1%. A wide range of fats and oils are used to process margarine. Other ingredients in margarine include salt, emulsifiers, and preservatives, and some margarines may contain milk solids. Other margarine products may contain 40% to 60% fat with a corresponding increase in moisture content. Margarine spreads have various oil contents and usually do contain milk solids.

7.2. Microbial concerns

The bacteria found in butter products reflect the initial microflora of the cream and the sanitary condition of the processing oper-

ation, as well as the sanitary condition of the environment and handling during packaging. The high water-phase salt content of salted butter precludes all but *S. aureus* as a pathogen of concern. As with butter, the salt content of the aqueous phase in margarine and its distribution through a fine water dispersion are critical to product stability and safety. Three percent salt results in 15.8% water phase salt in the product that is inhibitory to all foodborne pathogens except *S. aureus*. On the other hand, 1% salt drops the level in the water phase to 5.9%, which would permit the growth of most foodborne pathogens. The need for time/temperature control depends on the pH and a_w of the product, and on whether other preservatives have been added to the formulation. A special concern would be the post-processing contamination by psychrotrophic pathogens such as *L. monocytogenes*, which have been demonstrated to survive processing if introduced after pasteurization.

7.3. Effects of processing

The high-temperature-short-time pasteurization of the cream used for butter destroys all but the most heat-resistant vegetative forms of microorganisms, including pathogens. Toxin formed by *S. aureus* prior to pasteurization in poorly handled cream will result in toxin carryover to the finished butter product. Contamination from lack of sanitation during processing can carry pathogens into the finely dispersed water droplets where, if nutrients are present, they could multiply. Post-processing contamination of the pasteurized cream and/or butter by *L. monocytogenes* and/or *Y. enterocolitica* is a concern since survival and growth of both microorganisms at refrigerated temperatures have been shown to be possible. This is true for both butter and margarine products containing added milk solids.

7.4. Time/temperature control

Traditional butter and margarine have had a long history of safety without time/temperature control. The few problems that have occurred are related to modified products. As these traditional products have been modified by reducing the fat levels, increasing the water content, and reducing the salt levels, the built in microbiological inhibitory factors can also be expected to change. For example, a *S. aureus* enterotoxin outbreak has been associated with a whipped butter that had been temperature abused over an extended time period. Therefore, as these traditional product compositions are changed, other microbial inhibitors such as preservatives may have to be considered to enhance the safety of the finished product during its intended use. The need for time/temperature control depends on the pH and a_w of the product, and on whether other preservatives have been added to the formulation.

8. Sugars and syrups

8.1. Types of products

A wide variety of products fall into the sugar and syrup category. Some of these products include beet and cane sugar, corn syrup, maple syrup, table syrups, and other specialty sugar syrups, such as cane syrup.

8.2. Microbial/pathogen concerns

Because of the high sugar content and resulting low a_w, pathogen survival and growth is not an issue with these products. Some may, however, require refrigeration to prevent yeast and mold growth after opening if the a_w is high enough to support growth. *Clostridium botulinum* may be a concern in light syrups, and acidulants are often used to inhibit growth and toxin production.

8.3. Effects of processing

Syrups are heated during processing to facilitate clarification and handling. Clarification steps involving precipitation and filtration serve to remove some of the microorganisms.

8.4. Time/temperature control

Traditional syrups do not need time/temperature control for safety because of high sugar content and low a_w. Traditional syrups may be modified by reducing the caloric or reducing the sugar content which could result in a change in the microbial inhibitory characteristics of these modified products. As traditional products are modified, the changes could result in variations in the sugar to water ratios that could provide opportunity for the growth of pathogens. Therefore, the use of other microbial inhibitors may be necessary to prevent pathogen growth at ambient temperature. Using such ingredients as acidulants and preservatives as microbial inhibitors may maintain the modified syrups as shelf-stable products.

9. Eggs and egg products

9.1. Types of products

"Eggs," as a product category, refers to eggs in the shell. "Egg products" refers to eggs that have been separated from their shells to produce liquid, concentrated, dried, crystallized, frozen, coagulated, and reduced cholesterol products (ICMSF 1998, p 495). In the United States, approximately 83% of the eggs are sold as shell eggs (ICMSF 1998, p 480). Liquid eggs are usually homogenized as whole eggs or separated into white and yolk. Sugar, salt, or acidulants may be added to yolks that will be further processed. All liquid eggs are usually pasteurized and require temperature control at refrigeration or frozen temperatures. Liquid egg products are used as ingredients in a wide variety of processed products including bakery products (meringues, custards, cream, angel food cakes, and egg washes), confectionary products, drinks, special dietary foods, infant products, sauces and dressings, mayonnaise, and noodles (ICMSF 1998, p 480).

9.2. Microbial concerns

Eggs can become contaminated through trans-ovarian or transshell infection (ICMSF 1998, p 481). Freshly laid eggs may be contaminated through the oviduct of an infected hen. The shell of a newly formed egg can become contaminated with a variety of microorganisms from the environment where the egg is laid. Although there are a number of antimicrobial barriers present in eggs (lysozyme, conalbumen, avidin, and alkaline pH), spoilage and pathogenicity are related to the ability of microorganisms to penetrate the shell and overcome these barriers (ICMSF 1998, p 479). The bacterial ecology of eggs is varied and consists of psychrotrophic (primarily pseudomonads) and mesophilic bacteria and can also include some pathogens. Federal regulations stating that shell eggs must be kept refrigerated prior to use have been recently implemented ("Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution," 65 FR 76092 [Dec. 5 2000]). When properly cooked or processed (pasteurized) and stored at appropriate temperatures, the bacterial loads in these products are greatly reduced. Heat treatments used for liquid eggs do not produce shelf-stable products, so proper temperature control and safe handling after opening or thawing are necessary to prevent post-process or cross contamination and growth of pathogens.

9.3. Pathogens of concern

The principal human pathogens of concern in eggs and egg products are of the genus *Salmonella* (primarily *Salmonella* Enter-

itidis). These pathogens can enter the egg either by trans-ovarian transmission or by penetrating the surface of the egg in a way similar to that of spoilage organisms (ICMSF 1998, p 492). *Listeria monocytogenes* is also a concern in processed eggs, particularly in products with extended shelf life.

9.4. Effects of processing

Shell eggs are usually fried, boiled, or baked. In these cooking methods, it is important that eggs reach appropriate temperature to destroy any salmonellae that may be present. Eggs boiled or cooked long enough to solidify the yolk (~ 10 min of boiling) are heated sufficiently to inactivate salmonellae, but other cooking procedures that leave the yolk in a liquid state (for example, soft boiled and fried eggs "over easy") are not always sufficient to inactivate *Salmonella* spp (ICMSF 1998, p 493). Liquid eggs, white, and yolk that do not contain chemical additives are usually pasteurized at temperatures that vary from 55.6 °C (132 °F) to 69 °C (156 °F) at processing times that vary from 10 to 1.5 min. Lower temperatures and shorter processing times increase the risk of survival of *Salmonella* spp., whereas higher temperature and longer processing times increase damage to the functional properties of the egg (ICMSF 1998, p 496). It should be noted that reduced a_w and longer heating times are required to achieve the same level of pathogen reduction. In the United States, pasteurization requirements are 60 °C (140 °F) for 3.5 min, which achieve more than a 3-log reduction of salmonellae (ICMSF 1998, p 497). Proper pasteurization reduces the initial level of other microorganisms; however, if the product is temperature abused, some bacteria, such as micrococci, staphylococci, *Bacillus* spp., enterococci, and catalase negative bacterial rods, survive the process and can grow.

9.5. Time/temperature control

Eggs and egg products will easily support the growth of spoilage and pathogenic microorganisms and clearly require time/temperature control to assure safety. Control methods require an integrated approach that begins at the egg production facility, and carries through to processing and further processing operations as well as to retail and food service facilities. Temperature control of shell eggs, followed by thorough cooking and proper handling, are essential in assuring safety.

As mentioned above, heat treatments used for liquid eggs do not produce shelf-stable products, so they should be kept refrigerated or frozen. These products should be safely handled to reduce the likelihood of post-process and/or cross contamination.

10. Milk and milk products (except cheeses)

10.1. Types of products

Milk, the lacteal secretion from warm-blooded animals, is commercially available most commonly from cows, goats, and sheep. Milk may be available to consumers as a single- or multiple-ingredient fluid pasteurized product. It can also be obtained in a concentrated form, such as evaporated or condensed milk, or in a dry form. Bacterial cultures can be used in making other products such as cultured milk, yogurts, and cheeses. Milk and milk products are also included as major ingredients in other food forms ranging from ice cream to prepared foods.

10.2. Microbial concerns

Milk is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrients for microbes, is high in moisture, and has neutral pH. Due to these factors, it is subject to microbial spoilage from the moment it is secreted from a healthy animal. Milk is exposed to the potential for microbial contamination during collection, storage, transportation, and processing. With-

out basic sanitary practices in place and temperature control during handling, the product will quickly spoil and become unacceptable for human consumption. Uncontrolled microbial growth affects the flavor and appearance of the product and can affect its safety. On the other hand, controlled use of microbial cultures can produce many flavorful products and can also preserve milk and milk products. Milk and milk products are normally consumed after the application of a processing step to reduce pathogenic microorganisms.

10.3. Pathogens of concern

The principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *L. monocytogenes*, *S. aureus*, enterohemorrhagic *E. coli*, *Campylobacter jejuni*, *C. botulinum*, and *B. cereus.*

10.4. Effects of processing

Non-spore-forming pathogens are reduced in fluid milk through pasteurization. Milk used as an ingredient in other products is normally pasteurized or thermally processed in some form to reduce possible pathogens. The exceptions would be some cheese-making processes that rely on microbial cultures and the effects of their growth in the milk medium over time to render the finished food safe.

While most milk and milk products are sold refrigerated to prevent spoilage, some dairy products are shelf stable due to a combination of moisture content, salts, and pH that control the growth of microbes. Canned milks are shelf stable due to thermal processing of the product within the individual containers. Some milk and milk products may be aseptically processed and packaged to enable the product to be shelf stable. Other dairy products may be thermally processed and packaged hot in conjunction with product formulations designed to inhibit the growth and survival of pathogenic organisms in products stored at room temperature. Microbial growth in dried milk is prevented by removing most of the moisture in fluid milk. Other dairy products, such as ice cream, are sold in a frozen state to limit the growth of microbes.

Protection from post-pasteurization contamination before the milk product is packaged is a critical factor in achieving a safe food. Multiple-ingredient dairy products may raise the concern of contamination depending on the characteristics of the product and the location where the ingredient may be added in the process. Ingredients added after pasteurization of the milk portion of the food can be a source of pathogens. The control of potential sources of contamination can be addressed by following production practices based upon Good Manufacturing Practices.

10.5. Time/temperature control

During handling, basic sanitation practices and temperature control are required to maintain acceptable sensory qualities of milk and milk products. Similarly, most milk and milk products are sold refrigerated to prevent spoilage. Exceptions include canned milks, dried milk, ice cream, aseptically processed and packaged products, and thermally processed products that are packaged hot in conjunction with specific product formulations. These milk products do not require refrigeration because of the combination of moisture content, salts, and pH that control the growth of microbes.

11. Cheeses

11.1. Types of products

Cheese is the product of milk coagulation, followed by curd separation and ripening. More than 500 cheeses are manufactured worldwide, with variations deriving from modifications in

the cheesemaking technique; for example, type of milk, coagulation method, starter culture, addition of salt or other additives, and ripening period. The changes, including microbiological changes occurring during cheesemaking, are complex. Cheeses types can be can be classified according to many different criteria, but a general classification divides cheeses into fresh or unripened, soft, semisoft, hard, and processed cheese.

11.2. Microbiological concerns

The survival and growth of pathogens in cheese depend on the many factors affecting the cheesemaking process, including time and temperature during the ripening process, variations in pH and a_w, competing microflora, biochemical changes during ripening, and addition of antimicrobials. The microbiological quality of the milk will also contribute to the microbial ecology of the final product, especially in cheeses where milk is not pasteurized.

11.3. Pathogens of concern

Salmonella spp., *L. monocytogenes* (mainly in soft, high moisture, high pH cheeses), enterohemorrhagic *E. coli* O157:H7 (due to post-process contamination), *S. aureus* (due to faulty cheesemaking process), *Shigella* spp. and *C. botulinum* (due to faulty process) have been implicated in outbreaks associated with the consumption of various types of cheeses.

11.4. Effects of processing

Cheeses made with pasteurized milk generally would not be a concern unless post-process contamination with pathogenic vegetative cells occurs. To minimize post-process contamination, strict plant sanitation and Good Manufacturing Practices need to be followed throughout the cheese making process. In the United States, cheeses made with raw milk need to be ripened for at least 60 d to control for pathogens. If ripened for more than 60 d, pH, a_w, salt, and other parameters were thought to inhibit the growth of pathogens. However, recent studies have shown that low levels of certain pathogens such as *E. coli* O157:H7 can survive beyond 60 d curing in hard cheeses (Reitsma and Henning 1996). In general, there have been very few documented illness outbreaks linked to consumption of properly ripened hard cheese. Therefore, time/temperature control of hard cheeses is primarily needed not for safety reasons but to maintain the organoleptic quality of cheese. However, if the cheesemaking process is faulty (for example, high pH) or if post-process contamination occurs, the potential growth of pathogens is possible and time/temperature control is needed for safety. Soft cheeses (ripened or unripened), which have a higher moisture content, do require time/temperature control for safety.

In processed cheese, heat and sanitary packaging are used to prevent microbial hazards unless the cheese is contaminated with heat-resistant pathogenic spores. If the product is contaminated with spore-formers such as *C. botulinum*, however, germination and toxin formation can cause serious public heath concerns, especially if the product is intended to be used at ambient temperature. In this case, pH, a_w, moisture content, and antimicrobials (for example, phosphate, salt) become critical parameters that may preclude pathogenic growth and toxin formation and will determine the need for time/temperature control. Post-process contamination after opening is also possible, and therefore, processed cheeses often need refrigeration after opening.

11.5. Time/temperature control

For traditionally made hard cheeses, unless pH is high or post-process contamination occurs, time/temperature control for safety reasons is not required. Time/temperature control is needed, however, for high moisture soft cheeses because of the potential growth of pathogens. With processed cheeses, there is a concern with the growth and toxin production of *C. botulinum*. If a processed cheese is intended for use at ambient temperature, pH, a_w, moisture content, and antimicrobials should be appropriately adjusted to inhibit botulin toxin formation.

12. Combination products

12.1. Type of products

The "combination products" category refers to products whose formula contains distinct food systems (for example, cheese with vegetable pieces), or products whose components are processed separately and assembled later (for example, pumpkin pie with crème topping). Examples of products that fall into this category are focaccia breads, meat salads, meat-filled pastry and other stuffed products, and prepared foods (for example, fettuccine alfredo with chicken).

12.2. Microbial concerns

These products present special challenges to their identification as "potentially hazardous foods." Combination foods present the added complexity of the various components' microbial ecology compared to the ecology of single-component foods. The microbial concerns associated with combination products greatly depend on the food components from which they are processed. (For microbial concerns on products, see the hazard analysis of dairy, eggs, fruits and vegetables, meat and poultry, seafood, and cereal products earlier in this chapter.)

The interactions among the various foods combined, which contribute to the uniqueness of each food product, also need to be considered. Components of significantly different pH or a_w produce an altered microenvironment at the interface of the components. An example of this scenario is a donut filled with an acidified filling. The donut has higher pH and lower a_w than the filling. The pH and a_w at the interface will be affected by this difference, which may result in the growth of microorganisms if the product has a long enough shelf life. Obviously, these changes may affect the survival and growth of microorganisms in a less predictable manner than they might in single component foods. In addition to pH and a_w, other food characteristics such as redox potential and the effectiveness of antimicrobials are likely to differ at the interfaces, possibly resulting in unexpected pathogen behavior.

Another feature of combination foods that may affect their microbiological safety is the fact that products often are handled by employees, resulting in an increased risk of microbial contamination. Opportunities for post-processing contamination during handling may result in safety hazards associated with *S. aureus*, *L. monocytogenes*, *Shigella*, *E. coli* O157:H7, *Salmonella* spp., and other enteric pathogens. *Clostridium botulinum* is also of concern, especially for certain modified atmosphere, controlled atmosphere, and vacuum packaged products.

12.3. Effects of processing

Often, the food composed of other products is subjected to processing before consumption. For example, focaccia bread and fruit pastries are baked and the meat in meat salads is cooked. (For the effect of processing methods in microbial reduction, see the hazard analysis of dairy, eggs, fruits and vegetables, meat and poultry, seafood, and cereal products.) When considering the effect of processing in the microbial load of the product, one needs to consider if the components have been processed separately or after assembly. Processing of the food after assembly decreases the chances for contamination and growth of pathogens as compared to assembling the different components before processing.

12.4. Time/temperature control

In combination foods, the need for time/temperature control depends on the nature of the product. Both the potential for the development of microenvironments and the existence of interface areas contribute to the difficulties in accurately measuring the intrinsic factors of the food. Because of the complex interactions in multiple component foods, one cannot rely on the pH, a_w, or other parameter measurements and, therefore, challenge studies are often performed to decide if the food requires time/temperature control for safety.

References

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. York PA: Association of Food and Drug Officials. 7 p.

[ICMSF] International Commission on Microbiological Specification for Foods. 1998. Microorganisms in foods. Volume 6, Microbial ecology of food commodities. New York: Blackie Academic & Professional. 615 p.

[IFT] Institute of Food Technologists, Dept. of Science and Technology Projects. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. In Comprehensive Reviews in Food Science and Food Safety Supplement. Beuchat LR, Busta FF, Farber JM, Garrett EH, Harris LJ, Parish ME, Suslow TV, editors. Chicago: IFT. 204 p.

Reitsma CJ, Henning DR. 1996. Survival of enterohemorrhagic *Escherichia coli* 0157:H7 during the manufacture and curing of cheddar cheese. J Food Prot 59(5):460-4.

Smittle RB. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. J Food Prot 63(8):1144-53.

[USDA] U.S. Dept. of Agriculture, Food Safety and Inspection Service. 1996 [with change 98-01 (Sept 1996 thru Feb 1998)]. Food standards and labeling policy book. Available from: Superintendent of Documents, PO Box 371954, Pittsburgh, PA 15250-7954; (202)512-1800.

Chapter V
Effect of Preservation Technologies on Microbial Inactivation in Foods

1. Introduction

Traditionally, the most popular preservation technologies for the reduction of microbial contamination of food, and pathogens in particular, have been the manipulation of the water activity and/or pH, heat treatments, the addition of chemical preservatives, and the control of storage temperature of foods. Lately, and mainly as a result of consumer demand for "fresher products," other technologies are emerging as alternatives for extension of product shelf life (for better quality products) and reduction of pathogenic organisms (for safer products). The process by which a product is manufactured is one of the factors to be considered when determining if a food needs temperature control for safety. The efficiency of the process is dependent on a number of parameters unique to each technology that will be described briefly in this chapter. To determine the pathogen reduction needed for the food to be safe at room temperature, other factors need to be considered as well, such as water activity and pH of the food, packaging, processing, formulation, and opportunities for post-process contamination.

Inactivation of microorganisms is influenced by a number of microorganism-related factors that are generally independent of the technology itself. These include the type and form of target microorganism; the genus, species, and strain of microorganism; growth stage; environmental stress selection mechanisms; and sub-lethal injury. Each factor influences the bacterial resistance independently of the apparent inactivation capacity of that particular process. For pasteurization purposes, one is mostly concerned with the inactivation of vegetative cells of disease-producing microorganisms. However, to have a commercially sterile product, the process must control or inactivate any microbial life capable of germinating and growing in the food under normal storage conditions.

2. Validation of processing parameters

Establishment of traditional thermal processes for foods has been based on two main factors: (1) knowledge of the thermal inactivation kinetics of the most heat-resistant pathogen of concern for each specific food product; and (2) determination of the nature of heat transfer properties of the food system. The validity of a thermal process must be confirmed by an inoculated challenge test conducted on the product under actual plant conditions using surrogate microorganisms as biological indicators to mimic pathogens. Thus, the two factors described above, which are well established for thermal processes, should be used for establishing and validating scheduled new thermal processes based on thermal effect on microorganisms, such as microwave heating.

For other preservation processes not based on heat inactivation, key pathogens of concern and nonpathogenic surrogates need to be identified and their significance evaluated. Surrogate microorganisms should be selected from well-known nonpathogenic populations, should mimic the target pathogenic microorganism in growth habits, should not be susceptible to injury, and should not exhibit irreversible inhibition (thermal or otherwise). Surrogate microorganisms should be genetically stable and exhibit uniform thermal and growth characteristics from batch to batch over several generations.

The durability to food and processing parameters should be similar to that of the target organism. Population of surrogates should be constant and maintain stable thermal and growth characteristics from batch to batch. Enumeration of surrogates should be rapid and should utilize inexpensive detection systems that easily differentiate them from natural flora. Genetic stability of surrogates is desirable to obtain reproducible results. It also is recommended that surrogates do not establish themselves as "spoilage" organisms on equipment or in the production area. The validation process should be designed so that the surrogate exhibits a predictable time-temperature process character profile that correlates to that of the target pathogen. Introduction of system modifications or variables, leading to inaccurate results should be avoided (for example, thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity, and so on).

3. Processing technologies

3.1. Water activity and pH

The manipulation of water activity and/or pH is the less complicated of technologies in terms of equipment, expense, and expert personnel needed. Although it may not reduce the microbial load per se, reducing water activity or pH may retard or impede microbial growth. (For a more extended description on how water activity and pH can be used as preservation technologies and for a list of the optimum range pH and water activity for various pathogens of concern see Chapter 3). When changing these characteristics of foods with the intention of safely storing a food at room temperature, those minimum pH and water activity values should be taken as guidance. At different temperatures and for different foods, these ranges may vary. For example, as the temperature moves away from optimum, a higher minimum pH is generally observed.

3.2. Technologies based on thermal effects

In addition to microbial inactivation by conventional methods of heating, microwave and ohmic and inductive heating are also considered to be heat-based processes that can inactivate microorganisms by thermal effects. Microwave and radio frequency

heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material through two mechanisms—dielectric and ionic. Ohmic heating is defined as the process of passing electric currents through foods or other materials to heat them. Ohmic heating is distinguished from other electrical heating methods by the presence of electrodes contacting the food, frequency, and waveform. Inductive heating is a process wherein electric currents are induced within the food due to oscillating electromagnetic fields generated by electric coils. No data about microbial death kinetics under inductive heating have been published.

For any of these heat-based processes, the magnitude of time/temperature history and the location of the cold points will determine the effect on microorganisms. The effectiveness of these processes also depends on water activity and pH of the product. Although the shape of the inactivation curves is expected to be similar to those in conventional heating, the intricacies of each of the technologies, however, need special attention if this technology is used for microbial inactivation. For instance, in microwave heating a number of factors influence the location of the cold points, such as the composition, shape, and size of the food, the microwave frequency, and the applicator design. The location of the coldest-point and time/temperature history can be predicted through simulation software, and it is expected that food processors may be able to use them in the future. For determining the kinetics and efficiency of inactivation of microorganisms for these technologies, surrogate/indicator microorganisms could be selected from those traditionally used in thermal-processing studies.

3.3. High pressure processing

High pressure processing (HPP), also described as high hydrostatic pressure (HHP) or ultra high pressure (UHP) processing, subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa. Process temperature during pressure treatment can be specified from below 32 °F (0 °C) to above 212 °F (100 °C). Commercial exposure times can range from a millisecond pulse to over 20 min. Chemical and microbiological changes in the food generally will be a function of the process temperature and treatment time. The various effects of high hydrostatic pressure on microorganisms can be grouped into cell-envelope-related effects, pressure-induced cellular changes, biochemical aspects, and effects on genetic mechanisms.

HPP acts instantaneously and uniformly throughout a mass of food independent of size, shape, and food composition. Compression will uniformly increase the temperature of foods approximately 5 °F (3 °C) per 100 MPa. Compression of foods may shift the pH of the food as a function of imposed pressure and must be determined for each food treatment process. Water activity and pH are among the critical process factors in the inactivation of microbes by HPP. An increase in food temperature above room temperature, and to a lesser extent, a decrease below room temperature increases the inactivation rate of microorganisms during HPP treatment. Temperatures in the range of 113 to 122 °F (45 to 50 °C) appear to increase the rate of inactivation of food pathogens and spoilage microbes. Temperatures ranging from 194 to 230 °F (90 to 110 °C) in conjunction with pressures of 500 to 700 MPa have been used to inactivate sporeforming bacteria such as *Clostridium botulinum*. Current pressure processes include batch and semi-continuous systems, but no commercial continuous HPP systems are operating.

The critical process factors in HPP include pressure, time at pressure, time to achieve treatment pressure, decompression time, treatment temperature (including adiabatic heating), initial product temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity, and concurrent processing aids. Interestingly, because HPP acts instantaneously and uniformly through a mass of food, package size, shape, and composition are not factors in process determination. High hydrostatic pressures can cause undesirable structural changes in structurally fragile foods such as strawberries or lettuce (for example, cell deformation and cell membrane damage). Food products that have been brought to market include raw oysters, fruit jellies and jams, fruit juices, pourable salad dressings, raw squid, rice cakes, *foie gras*, ham, and guacamole.

A biphasic pressure inactivation curve is frequently encountered for both vegetative bacteria and endospores indicating the residence of a small pressure-resistant subpopulation. Tailing phenomena should be investigated carefully in challenge studies. The use of pathogens rather than surrogates for highly infective pathogens may be advised.

The elimination of spores from low-acid foods presents food processing and food-safety challenges to the industry. It is well established that bacterial endospores are the most pressure-resistant life forms known. One of the most heat-resistant pathogens, and one of the most lethal to human beings, is *C. botulinum*, primarily types A, B, E, and F. As such, *C. botulinum* heads the list of most pressure-resistant and dangerous organisms faced by HPP. Spore suspensions of strains 17B and Cap 9B tolerated exposures of 30 min to 827 MPa and 167 °F (75 °C) (Larkin and Reddy 1999; personal communication; unreferenced). Because some types of spores of *C. botulinum* are capable of surviving even the most extreme pressures and temperatures of HPP, there is no absolute microbial indicator for sterility by HPP. Among the sporeformers of concern, *Bacillus cereus* has been the most studied because of its facultative anaerobic nature and very low rate of lethality.

Normally, Gram-positive vegetative bacteria are more resistant to environmental stresses, including pressure, than vegetative cells of Gram-negative bacteria. Among the pathogenic nonsporeforming Gram-positive bacteria, *Listeria monocytogenes* and *Staphylococcus aureus* are the two most well studied regarding the use of HPP processing. *Staphylococcus aureus* appears to have a high resistance to pressure.

There appears to be a wide range of pressure sensitivity among the pathogenic Gram-negative bacteria. Patterson and others (1995) have studied a clinical isolate of *Escherichia coli* O157:H7 that possesses pressure resistance comparable to spores. Some strains of *Salmonella* spp. have demonstrated relatively high levels of pressure resistance. Given these pressure resistances and their importance in food safety, *E. coli* O157:H7 and *Salmonella* spp. are of key concern in the development of effective HPP food treatments. For vegetative bacteria, nonpathogenic *Listeria innocua* is a useful surrogate for the foodborne pathogen, *L. monocytogenes*. A nonpathogenic strain of *Bacillus* may be useful as a surrogate for HPP-resistant *E. coli* O157:H7 isolates.

3.3.1. Commercial implications

Current practical operating pressures for commercial HPP food treatment intensifiers and pressure vessels are in the order of 580 MPa (85,000 psi). If this pressure is specified, then the following process times may be considered as first estimates for initial process planning. It must be understood that actual process parameters must be developed from challenge test packs.

Experience with acid foods suggests that shelf-stable (commercially sterile) products, having a water activity close to 1.0, and pH values less than 4.0, can be preserved using a pressure of 580 MPa and a process hold time of 3 min. This treatment has been shown to inactivate 10^6 cfu/g of *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., or *Staphylococcus* spp. in salsa and apple juice.

Acid foods with pH values between 4.0 and 4.5 can be made commercially sterile using a pressure of 580 MPa and a hold time of 15 min. Products would have an initial temperature of about

71.6 °F (22 °C). Shorter hold times are possible if the product is to be refrigerated. Actual hold-time values must be determined from challenge packs and storage studies perhaps twice the length of the intended shelf life of the product.

Low-acid products can be rendered free of pathogens or pasteurized by HPP ; however, satisfactory guidelines for hold times at 580 MPa for low-acid food pasteurization have not emerged. For example, the post-package pasteurization of vacuum-packed cured meat products to eliminate *Listeria* spp. represents a useful application of HPP. Ground beef can be pasteurized by HPP to eliminate *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., or *Staphylococcus* spp. Much more work is required to develop a suggested hold time at 580 MPa due to the potential for tailing. Changes in product color and appearance may limit the usefulness of HPP treatment pressures above 200 to 300 MPa.

3.4. Pulsed electric fields

High-intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20-80 kV/cm) to foods placed between two electrodes. PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s. Use of PEF can reduce energy usage compared to thermal processes, as less energy is converted into heat, which also reduces detrimental changes to the sensory and physical properties of the food.

To date, PEF has been applied mainly to extend the shelf life of foods. Application of PEF is restricted to food products that can withstand high electric fields, have low electrical conductivity, and do not contain or form bubbles. The particle size of the food in both static and flow treatment modes is a limitation. Also, due to the variations in PEF systems, a method to accurately measure treatment delivery is still needed.

Factors that affect the microbial inactivation with PEF are process factors (electric field intensity, pulse width, treatment time and temperature, and pulse waveshapes), microbial entity factors (type, concentration, and growth stage of microorganism) and media factors (pH, antimicrobials and ionic compounds, conductivity, and medium ionic strength).

Many researchers have studied the effects of pulsed electric fields in microbial inactivation; however, due to the numerous critical process factors and broad experimental conditions used, definite conclusions about specific pathogen reductions cannot be made. Research that provides conclusive data on the PEF inactivation of pathogens of concern is clearly needed. Castro and others (1993) reported a 5-log reduction in bacteria, yeast, and mold counts suspended in orange juice treated with PEF. Zhang and others (1995) achieved a 9-log reduction in *E. coli* suspended in simulated milk ultrafiltrate treated with PEF by applying a converged electric field strength of 70 KV/cm for a short treatment time of 160 ms. This processing condition may be adequate for commercial food pasteurization that requires 6- to 7-log reduction cycles (Zhang and others 1995). However, numerous critical process factors exist and carefully designed studies need to be performed to better understand how these factors affect populations of pathogens of concern. Currently, there is little information on the use of surrogate microorganisms as indicators of pathogenic bacteria when PEF is used as a processing method. Selection of surrogates will require the prior identification of the microorganism of concern in a specific food and PEF system. The selection of the appropriate surrogate(s) will depend on the type of food, microflora, and process conditions (that is, electric field intensity, number of pulses, treatment time, pulse wave), and should also follow the general guidelines listed in the validation section.

3.5. Irradiation

Irradiation of food refers to the process by which food is exposed to enough radiation energy to cause ionization. Ionization can lead to the death of microorganisms due to genetic damage, which prevents cellular replication. For the treatment of foods, FDA has approved the use of gamma rays from decaying isotopes of cobalt-60 or cesium 137, x-rays with a maximum energy of five million electron volts (MeV), and electrons with a maximum energy of 10 MeV. An electron volt is the amount of energy acquired by an electron when accelerated by one volt in a vacuum. X-rays are produced when high-energy electrons strike a thin metal film. Lethality of irradiation depends on the target (microorganism), condition of the treated item, and environmental factors. Addition or removal of salt or water, time/temperature of the treatment, or oxygen presence are factors that will influence the antimicrobial effect of irradiation.

Irradiation is considered an additive in the U.S. and as such, it needs to be approved by the FDA office of premarket approval for each new application and labeled. Two terms have been used to define the extent of pathogen reduction with irradiation. Radiation pasteurization refers to the destruction of pathogenic, non-spore-forming foodborne bacteria. In radiation pasteurization, medium dose treatments (1 to 10 kGy) reduce microbial populations, including pathogens in foods. Elimination of pathogens on meat, seafood, and poultry by medium dose irradiation has been studied. Sterilization radiation is used for radiation processes that will render the food commercially sterile or for foods that are both sterile and shelf stable. In this last case, sterilization must ensure the elimination of the most resistant pathogen, endospores of *Clostridium botulinum.* In order to achieve this, higher doses (42-71 kGy depending on the product) than the ones currently permitted for foods (up to 10 kGy, except for spices) are needed. Only frozen meats consumed by NASA astronauts have been permitted by FDA to be sterilized through irradiation. They are, however, in the market in other countries.

Ionizing radiation is used as a means of extending the shelf life of produce (Diehl 1995; Thayer and others 1996). FDA has approved the use of ionizing radiation with a range dose 0.3-1 kGy for growth and maturation inhibition. Not much effort has been applied to the control of foodborne pathogens on fresh foods, mainly because most medium and high level doses are not appropriate for produce since they can cause sensory defects (visual, texture, and flavor) and/or accelerated senescence (Thomas 1986; Barkai-Golan 1992). Ionizing irradiation has recently been used to eliminate *E. coli* O157:H7 from apple juice, and *E. coli* O157:H7 and salmonellae from seed and sprouts. Doses in the range of < 1 to 3 kGy have been shown to reduce or eliminate populations of foodborne pathogens, postharvest spoilage organisms, and other microorganisms on produce (Moy 1983; Urbain 1986; Farkas 1997). Strawberry shelf life can be extended with treatments in the range of 2 to 3 kGy (Sommer and Maxie 1966; Zegota 1988; Marcotte 1992; Diehl 1995). Research conducted since that time suggests that irradiation can be an important treatment to enhance safety of other types of produce.

FDA and the U.S. Department of Agriculture Food Safety and Inspection Service have also approved irradiation to control foodborne pathogens in raw poultry with a dose range of 1.5 to 3.0 kGy. Recently, the irradiation of raw refrigerated and frozen meat has been approved with maximum doses of 4.7 and 7 kGy, respectively. Radiation doses of 2.5 kGy in beef will result in 6 log reduction of *Campylobacter*, 5 log reduction of *E. coli* O157:H7, 3 log reduction in *Salmonella* spp., and 5 log reduction of *Staphylococcus* cells (CAST 1996). Although the potential for consumer infection by pathogens is decreased greatly and shelf life is extended by radiation pasteurization of meat and poultry, the room temperature storage of raw meat products would be highly dis-

couraged.

Other products, such as shell eggs (up to 3 kGy), have recently been approved for irradiation for safety reasons. Shell eggs can be irradiated with the intention of significantly reducing populations of *Salmonella* spp. Reduction levels depend upon radiation dose, initial level of pathogen contamination, or other treatment-related conditions.

The effect of irradiation on microbial populations suggests that it could also be used to decrease pathogens in a product with the intention of allowing microbiologically safe storage at ambient temperature for a specific time. However, the foods currently allowed to be irradiated are very limited. One could envision that in the future a food such as pumpkin pie could be irradiated to allow for safe ambient temperature storage. As with other technologies, organoleptic changes in the food would need to be considered. More important, the effectiveness of the technology will need to be validated for the specific application.

3.6. Other technologies

Some of the technologies present greater limitations or are at a development stage that require extensive further scientific research before they can be commercially used. For instance, high voltage arc discharge (application of discharge voltages through an electrode gap below an aqueous medium) causes electrolysis and highly reactive chemicals. Although microorganisms are inactivated, improved designs need to be developed before consideration for use in food preservation. Likewise, oscillating magnetic fields have been explored for their potential to inactivate microorganisms; however, the results are inconsistent. Data on inactivation of food microorganisms by ultrasound (energy generated by sound waves of 20,000 or more vibrations per second) are scarce, and limitations include the inclusion of particulates and other interfering substances. Ultraviolet (UV) light is a promising technique, especially in treating water and fruit juices. A 4-log bacterial reduction was obtained for a variety of microorganisms when 400 J/m^2 was applied. Apple cider inoculated with *E. coli*

O157:H7 treated in that manner achieved a 5-log reduction (Worobo 2000). Critical factors include the transmissivity, the geometric configuration of the reactor, the power, wavelength, and physical arrangement of the UV source, the product flow profile, and radiation path length.

References

Barkai-Golan R. 1992. Suppression of postharvest pathogens of fresh fruits and vegetables by ionizing radiation. In: Rosenthal, editor. Electromagnetic Radiations in Food Science, I. Berlin: Springer-Verlag. p 155-94.
[CAST] Council for Agricultural Science and Technology. 1996. Radiation pasteurization of food. Issue paper no. 7. Ames IA: CAST. p 10.
Castro AJ, Barbosa-Canovas GV, Swanson BG. 1993. Microbial inactivation of foods by pulsed electric fields. J Food Process Pres 17:47-73.
Diehl JF. 1995. Safety of irradiated foods. 2nd revised ed. New York: Marcel Dekker, Inc.
Farkas J. 1997. Physical methods of food preservation. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington DC: ASM Press. p 497-519.
Marcotte M. 1992. Irradiated strawberries enter the U.S. market. Food Technol 46(5):80-6.
Moy JH. 1983. Radurization and radication: fruits and vegetables. In: Josephson ES, Peterson MS, editors. Preservation of food by ionizing radiation. Boca Raton FL: CRC Pr. p 83-108.
Patterson MF, Quinn M, Simpson R, Gilmour A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. J Food Prot 58(5):524-9.
Sommer NF, Maxie EG. 1966. Recent research on the irradiation of fruits and vegetables. In. Food Irradiation. Vienna: International Atomic Energy Agency. p 571.
Thayer DW, Josephson ES, Brynjolfsson A, Giddings GG. 1996. Radiation pasteurization of food. Council for Agricultural Science and Technology Issue Paper Nr. 7(Apr):1-12.
Thomas P. 1986. Radiation preservation of foods of plant origin. V. Temperate fruits: pome fruits, stone fruits, and berries. Crit Rev Fd Sci Technol 24:357-400.
Urbain WM. 1986. Fruits, vegetables, and nuts. In: Schweigert BS, editor. Food Irradiation. (Food Science and Technology, A series of monographs) Orlando FL: Academic Press. p 170-216. .
Worobo RW. 2000. Efficacy of the CiderSure 3500 ultraviolet light unit in apple cider. Ithaca NY: Cornell University, Department of Food Science and Technology. 1-6 p.
Zegota H. 1988. Suitability of Dukat strawberries for studying effects on shelf life of irradiation combined with cold storage. Z Levensm Unters Forsch 187:111-4.
Zhang QH, Qin B-L, Barbosa-Canovas GV, Swanson BG. 1995. Inactivation of E. coli for food pasteurization by high-strength pulsed electric fields. J Food Process Pres 19(2):103-18.

Microbiological Challenge Testing

1. Introduction

Microbiological challenge testing has been and continues to be a useful tool for determining the ability of a food to support the growth of spoilage organisms or pathogens. Microbiological challenge tests also play an important role in the validation of processes that are intended to deliver some degree of lethality against a target organism or group of target organisms. Quite often, with this latter purpose, there is an associated performance standard that the process must deliver (for example, a 5 log reduction of *Escherichia coli* O157:H7 for fermented meats). An appropriately designed microbiological challenge test will validate that a specific process is in compliance with the predetermined performance standard. The design, implementation, and assessment of microbiological challenge studies is a complex task that depends on factors related to how the product is formulated, manufactured, packaged, distributed, prepared, and consumed. An expert microbiologist must consider the relevant factors and design a study that best assesses the food safety of the product. Failure to account for specific product and environmental factors in the design of the test could result in flawed conclusions.

Microbiological challenge studies are also useful in determining the potential shelf life of certain refrigerated or ambient-stored foods. The determination of whether challenge studies are appropriate or useful must be made by considering such factors as the likelihood of the product to support growth of spoilage organisms or pathogens, or a knowledge of the previous history of the product. For example, it is not useful to conduct challenge studies on frozen foods that would not support growth under proper storage conditions; nor would it be especially useful to conduct challenge tests on commercially sterile retorted canned foods. However, in the canned food example, it may be appropriate to conduct inoculated pack studies as part of the protocol for process validation. Microbiological challenge testing is very useful for food products that may sustain the growth of pathogenic organisms and that are stored under refrigeration, elevated temperature, or at ambient temperature and vulnerable to the growth of microorganisms.

When conducting a microbiological challenge study, a number of factors must be considered (Vestergaard 2001). These include (1) the selection of appropriate pathogens or surrogates, (2) the level of challenge inoculum, (3) the inoculum preparation and method of inoculation, (4) the duration of the study, (5) formulation factors and storage conditions, and (6) sample analyses. The interpretation of the data and pass/fail criteria are critical in evaluating whether a food needs time/temperature control for safety.

While microbiological challenge testing is useful for determining the spoilage potential of a product formulation, the remainder of the discussion in this chapter will focus on pathogens relevant to foods that need time/temperature control for safety.

2. Selection of challenge organisms

Table 6-1 shows some pathogens that may be used in challenge studies for various types of foods (Vestergaard 2001). Knowledge of the food formulation and history of the food (for example, association with known illness outbreaks and/or evidence of potential growth) is essential when selecting the appropriate challenge pathogens. For example, *Clostridium botulinum* would be of concern with certain modified atmosphere packaged (MAP) products, and *Staphylococcus aureus* may be of concern in foods with little competitive microflora and in products with reduced a_w.

The ideal organisms for challenge testing are those that have been previously isolated from similar formulations. Additionally, pathogens from known foodborne outbreaks should be included to ensure the formulation is robust enough to inhibit those organisms as well.

Multiple specific strains of the target pathogens should be included in the challenge study. It is typical to challenge a food formulation with a "cocktail" or mixture of multiple strains in order to account for potential strain variation. It is not unusual to have a cocktail of 5 or more strains of each target pathogen in a challenge study. For example, botulinal challenge studies typically in-

Table 6-1—Pathogens that may be considered for use in challenge studies for various food products

Food Type	Type of Organism
Salad dressings	Salmonellae, *Staphylococcus aureus*
Modified atmosphere packaged products (that is, vegetables, meats, poultry, fish)	*Clostridium botulinum* (proteolytic and nonproteolytic strains) and other pathogens (for example, salmonellae, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli*.
Bakery items (that is, fillings, icings, nonfruit pies)	Salmonellae, *S. aureus*
Sauces and salsas stored at ambient temperature	Salmonellae, *S. aureus*
Dairy products	Salmonellae, *S. aureus*, *C. botulinum*, enterohemorrhagic *E. coli*, *L. monocytogenes*
Confectionery products	Salmonellae
Formula with new preservatives	Salmonellae, *S. aureus*, *C. botulinum*, enterohemorrhagic *E. coli*, *L. monocytogenes*

Source: Adapted from Vestergaard 2001.

clude several strains of proteolytic types A and B as well as representative nonproteolytic strains (if appropriate). A single challenge strain with specific well defined characteristics may be used to screen products similar in nature to those formulations that have been extensively challenged with multiple strains in the past.

Prior to conducting the challenge study, selected strains should be screened for mutual antagonism. Antagonism between certain strains of *Listeria monocytogenes* has been reported, as well as between certain strains of *C. botulinum* and between bacteriocin-producing lactic acid bacteria. If these are not compatible when used as part of a challenge cocktail, then erroneous results may ensue.

It is also important to incubate and prepare the challenge suspension under standardized conditions and format. Shifts in the incubation temperature used to propagate the challenge organisms and the storage temperature of the product have been shown to change the length of the lag period of the challenge study itself (Curiale 1991). Consideration must also be given to adapting the challenge suspension to the environment of the food formulation prior to inoculation. For example, acid-adaptation of *E. coli* O157:H7 cells or salmonellae cells prior to inoculation can greatly influence their ability to survive when inoculated into an acidic food.

Finally, the use of genetic characterization tools may greatly aid the determination of which strains (if any) used in the challenge study are the most dominant over the length of the study. Tools such as ribotyping and pulsed-field gel electrophoresis can help distinguish among strains, whether inoculated or naturally occurring (Farber and others 2001). Challenge organisms may also be genetically modified to carry a marker that aids in distinguishing them from other strains and helps in their detection in the food matrix itself. Obviously, the genetically modified organism needs to have physiological characteristics comparable to the wild-type or parent strain.

For certain applications, surrogate microorganisms may be used in challenge studies in place of specific pathogens. For example, it usually is not possible to introduce pathogens into a processing facility; therefore, it is desirable to use surrogate microorganisms in those cases. An ideal surrogate is a strain of the target pathogen that retains all other characteristics except its virulence. In practice, however, many surrogates are closely related to but not necessarily the same species as the target pathogen. Traditional examples include the use of *Clostridium sporogenes* as a proxy for *Clostridium botulinum* in inoculated pack studies, *Listeria innocua* as a surrogate for *L. monocytogenes*, and generic strains of *Escherichia coli* as substitutes for *E. coli* O157:H7. Caution must be used with the latter, however, since generic strains of *E. coli* do not have the same level of acid resistance as *E. coli* O157:H7. Therefore, while it may be appropriate to use generic strains of *E. coli* in a challenge study to assess the impact of a heat process or preservative system in a high-pH food, it would be inappropriate to use these generic strains for evaluating acidic foods. Generally, surrogates are selected from a group of well characterized organisms and have the following desirable attributes (IFT 2000):

- Nonpathogenic.
- Inactivation characteristics and kinetics that can be used to predict those of the target pathogen.
- Behavior similar to the target pathogen when exposed to formulation and/or processing parameters (for example, pH stability, temperature sensitivity, and oxygen tolerance).
- Stable and consistent growth characteristics.
- Easily prepared to yield high-density populations.
- Once prepared, population remains stable until utilized.
- Easily enumerated using rapid, sensitive, and inexpensive detection systems.

- Easily differentiated from background microflora.
- Attachment characteristics that mimic those of the target pathogen.
- Genetically stable so that results can be replicated independently of laboratory or time of experiment.
- Will not establish itself as a "spoilage" organism if used in a production area.
- Susceptibility to injury similar to that of the target pathogen.

If a surrogate strain is to be used in a microbiological challenge study, preliminary work should be done to well characterize the strain before use in the study. Characteristics such as those discussed above should be determined and confirmed through preliminary laboratory work to assure that the surrogate strain is suitable for the intended purpose. The use of surrogates should be limited to only those cases where specific pathogens absolutely cannot be used for product or personnel safety reasons.

3. Inoculum level

The inoculum level used in the microbiological challenge study depends on whether the objective of the study is to determine product stability and shelf life or to validate a step in the process designed to reduce microbial numbers. Typically, an inoculum level of between 10^2 and 10^3 cells/g of product is used to ascertain the microbiological stability of a formulation. Higher inoculum levels may be appropriate for other products. Depending on the product formulation, some of the inoculum may die off initially before adapting to the environment. If too low of an inoculum level is used, the incorrect assumption could be made that the product is stable when it is not. Conversely, if the inoculum level is too high for this purpose, the preservation system or hurdles to growth may be overwhelmed by the inappropriate inoculum size, leading to the incorrect conclusion that the formulation is not stable. When validating a process lethality step such as heat processing, high pressure processing, or irradiation, however, it is usually necessary to use a high inoculum level (for example, 10^6 to 10^7 cells/g of product) to demonstrate the extent of reduction in challenge organisms. For example, in the United States, juice processors are now required to demonstrate a 5 log reduction of relevant hazardous microorganisms in their products (5 D performance standard). These log-reduction validation protocols usually require the use of plating methods. In order to measure this level of reduction within the statistical limits of the enumeration method, the inoculum level must be at least 10^6 CFU/g.

4. Inoculum preparation and method of inoculation

The preparation of the inoculum to be used in microbiological challenge testing is an important component of the overall protocol. Typically, for vegetative cells, 18 to 24 h cultures revived from refrigerated broth cultures or slants or from cultures frozen in glycerol are used. The challenge cultures should be grown in media and under conditions suitable for optimal growth of the specific challenge culture. In some studies, specific challenge organisms may be adapted to certain conditions. Such adaptation will be tailored to the specific food. For example, *E. coli* O157:H7 may be acid adapted with the appropriate acidulant prior to use in the challenge studies on acidic products. Bacterial spore suspensions may be stored in water under refrigeration or frozen in glycerol. Spore suspensions should be diluted in sterile water and heat-shocked immediately prior to inoculation. Spores of *C. botulinum* should be washed thoroughly prior to use to ensure that no free botulinal toxin is carried over into the product undergoing challenge testing, and, if possible, the spores should be heat-shocked in the food to be studied. Quantitative counts on the challenge suspensions may be conducted to aid in calculating the

dilutions necessary to achieve the target inoculum in the challenge product. Appropriate procedures and containment facilities should be used when carrying out challenge tests with certain pathogens.

The method of inoculation is another extremely important consideration when conducting a microbiological challenge study. Every effort must be made not to change the critical parameters of the product formulation undergoing challenge. There are a variety of inoculation methods that can be used depending upon the type of product being challenged. In aqueous liquid matrices such as sauces and gravies with high a_w (> 0.96), the challenge inoculum may be directly inoculated into the product with mixing, using a minimal amount of sterile water or buffer as a carrier. Use of a diluent adjusted to the approximate a_w of the product using the humectant present in the food minimizes the potential for erroneous results in intermediate a_w foods. In studies where moisture level is one of the experimental variables, the inoculum may be suspended in the water or liquid used to adjust the moisture level of the formulation. For batch type inoculations, the inoculum may be added directly to the product in a mixing bowl or container. For individual package or pouch type applications, the inoculum may be aseptically injected using a sterile syringe through the package wall containing a rubber septum. In solid matrices with $a_w > 0.96$, such as cooked pasta or meat surfaces, an alternative to the syringe method may be the use of an atomizer. An atomizer sprays the inoculum, which is suspended in sterile water or buffer, into the ground product or onto the surface of product. Spraying should be done in a containment hood or using other protective devices to avoid worker safety issues related to creation of pathogenic. In all these applications, the smallest amount of water or buffer practical for suspension of the inoculum should be used. Inoculum may also be transferred using a velvet pad, paint pad, or similar fibrous cloth provided the method is calibrated and reproducible levels of inoculum can be delivered with minimum moisture transfer. Preliminary analyses should be done to ensure that the a_w or moisture level of the formulation is not changed after inoculation.

Products or components with $a_w < 0.92$ may be inoculated using the atomizer method with a minimal volume of carrier water or buffer. Again, the product should always be checked to ensure that the final product a_w or moisture level has not been changed. A short, post-inoculation drying period for some products may be needed prior to final packaging. Alternatively, they may be inoculated with challenge organisms that have been suspended in carrier water or buffer that has been added to sterile sand, flour, or a powdered form of the product (for example, dried pasta), and allowed to dry. Lyophilized culture may also be used for some applications. Inoculum viability and population levels should be determined in advance of the study. The dried inoculum preparation should be added aseptically to the test product and shaken or agitated thoroughly for even distribution of the inoculum.

Enough product should be inoculated so that a minimum of three replicates per sampling time is available throughout the challenge study. In some cases, such as in certain revalidation studies and for uninoculated control samples, fewer replicates may be used.

5. Duration of the study

It is prudent to conduct the microbiological challenge study over, at least, the desired shelf life of the product. It is even more desirable to challenge the product for its entire desired shelf life plus a margin beyond the desired shelf life because it is important to determine what would happen if users would hold and consume the product beyond its intended shelf life. Some regulatory agencies require a minimum of data on shelf life plus at least one-third of the intended shelf life.

Another consideration impacting the duration of the challenge study is the temperature of product storage. Refrigerated products may be challenged for their entire shelf life under the target storage temperature, but under abuse temperatures they are typically held for shorter time.

In certain foodservice venues, it may be convenient for the food establishment to hold specific refrigerated products at room temperature for short periods of time. For example, some fast food operations may find it convenient to hold processed cheese slices at room temperature for up to 8 h. This allows the cheese to temper and melt faster when preparing food items such as hot sandwiches. However, pathogens may be present on the cheese slices due to cross-contamination through handling in the restaurant, and therefore challenge testing will be needed to provide evidence that this practice is safe. If the restaurant would like to hold the cheese slices at room temperature for an 8 h shift, the duration of the challenge study should be at least 12 h. This challenge study is performed to ensure that the rapid growth of pathogens does not occur if the cheese slices are cross-contaminated in the restaurant through handling.

It is also desirable to test the product over and significantly beyond its entire shelf life because sublethal injury may occur in some products. This can lead to a long lag period, where it may not be possible to culture the inoculum, but over time, a small number of the injured cells recover and grow in the product. This rebound, or "Phoenix" phenomenon, has been observed in a number of products (Jay 1996). If the product is not tested for at least its entire shelf life, it is possible to miss the recovery and subsequent growth of the challenge organism late in its shelf life.

The frequency of testing is governed by the duration of the microbiological challenge study. It is desirable to have a minimum of 5 to 7 data points over the shelf life in order to have a good indication of the inoculum behavior. Typically, if the shelf life is measured in days, the frequency of testing should be at least daily, if not multiple times per day. If the shelf life is measured in weeks or months, the test frequency is typically no less than once per week. All studies should start with "zero time" testing, that is, analysis of the product right after inoculation. For some types of products, it may be desirable to also allow an equilibration period for the inoculum to adapt to the product before testing. It may be desirable to test more frequently (for example, daily or multiple times per day) early in the challenge study (that is, for the first few days or week), and then reduce the frequency of testing to longer intervals.

6. Formulation factors and storage conditions

When evaluating a formulation, it is important to understand the range of key factors that control its microbiological stability. Intrinsic factors such as pH, a_w, or preservative level may be key to preventing the growth of pathogens or to preventing spoilage that would influence the safety of the product during its intended shelf life. It is, therefore, important to test each key variable singly and/or in combination in the formulation under worst-case conditions. For example, if the target pH is 4.8 ± 0.2 and the process capability is within that tolerance range, it is important to challenge the product on the high side of that range (that is, pH 5.0). Similarly, if sorbic acid is used at a level of 0.15 ± 0.05%, the product should be challenged at the low concentration of 0.10%. This is recommended to ensure that the challenge study covers the process capability range for each critical factor in the formulation. Relevant intrinsic properties such as pH, a_w, and salt level should be documented for each study for future comparison and reference.

Test samples should ideally be stored in the same packaging as

intended for the commercial marketplace. If the commercial product is vacuum- or MAP-packaged, then the samples used in the microbiological challenge study should be packaged under the same conditions using the same packaging film.

The storage temperature used in the microbiological challenge study should include the typical temperature range at which the product is to be held and distributed. A refrigerated product that may be subject to temperature abuse should be challenged under representative abuse temperatures. Products that may encounter high humidity environments should also be challenged under those conditions (Notermans and others 1993). Some challenge studies may incorporate temperature cycling into their protocol. For example, the manufacturer may distribute a refrigerated product under well controlled conditions for a portion of its shelf life, after which the product may be subjected to elevated temperatures immediately prior to and during use.

7. Sample analysis

Typically, in a microbiological challenge study, the levels of live challenge microorganisms are enumerated at each sampling point. Usually, it is desirable to have at least duplicate and, preferably, triplicate samples for analysis at each time point. In cases where higher levels of certainty are needed, a larger number of replicates should be used or the study should be replicated. The selection of enumeration media and method (for example, direct plating versus Most Probable Number) is dependent on the type of pathogens or surrogates used in the study. If the product does not have a substantial background microflora, nonselective media for direct enumeration may be used. In cases where toxin-producing organisms are used (for example, *Staphylococcus aureus or C. botulinum)*, appropriate toxin testing should be performed at each time point using the most current validated method. Toxin levels may not always be tested at each time point in the study, but should be done at frequent enough intervals throughout the desired shelf life of the product to determine if that shelf life is acceptable. Where appropriate, resuscitation methods may be used to avoid erroneous results.

It is prudent to analyze the product, including uninoculated control samples, at each or selected sampling points in the study to see how the background microflora is behaving over product shelf life. For example, if a product has a high background microflora, it may suppress the growth of the challenge inoculum. In some cases, this is useful and desirable because the product spoils before pathogens can grow. In other situations, the background microorganisms may not be universally present, leading to a potentially false sense of security. Also, under some circumstances, the background microorganisms can change the formulation parameters in the product to favor or inhibit growth of the inoculum over time (for example, molds can raise product pH; lactobacilli can decrease product pH).

It is also important to track pertinent physicochemical parameters of the product over shelf life to see how they might change and influence the behavior of the pathogen. Understanding how factors such as a_w, moisture, salt level, pH, MAP gas concentrations, preservative levels, and other variables behave over product shelf life is key to understanding the microbiological stability of the product.

8. Data interpretation

Once the microbiological challenge study is completed, the data should be analyzed to see how the pathogens behaved over time. Trend analysis and appropriate graphical plotting (that is, semi-log plots) of the data will show whether the challenge organisms died, remained stable, or increased in numbers over time. In

the case of toxin-producing pathogens, no toxin should be detected over the designated challenge period. Combining the quantitative inoculum data for each time point with data on the background microflora and the relevant physicochemical parameters gives a powerful and broad representation of the microbiological stability of the formulation under evaluation. Based on these data, a reasonable shelf life can be established or adjustments can be made to the formulation so that it is less susceptible to pathogen growth.

When using microbiological challenge testing, as part of a process validation protocol, analysis of the data will show whether the process is capable of delivering the required level of lethality (that is, conforms with the predetermined performance standard). Based on this information, adjustments can be made to the process, if necessary, in order to meet the lethality requirements.

The data from microbiological challenge testing can be used in developing predictive microbiological models or in validating existing ones. Predictive models are computer-based programs that simulate or predict how specific microorganisms will behave in a formulation under specific conditions (for example, pH, a_w, moisture, salt, and preservatives). Microbiological challenge tests are used both to generate these types of empirical models and to validate their applicability.

Overall, well designed challenge studies can provide critical information on the microbiological safety and stability of a food formulation. They are also invaluable in validating key lethality or microbiological control points in a process. Challenge studies can be an invaluable aid in determining if a food product requires temperature control throughout its shelf life or if it can tolerate storage at room temperature for a portion or all of its shelf life.

9. Pass/fail criteria

Selection of microorganisms to use in challenge testing and/or modeling depends on the knowledge gained through commercial experience and/or on epidemiological data that indicate that the food under consideration or similar foods may be hazardous due to pathogen growth. In addition, the intrinsic properties (for example, pH, water activity, and preservatives) and extrinsic properties (for example, atmosphere, temperature, and processing) should be considered. The significance of a population increase varies with the hazard characterization of each microorganism. For example, the growth of infectious pathogens should always be controlled, whereas most toxin production requires substantial growth before a hazard exists. In this case, growth of the toxigenic organism alone does not result in a health hazard, but toxin production will.

The following list identifies microorganisms that can be used in a microbiological challenge study along with the panel's recommendations and rationale for selection and assessment of tolerable growth.

Toxigenic molds such as *Aspergillus, Penicillium,* and *Fusarium* spp. Challenge studies related to the need for time/temperature control for safety are not recommended because mold provides a visual clue to prevent consumption of the spoiled product.

Bacillus cereus. The absence of toxin formation is the preferred criterion. However, since toxin measurement is difficult, a 3 log increase over inoculum levels would indicate the need for time/temperature control. This growth limit determination is based on the following:
- Typical initial levels of *B. cereus* are low; therefore, 1000 CFU/g would be a conservative initial level based on the literature.
- Populations of $> 10^6$ CFU/g are needed to produce toxin at levels hazardous to health (FDA 2001).
- The emetic toxin of *B. cereus* is heat stable; therefore, no reduction is likely.

Other considerations:

- *Bacillus cereus* spores are relatively heat sensitive. Baking is likely to destroy low levels found in flour used in bread products (Kaur 1986); however, unusual high heat resistance has been reported in pumpkin pie (Wyatt and Guy 1981). The potential for survival of *B. cereus* should be evaluated for specific products.

- Rice and potatoes have a history of association with *B. cereus* foodborne illness and therefore products containing rice or potatoes should be evaluated for time/temperature control requirements.

Campylobacter spp. No challenge testing is recommended because other organisms such as *Salmonella* have similar routes of contamination, are less fastidious, and are easier to culture. Furthermore, its minimal growth temperature and water activity of 32 °C (90 °F) and 0.98, respectively, make *Campylobacter* spp. an unlikely candidate for challenge studies.

Clostridium botulinum. The absence of toxin formation based on current methodology is the recommended requirement. Other considerations: *C. botulinum* is appropriate to consider for certain cooked products, particularly those packaged under anaerobic and micro-aerophilic conditions such as MAP products; and those with a history of associated illness, such as products under oil and baked potatoes.

Clostridium perfringens. A 3-log increase is recommended based on the following facts:

- Although other products may contain surviving spores, *Clostridium perfringens* is relevant mainly to meat and poultry products, including sauces and gravies. Most products subject to the Food Code requirements will be either raw or freshly cooked.

- Vegetative cells of *C. perfringens* are easily destroyed by cooking meat and poultry products, and spore levels are typically low due to demanding sporulation requirements. An initial population of 100 CFU/g was considered to be a conservative worst case by the panel. A population of $>10^5$ CFU/g is needed to result in illness; therefore, a 3-log increase would control the hazard.

Enterohemorrhagic E. coli. If modeling programs are used to predict the growth of the pathogen, time/temperature holding conditions should maintain enterohemorrhagic *E. coli* in lag phase due to the infectious nature of the microorganism. However, if laboratory challenge studies are used, the inherent variability in quantitative methods necessitates the use of a progressive increase of < 1 log as indicative that growth is controlled.

Listeria monocytogenes. Recent risk assessments (FDA/USDA 2001) indicate that low numbers of *L. monocytogenes* present a low risk to public health. In recognition of this, some countries such as Canada and Germany have established a tolerance for low levels of this organism in certain ready-to-eat foods that will not support growth to high levels. However, a tolerance for *L. monocytogenes* has not been established in the United States for these types of foods. It is also recognized that products that support the growth of the microorganism present an increased risk. A *L. monocytogenes* level of 100 CFU/g at the time of consumption may provide an acceptable level of consumer protection (Ross and others 2000). However, data are insufficient to determine general worst-case initial levels. Overall, the panel concluded that a 1 log increase was an appropriate level of control for *L. monocytogenes.* This level accounts for variability in enumeration techniques and represents a view that growth of this organism to high levels represents a risk to public health that must be controlled.

Salmonella spp. Appropriately validated pathogen modeling programs for growth can be used to verify that *Salmonella* spp. is maintained in the lag phase. Otherwise, population growth should be limited to < 1 log, following the same rationale as for enterohemorrhagic *E. coli.*

Shigella spp. No challenge studies are recommended for *Shigella* spp. because it has the same potential source as *Salmonella* spp. and has more fastidious growth and survival requirements.

Staphylococcus aureus. No detectable toxin should be formed under the time/temperature studies evaluated. As with *C. botulinum*, current methodology should be used for toxin detection and specific toxin levels should be determined. In lieu of testing for toxin, limiting growth to < 3 logs may be used. This limiting growth level is based on an initial population of 1000 CFU/g, and a minimum of 10^6 CFU/g to produce toxin.

Other considerations: *Staphylococcus aureus* is appropriate to study in foods that receive extensive handling because of the human source of the microorganism. *S. aureus* does not compete well with other microorganisms; therefore, it is not appropriate to consider in foods with high levels of other organisms, such as raw vegetables or properly fermented products.

Vibrio spp. Appropriately validated pathogen modeling programs for growth can be used to verify that *Vibrio* spp. are maintained in the lag phase. Otherwise, population growth should be limited to < 1 log, following the same rationale as for *E. coli.*

Vibrio parahaemolyticus can be used as a surrogate for other *Vibrio* spp. *V. parahaemolyticus* studies are only appropriate for marine foods. It should also be noted that most fish are highly perishable and therefore will be temperature controlled for spoilage reasons.

Yersinia enterocolitica. Challenge studies are not recommended as *Salmonella* spp. and *Y. enterocolitica* have similar sources and salmonellae are easier to culture.

References

Curiale MS. 1991. Shelf-life evaluation analysis. Dairy Food Environ Sanit 11(4):364-9.

Farber JM, Gendel SM, Tyler KD, Boerlin P, Landry WL, Fritschel SJ, Barrett TJ. 2001. Chapter 11: Molecular typing and differentiation. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington (DC): American Public Health Assoc.

[FDA] Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. The "Bad Bug Book" [Foodborne pathogenic microorganisms and natural toxins handbook]. http://www.cfsan.fda.gov/~mow/intro.html. Accessed 2001 Dec 10.

[FDA/USDA] Food and Drug Administration, Center for Food Safety and Applied Nutrition; U.S. Dept. of Agriculture, Food Safety and Inspection Service. 2001. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. http://www.foodsafety.gov/~dms/lmrisk.html. Accessed 2001 Dec. 13.

[IFT] Institute of Food Technologists, Dept. of Science and Technology Projects. 2000. Special supplement: Kinetics of microbial inactivation for alternative food processing technologies. Barach JT, Barbosa-Canovas GV, Busta FF, Datta AK, Davidson PM, Farkas DF, Heldman DR, Hoover DG, Kokini JL, Pflug IJ, Pierson MD, Sastry SK, Schaffner DW, Zhang QH, editors. Chicago: IFT. [108] p. (Journal of Food Science; vol. 65, no.8, suppl).

Jay JM. 1996. Modern food microbiology. 5th ed. New York: Chapman & Hall. p???

Kaur P. 1986. Survival and growth of Bacillus cereus in bread. J Appl Bacteriol 60:513-6.

Notermans S, Veld P, Wijtzes T, Mead GC. 1993. A user's guide to microbiological challenge testing for ensuring the safety and stability of food products. Food Microbiol 10(2):145-57.

Ross T, Todd E, Smith M. 2000. Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods: preliminary report for joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. Rome: Food and Agriculture Organization of the United Nations. Report nr MRA 00/02. 242 p.

Vestergaard EM. 2001. Building product confidence with challenge studies. Dairy Food Environ Sanit 21(3):206-9.

Wyatt CJ, Guy VH. 1981. Incidence and growth of *Bacillus cereus* in retail pumpkin pies. J Food Prot 44(6):422-4.

Chapter VII

Comparison of NSF and ABA Protocols to Determine Whether a Food Requires Time/ Temperature Control for Safety

1. Introduction

Both the American Bakers Association (ABA) and the NSF International (NSF) have written protocols describing microbiological testing to determine whether certain foods require time/temperature control for safety (ABA 2000; NSF 2000). The ABA document is strictly devoted to testing pumpkin pie, while the NSF document addresses breads with vegetables or cheese added before baking, breads filled after baking, pies filled before baking, and toppings destined for use in other products. The ABA protocol can be obtained by calling ABA at 1-202-789-0300. The NSF protocol may be ordered by calling NSF at 1-800-NSF-MARK or via the website at www.nsf.org.

Both the ABA and the NSF testing protocols suffer from significant weaknesses that hamper their usefulness in determining whether a food can be safely stored at room temperature. The NSF protocol takes an overly stringent approach, whereas the ABA protocol is sometimes overly permissive. The two most significant differences between the two protocols are (1) the consideration—or lack of consideration—of the process the food did or will undergo, and (2) the selection of microorganisms used or not used to inoculate the food. Table 1 of this chapter presents a comparison between the features of the testing protocols, including the protocol developed by the panel (see Chapter 6).

2. Consideration of process

A significant difference between the two protocols is the consideration given to the processing method in the ABA protocol and the lack of consideration of process in the NSF protocol. A given process/packaging combination may serve to eliminate a particular pathogen from a food product. The post-process reintroduction of this pathogen in a challenge test may represent an artificial situation and not what may actually happen. A challenge test that inoculates a pathogen into a processed food may be unduly challenging if post-processing contamination is not likely. It should be noted that some non-PHF foods on the market today might not be able to pass such stringent test criteria. For example, while currently excluded from consideration as a PHF under the NSF protocol, if required to undergo the NSF protocol, white bread might not be able to pass such test criteria despite a well established safety record.

3. Microorganisms used

A second significant difference between the two protocols is the use of an inoculum. The ABA protocol uses only the natural microflora present in the product, and requires testing for aerobic plate count (APC), coliforms, *Staphylococcus aureus,* and *Salmo-*

nella spp. The NSF protocol requires the use of five strains each of *Bacillus cereus, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* spp., *S. aureus,* and *Clostridium perfringens,* depending on the pH and a_w of the product. The advantage of the ABA approach is its simplicity, but it is probably too simple, and relies on natural or accidental contamination events occurring in those batches of product produced for testing in order to detect a problem. Let us assume a product being produced for evaluation by the ABA protocol is inappropriately handled such that it is recontaminated with *S. aureus* on a recurring but infrequent basis (for example, 1/100 containers).

The ABA protocol will not appropriately evaluate this potential problem since the product used for the study is not deliberated inoculated and the chance of using the contaminated product is very low (for example, 1/100). If thousands of containers are produced on a daily basis, this may be enough to present a health concern to consumers.

The NSF protocol can be criticized from the opposite standpoint: it is unduly stringent. A food must be inoculated with the appropriate pathogens among those listed above, depending on the pH and a_w of the food. Inoculation is required even if none of the pathogens are commonly found in any of the product ingredients, or if one or several would be eliminated by processing. Both approaches, however, suffer from the lack of inclusion of *Clostridium botulinum* as a test organism. Inclusion of *C. botulinum* in a challenge study greatly increases its cost and complexity, but with these increases there is a concomitant increase in confidence that the appropriate organism is being used. While the NSF protocol includes *C. perfringens* for certain products due to concerns in baked goods, it is not meant to be used as a surrogate for *C. botulinum.* The panel agrees that because these organisms differ in cold sensitivity, heat resistance, rate of growth at various temperatures, oxygen tolerance, and toxin mode of action, *C. perfringens* should not be used as a surrogate for *C. botulinum.*

4. Pass/fail criteria

Given the differences in microbial testing between the ABA and NSF protocols, differences in pass/fail criteria are expected. A product will fail the ABA protocol if it contains detectable *S. aureus, Salmonella* spp. or coliforms, or if it contains more than 1000 CFU/g within 24 h of packaging, or more than 100,000 CFU/g at the end of its shelf life. Testing for the presence of pathogens in an uninoculated product is not sufficient to determine whether the product requires time/temperature control for safety. Aerobic plate count (APC) data may be useful in determining product quality during shelf life, but these data are of limited value as indicators of safety. APCs may be useful if there is enough

Table 1—Summary of comparison of NSF, ABA and expert panel protocols to determine if a food requires time/temperature control for safety.

Item	ABA	NSF	Panel's Alternative Protocol
Type of product	Pumpkin pie	Four groups: bread with vegetables and cheese pre-bake, filled post-bake, filled pre-bake, toppings. Traditional and other products excluded.	Any food product proposed to be stored outside temperature control.
Consideration of process	Yes (Good Manufacturing Practices, [GMPs], baking temperature, cooling, and packaging)	No	Yes. Additional information for validation of process also required.
Microorganisms tested	Aerobic Plate Counts (APC), *Staphylococcus aureus*, coliforms, *Salmonellae*	*Bacillus cereus, Escherichia coli* O157:H7, *Listeria monocytogenes*, Salmonella spp., *S. aureus*, *Clostridium perfringens*, depending on pH and a_w	Organisms should be selected based on history of safety, formulation, storage atmosphere environment and packaging of the food.
Inoculation type	None (indigenous only)	Composite of 5 strains of each organism. Each composite inoculated into the product separately.	Composite of multiple strains of each organism. Each composite inoculated into the product separately.
Inoculation method	Not applicable	Prescribed in phosphate buffer.	Prepared in system that mimics the product: Previously mixed with buffer or water, directly added to product, aseptically injected, mixed powder product, or lyophilized, depending on the product
Inoculum preparation	Not applicable	Aerobes cultured in tryptic soy broth, *C. perfringens* cultured in fluid thioglycolate broth	Cultures grown in suitable media under either optimal or food-adapted conditions. Spores are washed and heat-shocked before or after inoculation.
Inoculum position	Not applicable	Each unique component and each unique interface between components at both internal and external surfaces	Each component, and each unique interface between components, but only where the organisms of concern would survive the process or be reintroduced post-processing.
Inadvertent product modification	Not applicable	Addition of the inoculum in buffer has a potential to change product water activity.	Additional measurements of a_w should be taken to insure that inoculation technique does not influence product a_w.
Inoculum technique	Not applicable	No consideration for relative component weights given when splitting inoculum between components	Not applicable

(Continued on next page)

product history to suggest that no, or minimal, increases in APC always indicate no pathogen growth, and all pathogens of concern would be counted on APC. Conversely, an increase of APC on uninoculated product does not indicate that pathogens could not grow if present.

Failure criteria for the NSF protocol are based on increases in counts of any of the six pathogens tested. A food fails in the NSF protocol if it supports more than 1 log CFU/g increase by the end of its shelf life, or more than a 1 log CFU/g increase for any two consecutive time points. The 1 log CFU/g increase criterion for infectious pathogens is appropriate for two different reasons. First, a 1 log CFU/g increase is likely to be detected in spite of the inherent variability known to exist in microbial testing methods today. To use a smaller value might invite concerns regarding whether a particular increase was of statistical significance. Second, given our current level of understanding regarding human dose-response for enteric pathogens, a 1 log CFU/g increase probably constitutes a measurable increase in risk. It is not clear why the NSF protocol allows a 1 log CFU/g increase that subsequently declines during the shelf life, but does not allow a 1 log CFU/g increase that is observed at two time periods, except perhaps to allow for inherent sample and analytical observation variations. The NSF protocols are too stringent with respect to *S. aureus, B. cereus* or *C. perfringens* because a 1 log CFU/g increase in any of these organisms is unlikely to result in a public health concern (see discussion in Chapter 6).

5. Number of sampling times

Sampling time differences also exist between the two protocols. Neither of the protocols proposes the presence of toxin in the food as a valid criterion. The ABA protocol advocates microbiological testing at only two times (within 24 h post bake and at the end of shelf life) while the NSF protocol advocates 1 to 10 testing times depending on shelf life. The appropriate number of test observation times is dependent upon the failure criteria. If the failure criterion is detectable toxin, it may be sufficient to simply test a suitably inoculated product at the end of its shelf life. If no toxin is

Table 1—(*Continued from previous page*) **Summary of comparison of NSF, ABA and expert panel protocols to determine if a food requires time/temperature control for safety.**

Item	ABA	NSF	Panel's Alternative Protocol
Sampling times	Two: within 24 h of packaging, and at end of shelf life	One to ten: depending on intended shelf life	A minimum of 5 times over shelf life, including zero time.
Pass criteria	No pathogens detected, APC less than 1,000 CFU after bake, and less than 100,000 CFU at end of shelf life	Less than 1 log CFU increase for any pathogen by the end of the study and not to exceed 1 log CFU for any pathogens at two consecutive time points before the end of the study.	Depending on the pathogen, less than 1 or 4 log increase at any point in shelf ife for vegetative pathogen(s) of concern and no detectable toxin at the end of the shelf life for toxin-forming microbes.
Other tests	Oxidation/Reduction potential, pH, a_W	pH, a_W	pH, a_w, pH/aw interaction
Process	Process is considered by use of natural inoculum.	Process is not considered, since pathogens are inoculated into the food after processing.	Process should be considered in the selection of appropriate microbes for use in the challenge study. Data to validate the process should be provided.
Methods	Association of Official Analytical Chemists/Bacteriological Analytical Manual	Compendium of Methods for the Microbiological Examination of Foods	Any reproducible, validated method is acceptable.
Duration of study	The study lasts until the use by date, which is calculated by multiplying 1.3 times the sell by date.	The study lasts 1.3 times the time the products will be out of temperature control.	The study should last for at least the shelf life of the product, but 1.3 times the intended shelf life is recommended.
Spoilage	Addressed indirectly with APC	Not applicable	Testing of inoculated sample for background bacteria
Replication	6 samples at beginning and 6 at end of one production run	3 lots, 2 samples/lots, over shelf life	Minimum of 3/sampling time unless this is a revalidation study or control sample (less samples are needed)
Anaerobes	Only an O/R potential measurement is made, no microbial tests are done.	*C. perfringens*	*C. botulinum* itself is used, with toxin production as the definitive measure of safety.
Microbial growth modeling	Not applicable	Not applicable	Properly validated growth models can be used alone or in combination with microbial challenge studies.
History of safe use	Not applicable	Not applicable	A long history of safe use can be considered in combination with appropriate scientific rationale instead of challenge studies.

detected, the product passes the challenge test. It might also be appropriate to sample at additional times and adjust the shelf life of the product such that toxin production does not occur during this time. The appropriate number of observations in challenge tests with vegetative cells is significantly more complex. A product should be tested at a sufficient number of time points to insure that a one log CFU/g increase has not occurred. It should also be noted that tests must continue until the end of the test period, even if the inoculated organism declines below the level of detection, to insure against the "Phoenix" phenomenon (Jay 1996).

6. Replication

Both protocols require six replicates: all from one production run for the ABA protocol; two samples each from three lots for the NSF protocol. The decision about the appropriate number of samples and lots must be based on the characteristics of the food and microbes in question, but two samples each from three lots is probably a reasonable minimum. In worst-case scenarios and considering variation and process capability and tolerance, it may be more appropriate to test a greater number of random samples from each lot.

7. Oxidation-reduction potential

The ABA protocol requires evaluation of oxidation-reduction potential (Eh) as a means of controlling risk of *C. botulinum,* whereas the NSF protocol does not. While the ABA protocol proposes a stringent value for Eh (+100 mv or greater), there are still some important limitations to this approach. Eh values of +100mv or greater are not inhibitory to *C. botulinum* type E. Although this organism is not expected to be found in pumpkin pie, it might be encountered in marine foods that require time/temperature control for safety. Eh is also notoriously difficult to measure accurately, and erroneous measurements may lead to a false sense of security. Finally, the Eh of the micro-environment may not be reflected by standard measurements. If *C. botulinum* is a concern, the only reliable means of determining the safety of a particular food are challenge studies using this organism.

8. Methodology

The ABA protocol advocates the use of FDA's *Bacteriological Analytical Manual* (BAM) and Association of Official Analytical Chemists (AOAC) methodology, while the NSF protocol uses the *Compendium of Methods for the Microbiological Ex-*

amination of Foods. The differences between these methodologies are largely inconsequential. It is critical not that one method be used over another, but that some reproducible, commonly accepted, and widely used method be employed. AOAC, BAM, and the Compendium methods all satisfy this requirement.

9. Inoculum

Since the ABA protocol does not use inoculated organisms, the question of inoculum preparation and position is irrelevant. The NSF protocol takes an overly stringent approach by requiring each component and each unique component interface to be inoculated. This requirement ignores the fact that in many cases a properly processed product should not contain contamination with vegetative cells on any internal surfaces. The NSF approach, however, may be appropriate for products in which post-processing contamination may occur at internal surfaces. Other problems include the use of a phosphate buffer that may modify the food microenvironment, and the use of high levels of challenge microbes that could locally overwhelm the preservative system.

10. Duration of test

The two protocols use similar criteria to establish the duration of the test. The ABA protocol tests the product up to the "use by" date, which is 1.3 times the "sell by" date, while the NSF protocol requires that a test last 1.3 times as long as the time period that the product will be outside temperature control. A useful and valid test protocol should last slightly longer than the time period of concern. In the absence of any scientifically valid documentation on this matter, 1.3 times as long as the time period that the product will be outside a temperature control seems as reasonable to use as any criterion.

11. Product categories

Neither protocol addresses all of the product categories the panel was asked to consider by the FDA. The ABA document has a narrow focus (evaluation of pumpkin pie), while the NSF document is somewhat broader (evaluation of breads with vegetables or cheese added before baking, breads filled after baking, pies filled before baking, and toppings destined for use in other products). Neither protocol includes such food items as cheeses or fruits and vegetable products. A testing protocol should be flexible and robust enough to use with any food product where safety out of time/temperature control is questioned. However, a universal protocol may be impossible to develop. In some instances, different challenge study protocols will need to be used for different foods. A well thought-out generic protocol should satisfy the desired criteria of flexibility and robustness to the greatest extent possible.

12. Summary

Both the ABA and NSF protocols have some significant weaknesses. An alternative protocol that considers the complementary strengths and weaknesses of the ABA and NSF methods, with the few minor additions noted above, can be used to determine which foods require time/temperature control for safety. The panel's recommendations, summarized in Table 1, can be seen as an alternative protocol.

References

[ABA] American Bakers Association. 2000 Jan 18. Industry protocol for establishing the shelf stability of pumpkin pie [final version plus executive summary]. Washington (DC): ABA. 18 p. Available from: lsanders@americanbakers.org.

Jay JM. 1996. Modern food microbiology. 5th ed. New York: Chapman & Hall. Chapter 10, Culture, microscopic, and sampling methods.

[NSF] NSF International. 2000 Nov. 10. Non-potentially hazardous foods. Ann Arbor (MI): NSF International. Report nr ANSI/NSF 75-2000. 12 p.

Framework Developed to Determine Whether Foods Need Time/Temperature Control for Safety

1. Description of framework

The variety and novelty of the foods currently available to consumers has resulted in a complex situation when determining whether a food needs time/temperature control for safety. Although there are many foods that need time/temperature control for safety (TCS), other foods require specific evaluation in order to determine their status as TCS or non-TCS foods. To facilitate the decision as to whether a food needs time/temperature control for safety, the panel developed a framework based on: in-depth evaluation of criteria used by industry, government, and trade organizations; survey data collected by the panel (see Appendix B); available scientific literature; and the panelists' own experience on this subject. The framework provides a stepwise process that considers holding time and temperature, product description, pH and a_w interaction, product assessment, challenge testing, and mathematical models. Decisions as to whether or not a food should be designated as TCS can be made at various steps of the framework. Performing the initial steps requires only limited experience and/or minimum training, while subsequent steps require knowledge of the product's pH and a_w. More technical expertise is needed for the analysis step which is based on product assessment, challenge studies, and predictive modeling. If it is determined that the product needs (or may need) time/temperature control for safety, a number of alternatives are presented in the framework that might be considered. For example, a decision might be made that a challenge study is so costly that the best alternative is to reformulate the product or control the time or temperature.

The following is a description of the proposed framework that the panel has developed to determine whether a food needs time/temperature control for safety (see section 2 of this chapter).

Before proceeding with Step 1 of the evaluation process, the evaluator needs to make a succinct review of the food product in question, including intrinsic and extrinsic factors that may affect microbial growth and potential hazards. (Detailed descriptions of factors and potential hazards that will help with this review are presented in Chapters 3 and 4.) The food may already be held hot or cold for safety reasons. In this case, and if there is no desire to store the food at ambient temperature, the trained decision-maker need not proceed any further. Product history, in combination with a robust scientific rationale that justifies such safe history of use, may also be used as criteria to designate a food as a non-TCS food not requiring further evaluation (see also Chapter 3, section 4.2.).

Step 1. The panel concluded that the appropriate scientific evidence exists to allow for the evaluation of a food according to its pH, water activity, and pH/a_w interaction. The panel also agreed that a product that is processed to eliminate vegetative cells needs to be addressed differently than an unprocessed product that received no treatment or a less robust treatment. The concern of possible post-process contamination also needs to be addressed. If a food is processed to inactivate bacteria and packaged so that there is no post-process contamination, the tolerable range conditions of a_w and pH are more permissive, since spores would become the only microbial hazard. For these reasons, the panel designed two pH/a_w tables: one for the control of spores (Table A), and one for the control of spores and vegetative cells (Table B). The rationale for the ranges of pH and a_w in determining whether a food is non-TCS versus TCS is based on minimum pH and a_w requirements for the pathogens of concern; that is, *Bacillus cereus* and *Clostridium botulinum* toxin production when controlling spores, and *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp, *C. botulinum*, and *B. cereus* when controlling both vegetative cells and spores (see Chapter 3, sections 2.1. and 2.2. and Appendix C). If process technologies other than heat are applied, then the effectiveness of the process needs to be validated. For this decision, the evaluator needs to have an understanding of both the process and the validation of its effectiveness in reducing pathogens of concern. It should be noted that for some products, the analysis of pH and a_w may be inaccurate, especially in the case of combination products (see Chapter 4, section 10). Consequently, for these products the pH and a_w would not be considered as controlling factors without supporting data from challenge studies.

Step 2. After the product's assignment to a box inside one of the tables, if the product is designated as non-TCS, it may be safely stored at room temperature. If the product is placed in a box indicating with a question mark (?) that it may require temperature control for safety, an analysis may be performed to assess the microbial risk of holding the product at ambient temperature. The evaluator may also decide not to perform the analysis, in which case the time and temperature of the product should be controlled for safety.

Product assessment. A comprehensive description of the product is the first task in this product assessment. This entails a detailed description of such factors as (1) potential pathogens, (2) intrinsic factors (for example, preservatives, antimicrobials, humectants, acidulants, and nutrients), (3) extrinsic factors (for example, packaging, atmosphere (MAP), use/shelf life, and temperature range of storage and use), (4) effectiveness of the processing for control of pathogens, and (5) possible post-process recontamination opportunities that may be present. If any of the factors precludes the growth of pathogens (for example, acetic acid as an acidulant at a reasonably low pH), the product may be designated non-TCS. Historical information regarding product safety should be considered by determining whether the food in question, or

any of its ingredients, has been previously implicated as a common vehicle of foodborne disease after temperature abuse. Of particular importance are the microbiological agents that are responsible for illnesses associated with the food and the reported contributing factors that have led to documented illnesses. Has adequate temperature control been clearly documented as a factor that can prevent or reduce the risk of illness associated with the food? Lastly, product history alone should not be used as the sole factor in determining whether or not a food needs time/temperature control for safety, unless a scientific basis for such safe use could be rationalized. As intrinsic or extrinsic factors change (for example, MAP or greatly extended shelf life), historical evidence alone is not appropriate in determining potential risk. Therefore, for a product to be identified as non-TCS based on history, the intrinsic and extrinsic factors affecting microbial growth need to have remained constant, and a scientific rationale needs to have been provided for the product's safe use (see also Chapter 3, section 4.2.).

Microbial growth models and challenge studies. In addition to the usual considerations, time of expected storage and display might also play a significant role in determining the classification of the food. Foods that have combinations of pH, a_w, preservatives, or other factors that are restrictive (but not prohibitive) to microbial growth and/or toxin production may not require refrigeration to protect public health. For example, if the duration of storage and/or display is less than that needed for microbial growth and/or toxin production, adequate control may be achieved through a variety of time and temperature combinations. Under certain circumstances, time alone at ambient temperatures can be used to control product safety. These factors can be considered in light of the product assessment and the microbial hazards of concern. The following is an example of how storage or holding time alone at ambient temperatures could be used to control product safety. If the microbiological concern for a specific food is the growth of *S. aureus*, the USDA Pathogen Modeling Program v. 5.1 could be used to estimate the time of storage where pathogen growth could occur. Using Table 8-1 with data generated from the model, a product with an $a_w = 0.88$ and pH = 5.5 could be safely

Table 8-1—Time estimates required for 3-log growth of *Staphylococcus aureus* at various pH and water activities (a_w) based on the USDA Pathogen Modeling Program v. 5.1

a_w	PH			
	6.0	5.5	5.0	4.6
0.94	Hours	Hours	Hours	Hours
0.92	Hours	Hours	Days	Days
0.90	Hours	Days	Days	Weeks
0.88	Days	Weeks	Months	Months
0.86	Weeks	Months	Months	Months

Days = 2–13 days
Weeks = 13–60 days
Months = > 60 days

stored at ambient temperature for weeks, assuming *S. aureus* would be the only microbial concern.

It must be emphasized, however, that general growth models such as the USDA Pathogen Modeling Program must be restricted in use because of limitations of the model parameters, microorganisms of concern, or other factors. Consequently, unless used conservatively, it is often more appropriate to use them in combination with challenge testing. Nevertheless, a general model can assist, for example, in selecting pathogens of concern for a challenge test. In the absence of an appropriate model, a challenge test alone could be used to determine whether pathogens of concern could grow under specified storage conditions (see Chapter 6 for guidelines on challenge testing). On the other hand, if an in-house model has been developed and validated for a particular food, it could be used to make such an assessment by itself or with challenge testing. At this point, a final decision needs to be made about the product's need to be time/temperature controlled. If the hazard analysis indicates that the product should be designated as non-TCS, the product can be stored at room temperature. If, on the contrary, the product is identified as TCS, the evaluator can either decide to modify the product, change the processing and handling it undergoes, control pathogen growth with time/temperature, or revisit the commercial feasibility of the product.

(See "2. Framework for determining if time/temperature control is required for safety" on next page)

2. Framework for determining if time/temperature conrol is required for safety

The food in question may already be held hot or cold for safety reasons. In this case, and if there is no desire for ambient temperature storage, an analysis using this framework is not needed. If the need to control the temperature of the product for safety reasons is unknown, a review of the food, its ingredients, and general methods of preparation should precede the evaluation of the food. If the food, as described, has a substantial and extensive history of safe use without time/temperature control, and there is enough scientific rationale that supports such safe history of use, then the food may continue to be classified as not requiring temperature control for safety, or non-TCS (see also Chapter 3, section 4.2.).

If there is no known history of safe use, proceed with Step 1.

Step 1—Was the food treated to destroy vegetative cells of potential pathogens and packaged to avoid recontamination? If yes, position your product in Table A according to its pH and water activity (a_w). If not, position your product in Table B according to its pH and a_w.
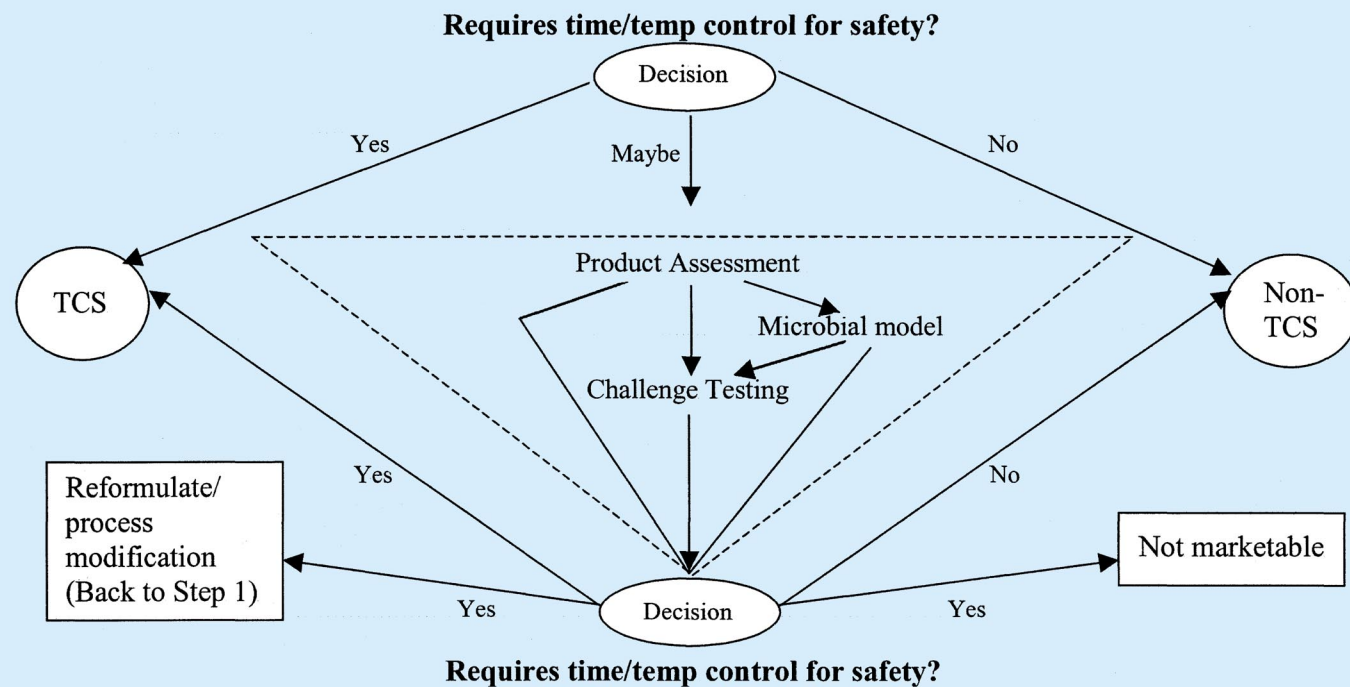
Table A—Control of spores: Product treated to control vegetative cells and protected from recontamination.

Critical a_w values	Critical pH values		
	4.6 or less	> 4.6 to 5.6	> 5.6
0.92 or less	Non-TCS	Non-TCS	Non-TCS
> 0.92 to .95	Non-TCS	Non-TCS	?
> 0.95	Non-TCS	?	?

Table B—Control of vegetative cells and spores: Product not treated or treated but not protected from recontamination

Critical a_w values	Critical pH values			
	< 4.2	4.2 to 4.6	> 4.6 to 5.0	> 5.0
< 0.88	Non-TCS	Non-TCS	Non-TCS	Non-TCS
0.88 to 0.90	Non-TCS	Non-TCS	Non-TCS	?
> 0.90 to .92	Non-TCS	Non-TCS	?	?
> 0.92	Non-TCS	?	?	?

Step 2—If the food is classified as a non-TCS food according to Step 1 above, it may be stored and held safely without regard to time or temperature. If the need for time/temperature control is questionable, the food should be held either hot or cold for safety, or subjected to a product assessment as the next step in determining the appropriate classification.

3. Critique of framework.
Application of framework to foods.

The panel's framework on time/temperature control of foods for safety was applied to the following foods as examples. Each step of the framework has been described as it applies to the food under consideration. Most of the data presented were from industry studies submitted to the panel in response to a survey of industry practices to determine whether a food needs time/temperature control (see Appendix B).

3.1. Salad dressings

Product: Viscous, non-particulate[1] pourable salad dressing. The product is not held hot or cold. The ingredients of the product are eggs, soybean oil, buttermilk, tomato paste, onion, garlic, spices, lemon juice, vinegar (2.5 – 5.4% salt), and potassium sorbate. Microbial hazards: *Clostridium botulinum*. The product is intended to be distributed and stored at ambient temperature for 7 to 9 mo. New product, so there is no history of use.

Step 1. Processing: Cold blended and filled in plastic or glass bottle. No heat applied.

Go to Table B.

Table: pH maximum of 4.2 and "high" (not specified) a_w.

Step 2. Decision: Product may be a temperature controlled for safety (TCS) food.

Product Assessment: Salad dressing is acidified with acetic acid. No microbiological hazard at pH 4.2.

Decision: Product is a Non-TCS.

[1]If salad dressing had particulate matter, then this product would need to be reevaluated.

3.2. Condiments: Mustard

Product: Viscous, non-particulate[1] mustard.

The product is not held hot or cold. The ingredients of the product are mustard seeds and vinegar (acetic acid). The product is intended to be distributed and stored at ambient temperature for extended shelf life. Microbial hazards: *Listeria monocytogenes, Salmonella* spp*., Escherichia coli* O157:H7, *C. botulinum.* There is history of safe use without time/temperature control[2].

Step 1. Processing: Ground and blended. Go to Table B.

Table: pH maximum of 4.0 and "high" (not specified) a_w.

Decision: Product is a Non-TCS.

[1] If mustard had particulate matter, then this product needs to be reevaluated.

[2] If pH of mustard was above 4.2 or if acidulant was not acetic acid, then this product would need to be reevaluated.

3.3. Butter

Example 1

Product: Salted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution product is held at low temperatures for quality reasons. The ingredients of the product are cream and salt. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There is no history of safety problems when the consumer does not control time/temperature of commercial salted butter.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 5.41 and a_w 0.897.

Step 2. Decision: Product may be a TCS food.

Product Assessment: Product characteristics prevent *L. monocytogenes* growth. Predictive model (p 8-3) suggests that holding the product for hours at ambient temperature is safe.

Decision: Challenge testing, predictive microbial model, reformulation to decrease a_w, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or product is not marketable.

Example 2

Product: Salted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution product is held at low temperatures for quality reasons. The ingredients of the product are cream and salt. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There is no history of safety problems when the consumer does not control time/temperature of commercial salted butter.

Step 1. Processing: Pasteurization of cream. Acidified by fermentation. No heat applied after butter is churned. Go to Table B.

Table: pH 4.25 and a_w 0.897.

Step 2. Decision: Product is a Non-TCS food.

Example 3

Product: Salted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution product is held at low temperatures for quality reasons. The ingredients of the product are cream and salt. The product is intended to be stored at ambient temperature. Microbiological hazard: *S. aureus, L. monocytogenes*. There is no history of safety problems when the consumer does not control time/temperature of commercial salted butter.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 5.94 and a_w 0.847.

Step 2. Decision: Product is a Non-TCS food.

Example 4

Product: Salted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution product is held at low temperatures for quality reasons. The ingredients of the product are cream, lactic acid bacteria, and salt. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There is no history of safety problems when the consumer does not control time/temperature of commercial salted butter.

Step 1. Processing: Pasteurization of cream. Acidified by fermentation. No heat applied after butter is churned. Go to Table B.

Table: pH 4.78 and a_w 0.863.

Step 2. Decision: Product is a Non-TCS.

Example 5

Product: Unsalted whipped butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution, the product is held at low temperatures for quality reasons. The ingredients of the product are cream and acidified natural flavoring. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There has been a report of unsafe handling of a whipped butter product.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 4.91 and a_w 0.921.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision: Challenge testing, predictive microbial model, reformulation to decrease a_w, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or product is not marketable.

Example 6

Product: Unsalted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution , the product is held at low temperatures for quality

reasons. The ingredients of the product are cream and natural flavoring. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There is no history of unsafe use without time/temperature control.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 5.98 and a_w 0.941.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision: Challenge testing, predictive microbial model, reformulation to decrease a_w, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or product is not marketable.

Example 7

Product: Unsalted butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution , the product is held at low temperatures for quality reasons. The ingredients of the product are cream and natural flavoring. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There is no history of unsafe use without time/temperature control.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned. Go to Table B.

Table: pH 5.42 and a_w 0.907.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision: Challenge testing, predictive microbial model, reformulation to decrease a_w, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or product is not marketable.

Example 8

Product: Salted light whipped butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution, the product is held at low temperatures for quality reasons. The ingredients of the product are cream, salt, water, tapioca, modified food starch, beta carotene, vitamin A, natural flavoring, lactic acid, vegetable mono and diglycerides, potassium sorbate, sodium benzoate. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There has been a report of unsafe handling of a whipped butter product.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 4.48 and a_w 0.985.

Step 2. Product may be a TCS food.

Product Assessment: Sodium benzoate and potassium sorbate may prevent pathogen growth.

Decision: Challenge testing, predictive microbial model, reformulation to decrease a_w, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or product is not marketable.

Example 9

Product: Salted whipped butter. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution, the product is held at low temperatures for quality reasons. The ingredients of the product are cream and acidified natural flavoring. The product is intended to be stored at ambient temperature. Microbiological hazards: *S. aureus, L. monocytogenes*. There has been a report of unsafe handling of a whipped butter product.

Step 1. Processing: Pasteurization of cream. No heat applied after butter is churned.

Go to Table B.

Table: pH 4.14 and a_w 0.822.

Step 2. Decision: Product is a Non-TCS food.

3.4. Margarine

Product: Margarine. The product is not held hot or cold for safety. However, during commercial handling, storage, and distribution, the product is held at low temperatures for quality reasons. The ingredients of the product are soybean oil (80%), water and milk protein (19%), salt (0.9%), and potassium sorbate (.1%). The product is intended to be distributed and stored at ambient temperature for 3 mo. Microbiological hazards: *S. aureus, L. monocytogenes*. There is history of safe use without time/temperature control.

Step 1. Processing: Emulsification of oil blend/water preservative mixture. No heat applied. Go to Table B.

Table: pH 4.8 and a_w unknown.

Step 2. Product may be a TCS food.

Product Assessment: Sorbic acid in formulation prevents pathogen growth. Historically product is safe and stable.

Decision: Product is a Non-TCS.

3.5. Garlic-in-oil[1]

Product: Garlic-in-oil. The product is not held hot or cold. The ingredients of the product are chopped fresh garlic and oil. The product is intended to be distributed and stored at ambient temperature for extended shelf life. Outbreaks have been associated with *C. botulinum* toxin in garlic-in-oil. Microbiological hazards: *C. botulinum* toxin production.

Step 1. Processing: Oil poured into chopped garlic in a bottle. Although no heat is applied, vegetative pathogens are not associated with this food. Go to Table A.

Table: pH > 4.6 and high a_w (not specified).

Step 2. Decision: Product may be a TCS food.

Product Assessment: No identified product characteristic that prevents spore-forming pathogen growth. Antimicrobial properties of garlic will prevent the growth of vegetative pathogens.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic or phosphoric acid to < 4.6, refrigerate (TCS food), store hot (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

[1]Flavored oil will present negligible hazard due to lack of *C. botulinum* survival or growth in 100% oil.

3.6. Cheeses

Example 1

Product: Cream cheese. The product is not held hot or cold during use. The ingredients of the product are milk, cream, salt, gums. The product is intended to be distributed and stored

at ≤ 7 °C (45 °F) for a maximum of 120 d. When in use, the tempered unopened product can be kept up to 48 h at ambient temperature. There is no history of botulism associated with cream-cheese products. Microbiological hazard: *C. botulinum.*

Step 1. Processing: Full fat, plain cream cheese, bulk packed and hot-filled > 68 °C (155 °F) in 3 lb/30 lb/ 50 lb tubs/blocks. Ready-to-eat after opening or baked. Go to Table A

Table: pH 4.7 to 5.1, a_w > 0.97.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic acid or phosphoric acid to < 4.6, keep refrigerated—that is, eliminate tempering at ambient (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: Separate products were inoculated with 100 – 500 spores/g of either proteolytic A & B or non-proteolytic B cocktails of *C. botulinum* and held at 30 °C (86 °F) for 10 d. No toxin was detected throughout the study. Conclusion is that the unopened product can be stored safely at ambient temperature for up to 7 d based on a safety factor of 1.3 times shelf life of the product. However, loss of product quality dictates storage at ambient temperature for no longer than 48 h. Without additional challenge studies on vegetative pathogens, opened product requires time/temperature control.

Example 2

Product: Process cheese sauce packed in 40 lb bag-in-box containers. The product is not held hot or cold during use. The ingredients of the product are cheddar cheese, milk, whey, milk fat, water, salt, sodium phosphate, sorbic acid, artificial color. The product is intended to be distributed and stored at ≤ 7 °C (45 °F) for a maximum of 9 mo. The tempered unopened product can be kept 24 h at ambient temperature in foodservice establishments prior to use. New product, so there is no history of use. Microbiological hazards: C. *botulinum.*

Step 1. Processing: Heated to 85 °C (185 °F) for 1 to 2 min and hot-filled at 68 to 69 °C (155 to 165 °F) into bag-in-box containers. Ready-to-eat or heated prior to consumption. Go to Table A.

Table: pH 5.7 (target) and a_w > 0.95.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No apparent product characteristic that prevents spore outgrowth. Possibly certain ingredients such as sodium phosphate and sorbic acid may inhibit pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic, lactic or phosphoric acid, refrigerate (that is, eliminate tempering at ambient temperature [TCS food]), or store at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Run formulation through a validated microbial model.

Predictive microbial modeling: Microbial model showed that the product would support the growth and toxigenesis of *C. botulinum.* A decision was made to reformulate by optimizing the controlling factors and their interactions. In this case, sorbic acid levels were adjusted from 0.08 % to 0.15 %. The reformulated product was run through the microbial model

which gave a prediction of safety. Conclusion is that the reformulated unopened product may be tempered at room temperature for 24 h maximum. Without additional challenge studies on vegetative pathogens, opened product requires time/temperature control.

Example 3

Product: Pasteurized process cheese slices, bulk packaged. The product is not held hot or cold during use. The ingredients of the product are milk, whey, cheese, milk fat, water, salt, sodium citrate, sorbic acid, artificial color. The product is intended to be distributed and stored at ≤ 7 °C (45 °F) for a maximum of 8 mo. The tempered 96-slice pack can be kept for an 8 h shift at ambient temperature prior to use near to the grill in foodservice establishments to facilitate peeling of slices and melting on sandwiches. No history of pathogenic growth associated with commercial pasteurized process cheese slices. Product is subject to recontamination after opening. Microbial hazards: *L. monocytogenes, S. aureus, Salmonella* spp., *E. coli* O157:H7, *C. botulinum* (product does not receive a proteolytic botulinal cook).

Step 1. Processing: Heated to ≥ 66 °C (150 °F) for ≥ 30 s and cooled over a chill roll. Slices are then bulk packed in units of 96 slices. Ready-to-eat directly out of package or used in melt applications. Go to Table B.

Table: pH 5.7 to 5.8 and a_w > 0.92.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No apparent product characteristic that prevents spore outgrowth. Possibly sorbic acid may inhibit pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic, lactic, or phosphoric acid, refrigerate (that is, eliminate tempering at ambient temperature [TCS food]), or store at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: Product was inoculated with 10^3 CFU/g *L. monocytogenes, S. aureus, E. coli* O157:H7, *Salmonella* spp., and *C. botulinum* (proteolytic strains only). Cocktails of each challenge organism were inoculated into separate samples. Inoculated product was incubated at 30°C (86°F) for 96 h. Results showed that *Salmonella* spp, *E. coli* O157:H7, and *L. monocytogenes* decreased in populations over the challenge period. *Staphylococcus aureus* levels remained constant during the challenge period, but were below levels that supported detectable enterotoxin production. No botulinal toxin was detected over the challenge period. From a safety perspective the opened product could be stored for 67 h at room temperature, based on a safety factor of 1.3 times shelf life of the product. Loss of product quality dictates that slices be tempered for no longer than 8 h.

Example 4

Product: Cheese blend for pizza topping. The product is not held hot or cold during use. The ingredients of the product are cheese, sodium chloride 1.81%, nitrite level < 1ppm. The product is intended to be stored at ambient temperature for a maximum of 10 h before being baked. This is a new intended use, so there is no history of safe use. The microbiological hazards are the heat-stable toxins of *S. aureus* and *B. cereus.*

Step 1. Processing: Baked, but heat-stable toxins may remain. Go to Table B.

Table: pH 5.56 and a_w 0.978.

Step 2. Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic, lactic, or phosphoric acid, refrigerate (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: 1,000 CFU/g of product inoculated with *S. aureus* and *B. cereus* and incubated at 27 °C (80 °F) for various lengths of time: No toxin was detected at 10 h. Product can be stored safely at room temperature for 7 h, based on a safety factor of 1.3 shelf life of the product.

Example 5

Description: Cheese-filled bread. The product is not held hot or cold during use. The ingredients of the product are process cheese, pastry covering, salt, glycerol. The product is intended to be distributed and stored at 4.4 to 7.3 °C (40 to 45 °F) for a maximum of 90 d, and then stored at ambient temperature for sale. New product, so there is no history of use. Microbiological hazard: *Bacillus cereus* and *Clostridium botulinum* toxin production.

Step 1. Processing: Baked to internal temperature of 88 °C (190 °F) and MAP packed with 100% N_2. Go to Table B.

Table: pH 5.6 to 5.7 and a_w 0.93.

Decision: Product may be a TCS food.

Product Assessment: No product characteristic that prevents pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic acid or phosphoric acid, refrigerate (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: Separated inocula of 500 spores of *C. botulinum* and 500 spores of *B. cereus* incubated at 13, 18.5, 30 °C (55, 65, 86 °F) for various lengths of time. No toxin production or *B. cereus* growth at 30°C (86 °F) for 14 d. Product can be stored safely at room temperature for at least 10 d, based on a safety factor of 1.3 times shelf life of the product.

Example 6

Product : Monterey cheese slices. The product is not held hot or cold during use. The ingredients of the product are Monterey Jack cheese, milk fat, water, citrate and phosphate emulsifiers, salt (1.9 to 2.5%), sorbic acid (2000 ppm max), color. The product is intended to be distributed and stored refrigerated for 180 to 210 d, but used at room temperature in food service. New product, so there is no history of use. Microbiological hazards: *L. monocytogenes, S. aureus, Salmonella* spp., *E. coli* O157:H7.

Step 1. Processing: 71 °C (160 °F) for 30 s, hot filled, and sliced, but recontamination is possible. Go to Table B.

Table: pH 5.7 to 6.0, and a_w 0.94 to 0.95.

Step 2. Decision: Product may be a TCS food.

Product Assessment: Sorbic acid as a preservative may prevent pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic acid or phospho-

ric acid, refrigerate (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: Inoculum with 1,000 CFU/g of *L. monocytogenes, S. aureus, Salmonella* spp., *E. coli* O157:H7 incubated at 30 °C (86 °F) for various lengths of time: No growth of any pathogen tested at 24 h, no *S. aureus* toxin, *E. coli, L. monocytogenes* and *Salmonella* spp. were detected at 48 h. Although *E. coli, L. monocytogenes* and *Salmonella* spp. levels remain the same up to 72 h, *S. aureus* toxin was detected at 72 h. Product can be stored safely at room temperature for no more than 33 h, based on a safety factor of 1.3 times shelf life of the product.

3.7. Filled bakery product

Product: Cream-filled éclairs. The product is not held hot or cold during use. The ingredients of the product are pastry shell (water, eggs, flour, hydrogenated vegetable oil, baking powder, sodium acid pyrophosphate, baking soda, corn starch, monocalcium phosphate, salt, malted barley); filling (water, sugar, modified corn starch, dextrose, vegetable oil, cottonseed, mono and diglycerides, salt, carrageenan, glucono delta lactone, sodium benzoate and potassium sorbate (0.02%), polysorbate 60, soy lecithin, natural and artificial flavors colored w/Yellow). The product is intended to be distributed at = 0 °C (32 °F) or refrigerated for a maximum of 180 d or 3 d, respectively, and stored at room temperature for a maximum of 4 h. This is a new product, so there is no history of use. Microbiological hazards: *L. monocytogenes, S. aureus, Salmonella* spp.

Step 1. Processing: Filling 88 °C (190 °F), cooled to 5 °C (41 °F) in 4 h; shell > 93 °C (200 °F), cooled to ambient but recontamination is possible. Go to Table B.

Table: pH 7.2 (shell), 5.1 to 5.8 (filling), a_w 0.87 (shell), 0.96 to 0.98 (filling).

Step 2. Decision: Product may be a TCS food.

Product Assessment: Benzoate, sorbate, and glucono delta lactone as preservatives may prevent pathogen growth.

Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic acid or phosphoric acid, refrigerate (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Decision: Challenge test.

Microbial Challenge Testing: Filling inoculated (and placed in shell) with 100 to 1,000 CFU/g with *L. monocytogenes, S. aureus, Salmonella* spp. incubated at 7, 12 and 26 °C (44.6, 53.6 and 78.8 °F) for various lengths of time. There was pathogen growth at 1 d. Product as processed and formulated cannot be stored safely at room temperature.

3.8. Breads

Example 1

Product: Pepper focaccia. The product is not held hot or cold during use. The ingredients of the product are bread, roasted sliced red peppers, oil, Romano cheese, garlic powder, oregano. This is a new product, so there is no history of use. The microbiological hazards are: *S. aureus, Salmonella* spp, and *C. botulinum*.

Step 1. Processing: Baked, but recontamination is possible. Go to Table B.

Table: pH (pepper and bread) 3.9 to 4.1[1] and a_w 0.99.
Step 2. Decision: Product is a non-TCS food.
[1]If only the bread or the peppers have low pH, then a challenge study should be performed.

Example 2
  Product: Plain focaccia. The product is not held hot or cold during use. The ingredients of the product are bread, oil, Romano cheese, garlic powder, oregano. This is a new product, so there is no history of use. The microbiological hazards are: *S. aureus, Salmonella* spp.
  Step 1. Processing: Baked, but recontamination is possible. Go to Table B.

Table: pH 5.5 to 5.3, and a_w 0.95 to 0.97.
Step 2. Decision: Product may be a TCS food.
Product Assessment: No product characteristic that prevents pathogen growth. Although product has properties similar to white bread, with a long history of safe use, some ingredients would not be in the formulation of white bread; therefore, the product may be a TCS food and should be further analyzed.
Decision options: Challenge testing, predictive microbial model, reformulation to lower pH with acetic acid or phosphoric acid, refrigerate (TCS food), or at ambient temperature for a limited time less than the estimated lag phase for the pathogens of concern, or not marketable.

Comprehensive Reviews in Food Science and Food Safety

Summary and Future Needs

Summary

The panel assembled by IFT was charged with evaluating the Food Code definition of "potentially hazardous foods" and proposing a new framework to determine which foods need time/temperature control for safety. Before critically reviewing the current definition, the panel completed a careful review of how the food safety community—including domestic and foreign government agencies, industry, and other organizations—identify foods that need time/temperature control for safety. In essence, some countries provide a list of foods that should always be time/temperature controlled, unless appropriate information is provided that demonstrates the safety of the specific food when held at ambient temperature. Other countries define foods according to their pH and water activity (a_w) in a manner similar to that of the United States' FDA Food Code definition. Some foreign regulatory agencies provide a list of potentially hazardous foods, exempting foods in which specific pH or a_w levels are met. In general, government agencies do not offer a standard procedure by which industry can demonstrate that time/temperature control requirements are not necessary. For instance, the regulations and guidelines of these various agencies include no mention of specific protocols for microbial challenge studies or microbial growth modeling programs that could aid in supporting a decision to store a food at ambient temperature.

In the United States, most states have adopted the Food Code definition of potentially hazardous foods. This definition relies solely on pH and a_w as the parameters for making decisions about the need for time/temperature control for safety. After an in-depth evaluation, the panel concluded that revisions are needed in order for the Food Code description to be meaningful and accurate in identifying foods that require time/temperature control. These reconsiderations are particularly important in light of the novelty and complexity of currently available foods along with the additional knowledge and scientific information gained in recent years.

The panel conducted a survey among industry and other organizations to gain knowledge on how food product manufacturers are tackling this issue. Data collected from the industry survey clearly show that some products currently identified as potentially hazardous foods could be stored at ambient temperature by virtue of the process method, formulation, time of storage, or other characteristics of the food. In most cases, microbial challenge studies were used to support such conclusions. In the absence of practical standardized protocols for applying the current definition to foods, two organizations, NSF International and American Bakers Association (ABA), developed protocols. Although not yet officially implemented, the NSF International and ABA protocols are being followed by some laboratories and companies as a guide to determine the time/temperature control status of a food. The panel concluded that both protocols present significant weaknesses in their approach to defining foods that do not need time/temperature control for safety. The data from the industry survey, the absence of a robust standardized method, and the experience of the panel further indicated that the current FDA Food Code definition needs to be revisited.

The panel developed a framework that would accurately identify which food products need time/temperature control. Several general approaches were proposed, reviewed, and critiqued by panel subgroups. Microbial growth factors that would affect the need for time/temperature control were discussed at length, including product history of safe use and processing methods (see Chapter 3). To critically evaluate pH and a_w values and their interactions, the panel reviewed in-depth microbial growth data from the scientific literature. Data obtained through validated predictive microbial growth models were used to confirm the panel's determinations.

The panel concluded that although research demonstrates that parameters such as packaging environment, antimicrobials, nutrient content, or competitive microflora influence growth of microbial pathogens, sufficient data to specify the limits of such parameters are not yet available. Therefore, specific criteria used in the framework were limited to a_w, pH, and their interaction. Although pH and a_w were the only criteria for which scientific-based values could be provided, the effects of many other parameters are addressed in subsequent steps in the framework. For instance, the panel recognized that historically, certain foods, such as white bread, have been safely stored at ambient temperatures. The panel provided a framework in which foods with scientific rationale that could justify such a safe history of use could continue to be stored and/or used at ambient temperatures.

The method used to process a food is another important factor considered in the proposed framework. The panel's framework indicates, for instance, that if a food has been processed to eliminate all vegetative pathogens (for example, with a properly validated heat or high hydrostatic pressure method) and packaged to avoid post-process contamination, only pathogenic spore-forming microorganisms would be of public health concern. This factor was handled in the framework by developing different critical pH/a_w limits, depending on whether spores or vegetative cells and spores are the likely hazards. In cases where the a_w and pH combination suggests the food needs time/temperature control for safety, a product assessment can be performed to make a more definite decision. Such a food product assessment may involve a detailed description of the product characteristics, such as antimicrobials or packaging environment that may support a history of safe use at ambient temperatures. On the basis of safe use and a

reliable product assessment, a product may be regarded as safe at ambient temperature.

Alternatively, a validated in-house, food-specific microbial growth model may be appropriately used to decide whether a food needs time/temperature control. Validation of these models is essential because many microbial growth models have been developed from data generated in media, and an extrapolation of those data to real food situations may not be appropriate. These models developed with media data may still be useful in selecting microorganisms for microbial challenge studies or limiting food parameters; if, however, after product assessment and/or microbial growth modeling a clear decision cannot be made, microbial challenge studies may provide the definite data to determine whether a food requires time/temperature for safety.

The panel described in detail the issues to be considered when designing challenge studies and interpreting data. In addition, pass/fail criteria for challenge tests were determined based on limited pathogen growth or toxin formation. The panel recognized that it was appropriate to develop different criteria for each pathogen because infectious doses and typical contamination levels vary for different pathogens. To critique their framework, the panel selected and assessed a list of food products.

In summary, the panel introduced a new approach for evaluating foods that may need time/temperature control for safety. This framework was based solely on scientific data from peer-reviewed publications that were further evaluated by the panel. The panel recognizes that the implementation of their approach in the field may not be an easy task. For example, although some of the considerations introduced in the proposed framework require careful evaluation and assessment by an expert microbiologist, this report does not attempt to propose who would be responsible for deciding the time/temperature status of a food. The panel also did not address the implications of the framework at the retail level. The panel believes, however, that in light of the complexity of the food systems and the confusion over the interpretation of the term "potentially hazardous foods," a science-based framework such as the one proposed here would be a more accurate, comprehensible, and clear alternative to the current definition and application of the term.

Future Needs

- Validate the framework for a broad variety of products, including those that are presently handled as TCS but have the potential to be non-TCS or are presently handled as non-TCS and may be TCS. Products from various sources should be used for framework validation.
- Develop educational and other required programs for implementing a validated TCS food framework at the federal, state, and local level.
- Develop general predictive models that include the effects of several parameters, such as packaging atmosphere, redox potential, a_w, pH, and selected ingredients, on the growth of pathogens of concern.
- Identify and validate appropriate pathogen and/or surrogate strains for use in challenge studies in different groups of foods.
- Investigate synergistic inhibitory effects of various strategies that combine more than one antimicrobial control parameter (hurdle technologies) as they relate to non-TCS foods.
- Identify improved methods for detection of *Clostridium botulinum*, *Staphylococcus aureus*, and *Bacillus cereus* toxins for evaluating the need for time/temperature control of foods.
- Validate the appropriateness of test frequency and method sensitivity as they relate to pathogen growth and pass/fail criteria for TCS foods;
- Collect epidemiological data to support the anecdotal evidence on safe or unsafe history of use for foods that may be considered TCS or non-TCS. Establish the scientific explanations for their safe or unsafe use.
- Determine the effect of alternative processing technologies on human pathogens in the production of non-TCS foods.
- Establish Food Safety Objectives for the production of non-TCS and TCS foods that may have potential to be non-TCS. Determine the performance criteria and process criteria for these systems.
- Develop methods, approaches, and frameworks to evaluate shelf-life open-dating for safety.
- Identify specific factors that control pathogen growth in products that appear to be TCS foods but do not support growth of the pathogens when challenged.
- Establish accurate measurement techniques for intrinsic and extrinsic factors in food microenvironments and interfaces in multicomponent foods. Determine with accurate and sensitive analytical methods the effects of these microenvironments and interfaces on microbial responses.

Appendix A:
Development of the Definition of "Potentially Hazardous Foods"

● U.S. Public Health Service. *An Ordinance Regulating Food and Drink Establishments* (December 1935). This ordinance regulates "perishable food or drink" in eating and drinking establishments. Specifically, Item 13 (Refrigeration) of this document recommends that "perishable food or drink [be] kept at or below 50 °F., except when being prepared or served."

● U.S. Public Health Service. *Ordinance and Code Regulating Eating and Drinking Establishments* (March 1938). Item 13 (Refrigeration) is retained. A scientific explanation of the "public-health reason" that perishable foods need to be kept cold (because there is a danger of pathogenic bacteria entering food and causing disease) is added along with a "code" for "satisfactory compliance."

● U.S. Public Health Service. *Ordinance and Code Regulating Eating and Drinking Establishments* (June 1940). Item 13 (Refrigeration) is retained. The regulation to keep perishable food or drink at or below 50 °F (10 °C) now includes *heat* as a deterrent (that is, keeping food warm) and is specifically includes "cream-filled pastries."

● U.S. Public Health Service. *Ordinance and Code Regulating Eating and Drinking Establishments* (1943; PHS Publication No. 37). Item 13 (Refrigeration) is retained. This item includes not only microorganisms but also their toxins as public health concerns. The regulation specifically includes custard- and cream-filled pastries, milk and milk products, egg products, meat, fish, shellfish, gravy, poultry stuffing, and sauces, dressings, and salads containing meat, fish, eggs, milk, or milk products.

● U.S. Public Health Service. *Food Service Sanitation Manual, Including a Model Food Service Sanitation Ordinance and Code* (1962; PHS Publication No. 934). This code includes extensive and detailed additions to the earlier ordinances. "Perishable food," for example, is defined as "any food of such type or in such condition as may spoil." The term "**potentially hazardous food**" is introduced and defined as: "any perishable food which consists in whole or in part of milk or milk products, eggs, meat, poultry, fish, shellfish, or other ingredients capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms." Specific recommendations for ensuring the safety of potentially hazardous food are stated along with sanitary practices recommended for the "storage, preparation, display, and service of food."

Comprehensive Reviews in Food Science and Food Safety

Appendix B:
Data from Industry and Trade Organizations

The panel conducted a survey among industry and trade organizations on their approaches to temperature control for safety (see below). More than 60 individuals and organizations submitted information, and from this data, the results of 35 challenge studies were graphed as shown below. Seven submissions included no data; 17 submissions did not include water activity measurements; 3 submissions included no pH or water activity measurements; and 2 submissions did not include appropriate time data. Of the respondents' observations, 8 were from failed challenge studies, and the remaining 27 observations were for products that passed challenge studies. It should be noted that the challenge test design and criteria were those proposed by the submitters, not the panel, and that some data is based on studies using total plate counts and not challenge organisms.

The tables on the following pages summarize the challenge study data submitted to the panel for consideration. Only the data for products where pH and a_w was available and challenge studies were performed are represented in the graph below. Products that failed or passed the microbial challenge study are indicated. The x and y coordinates show the corresponding pH and water activity values associated with the foods used in those challenge tests.

It is clear that factors other than pH and water activity influence the safe storage times of these foods at room temperature. For instance, some of the formulations include preservatives such as sorbic acid, sodium propionate, and phosphoric acid which may result in a product that does not need time/temperature control for safety, even though its pH and a_w may suggest differently. For example, one product failed its challenge study after only 24 h, whereas another product, with more permissive pH and water activity values, was judged safe for 4320 h. It is also evident that many (14 out of 21) products with pH and water activity values greater than 4.6 and 0.85, respectively, can be safely held at ambient temperatures for lengthy periods of time.

Note that the challenge test design and criteria were those proposed by the submitters, not the panel, and that some data is based on studies using total plate counts and not challenge organisms.

Evaluation & Definition of Potentially Hazardous Foods

IFT Scientific & Technical Panel for FDA Task Order No. 4
Request for Information

The IFT Scientific & Technical Panel on Evaluation & Definition of Potentially Hazardous Foods seeks information to support a thorough scientific evaluation of the FDA Food Code 1999 definition of Potentially Hazardous Foods. Specific interest is in foods that might be considered potentially hazardous under the definition, but are demonstrated to be safe at room temperature through testing or other means.

We are interested in contributions in any format. You may answer the General Considerations below, provide more specific information and data using the attached form, and/or provide a copy of results or protocols that you have on file. All information will be blinded prior to delivering to the panel to maintain confidentiality unless requested otherwise.

We would appreciate your response within 30 days; however if more time is necessary due to extenuating circumstances, please let us know. We would be happy to accept data up to March 30th.

Send information to the soliciting organization (e.g., trade association, testing lab, etc.) or directly to IFT at the following address:

Frank Busta
Department of Science & Technology Projects
Institute of Food Technologists
1025 Connecticut Avenue NW, Suite 503
Washington DC 20036
For questions contact:
Maria Oria
Phone: 202-466- 5980 or mporia@ift.org

General Considerations (Attach additional information if needed)

 1. How do you determine if a specific food product requires refrigeration for safety? List specific criteria (e.g., pH, water activity, time, history, etc.) and provide examples as appropriate.

 2. Do you use computer modeling (e.g., USDA Pathogen Modeling Program, Food Micro Model, etc.) in determining the need for refrigeration for safety? If so, how? What pass/fail criteria do you use? Please attach an example if available.

 3. Do you use challenge testing in determining the need for refrigeration for safety? If so, how? What pass/fail criteria do you use? Attach general or specific protocol(s) if available.

 4. How do you determine appropriate pathogens to consider for challenge testing or modeling? Do you use surrogates for specific organisms?

 5. If you use challenge testing or modeling, how do you determine which pathogens or surrogate organisms to use?

Specific Product Example

Example type: ☐ Challenge study ☐ Computer modeling ☐ Both

Product Description _____

Ingredients (e.g., package ingredient declaration) _____

Intrinsic factors:

- pH _____

- Water activity _____

- Preservatives _____

- Other _____

Extrinsic factors:

- Processing _____

- Packaging _____

- Distribution temperature _____

- Other _____

Intended use _____

Shelf-life _____

Data validating safety of room temperature use or storage: Attach data (table or chart) and describe the following, as appropriate. Method protocol may also be attached.

- Initial inoculum level _____

- Inoculation method & preparation _____

- Organism(s) _____

- Incubation temperature(s) _____

- Enumeration methods _____

- Pass/fail decision criteria _____

Challenge studies—"Potentially Hazardous Foods" *(also continued on following pages)*

Product	Micro-organism	Rep	Method (Inoculation)	Level	End-point	T	t	other	Enumeration methods	Pass/fail criteria	Other
Italian salad dressing (paper 4)	S. enteritidis S. typhimurium	no no	— —	3 log/g 3 log/g	>10 >10	35 °C 35 °C	5 min 5 min	covered covered	FDA's BAM	no growth/ pass	pass
67 Salad dressings (5A)	Salmonella spp (3 ck)	—	—	500 cfu/g	not detected	—	BA protocol	—	XLD agar, enrich in lac-tose broth/FDA	no Salmonella within 72 h	pass
3 Barbecue sauces (5B)	Salmonella spp (3 ck)	—	—	500 cfu/g	not detected	—	—	BA protocol	XLD agar, enrich in lac-tose broth/FDA	no Salmonella within 72 h	pass
Pumpkin pie (13)	S. aureus	3	on and in filling	106,107,108, 109/g (1 ml)	103,103,106, 106/no toxin*	Room T	6 d (3 samp) or box	bagged, open air, staph test	BHI agar/oxoid pass	no toxin production/	*> 1ng/g
Pumpkin pie (14)	S. aureus	3	in pie filling	140/g (0.05 ml) 140cps	<100 cps/g	25 °C	3 d (2 samp)	—	Baird-Parker /coagulase +	no growth /g/pass	—
Lemon meringue pie (15) (2 cultures)	S. aureus (2 cultures)/ L. monocytogenes	3	rows in filling, intersection, and meringue	3,000/ 1,500/ sample	<10/ sample	Room	5 d (3 samp)	—	Baird-Parker and Mox agar	no growth/ pass	TPC, Salmonella
Hush puppy mix (16A)	B. cereus S. aureus S. senftenberg	2 2 2	—	~3.5log/g ~4log ~2.3log	~3log ~5log,~6.5log ~2.5log,~6log	35 °C 35 °C 35 °C	8 h 4h,8h 8h,19h	—	ICMSF and FDA's BAM	no growth	—
Hush puppy mix w/ onions (16B)	S. aureus	2	—	~2.3log	~2.3log, ~3.3log	35 °C	4h 8h	—	ICMSF and FDA's BAM	no growth/ pass at 4 h	—
Apple-cheese Danish (17A)	S. aureus ck, (5) L. monocytogenes, (5) E. coli ck (2) S. enteritidis/ typhimurium ck (5)	2 2 2 2	in 4 places in 4 places in 4 places in 4 places	360/g 435/g 1900/g 145/g	320,395,10/g 235,160,10/g 470,10,10/g 15,10,10/g	25 °C 25 °C 25 °C 25 °C	24 h, 4 d,11 d 24 h,4 d, 11 d, 24 h, 4 d, 11 d, 24 h, 4 d, 11 d	in sterile bags in sterile bags in sterile bags in sterile bags	Baird-Parker Mox E. coli petrifilm XLD	no growth at 14 to 18 h/ pass	—
Blueberry-cheese Danish (17B)	S. aureus ck, (5) L. monocytogenes, (5) E. coli ck (2) S. enteritidis/ typhimurium ck (5)	2 2 2 2	in 4 places in 4 places in 4 places in 4 places	360/g 435/g 1900/g 145/g	340, 215, 10/g 190, 235, 50/g 300, 35, 10/g 70,10,10/g	25 °C 25 °C 25 °C 25 °C	24 h, 4d, 11d 24 h, 4d, 11d 24 h, 4d, 11d 24 h, 4d, 11d	in sterile bags in sterile bags in sterile bags in sterile bags	Baird-Parker Mox E. coli petrifilm XLD	no growth at 14 to 18 h/ pass	—
Cherry-cheese Danish (17C)	S. aureus ck, (5) L. monocytogenes, (5) E. coli ck (2) S. enteritidis/ typhimurium ck (5)	2 2 2 2	in 4 places in 4 places in 4 places in 4 places	360/g 435/g 1900/g 145/g	170, 170, 170/g 275, 25, 30/g 155, 30,10/g 25,10,10/g	25 °C 25 °C 25 °C 25 °C	24 h, 4 d, 11 d 24 h, 4d, 11d 24 h, 4 d, 11 d, 24 h, 4 d, 11 d	in sterile bags in sterile bags in sterile bags in sterile bags	Baird-Parker Mox E. coli petrifilm XLD	no growth at 14 to 18h/ pass	—
Eclaires (18)	Salmonella spp ck (4)— S. aureus ck (5) L. monocytogenes ck		in filling in filling in filling	90/g 2.3 × 102/g 4.8 × 102/g	<100, <100, <100 <5 log, 2 log, <100 <4 log, 4 log, 4 log	26,12,7C 26,12, 7C 26,12, 7C	4 d 4 d 4 d	in sterile bags in sterile bags in sterile bags	XLD Baird-Parker Mox	no growth or toxin at 4.5 d/ fail	—

Challenge studies—"Potentially Hazardous Foods" (continued from previous page)

Product	Rep	Micro-organism	Inoculation			Incubation			Enumeration methods	Pass/fail criteria	Other
			Method	Level	End-point	T	t	other			
RTE chocolate pie and donut filling (19)	—	S. aureus ck (5) L. monocytogenes (2) B. cereus (2) S. enteritidis/typhimurium (6) C. sporogenes (2)	— — — — —	8×2 log 1.5×3 log 3×2 log ~2.5×2 log 2 log	not detected ~200 not detected not detected not detected	10, 32 °C 10, 32 °C 32 °C 10, 32 °C 32 °C	21 d 21 d 21 d 21 d 21 d	in sterile bags in sterile bags in sterile bags in sterile bags/ ana	Baird-Parker Mox MYP XLD SFP	no growth or toxin at $1.5 \times$ shelf-life/ pass at 2 weeks	—
Fettuccine Alfredo with chicken (31)	—	C. botulinum ck	—	4log cfu/ml	no toxin	12, 21 °C	60 d, 5 d	—	—	no toxin for 5 d at 21 °C or 60 d at 12 °C pass	—
Chopped lettuce (35)	2	S. enteritidis, cholerasuis, and typhimurium, L. monocytogenes ck (3)	—	~3 log ~3 log	~5 log ~4 log	24 °C 24 °C	24 h 24 h	sterile containers sterile containers	XLD MOX	?	—
Chopped onions (36)	2	S. enteritidis, cholerasuis, and typhimurium, L. mono cytogenes ck (3)	—	~3 log ~3 log	~2 log ~4 log	24 °C 24 °C	24 h 24 h	sterile containers sterile containers	XLD MOX	?	—
Tomatoes (37)	—	Salmonella spp	—	50	~1 log, ~7 log	30 °C	4 h, 24 h	—	—	?	Distr. at 5 °C
Poultry/Bacon/Cheese-filled Bread (38)	—	C. botulinum prot. A + B, C. botulinum non-prot. A	added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14 d no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant
Chicken/cheese-filled bread (39)	—	C. botulinum prot. A + B, C. botulinum non-prot. A	added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14d no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant
Chicken/cheese-filled bread (40)	—	C. botulinum prot. A + B, C. botulinum non-prot. A	added to ground product/anaero	100 to 500 spores/g	no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	
Ham/cheese-filled bread (41)	—	C. botulinum prot. A + B, C. botulinum non-prot. A	added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14d no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant
Precooked ground beef/cheese/ham-filled bread (42)	—	C. botulinum prot. A + B, C. botulinum non-prot. A	added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14d no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant

Challenge studies—"Potentially Hazardous Foods" (continued from previous page)

Product	Micro-organism	Rep	Inoculation			Incubation			Enumeration methods	Pass/fail criteria	Other
			Method	Level	End-point	T	t	other			
Precooked ground beef/cheese/ham-filled bread (42)	C. botulinum prot. A + B, C. botulinum non-prot. A		added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14d, no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14 d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant
Cheese-filled bread (43)	C. botulinum prot. A + B, C. botulinum non-prot. A	—	added to ground product/anaero	100 to 500 spores/g	toxin at a_w .98 at 26 °C/14d, no toxin at a_w .89 to 94	26/18/13 °C	14 d/1 wk/8 wk	—	mouse bioassay	no toxin for 14d at 26 °C, 1 wk at 18 °C, and 14 wk at 13/	pass or fail a_w dependant
Process cheese slices (Monterey—44A)	L. monocytogenes, S. aureus, Salmonella, E. coli 0157:H7	—	surface inoculated	1000 cfu/g	3.3 log, 7 log, 1 log, 2.7 log	30 °C	(24, 48, 72,) 96 h	—	selective media	no growth	failed (pass at 24 only)
Process cheese slices (Swiss—44B)	L. monocytogenes, S. aureus, Salmonella, E. coli 0157:H7	—	surface inoculated	1000 cfu/g	2.8 log, 3.2 log, 1 log, 2.5 log	30 °C	(24, 48, 72,) 96 h	—	selective media	no growth	pass
Process cheese slices (American—44C)	L. monocytogenes, S. aureus, Salmonella, E. coli 0157:H7	—	surface inoculated	1000 cfu/g	—	30 °C	(24, 48, 72,) 96 h	—	selective media	no growth	no data
Cottage cheese hot pack (45)	C. botulinum prot. A + B, C. botulinum non-prot. A	—	added to ground product/anaero	100 to 500 spores/g	—	26 °C	108 h	—	mouse bioassay	no toxin	pass
	S. aureus	—	—	4 log	5 log (no toxin), 5 log (no toxin), 5 log (no toxin), 2 log (no toxin)	140 °F to 45 °F, 140 °F to 45 °F and 98 °F, 140 °F to 45 °F, 45 °F	30 h, 30 h, 8 h, 4 h, 60 d	—	Baird-Parker agar or SET-EIA kit for toxin	no toxin	pass
18 Process cheese slices (Cream - 47)	C. botulinum (mixed strains)	—	added to heated cheese/agitation into tubes	1000 spores/g	—	86 °F	4 wk, 8 wk	—	mouse bioassay	no toxin	depending on formulation (salt?)
14 Process cheese sauce (50)	C. botulinum (mixed strains)	—	added to heated cheese/agitation into tubes	1000 spores/g	—	45, 55, 65, 86 °F	4 wk, 8 wk	—	mouse bioassay	fail	
Process cheese sauce (with spices) (51)	C. botulinum (mixed strains)	—	—	20 or 70 spores/g	—	86 °F	13 mo (13 sampling times)	—	mouse bioassay	no toxin	pass
Process cheese spread (52)	C. botulinum (mixed strains)	—	added uniformly to cheese	1000 spores/g	—	86 °F	—	—	mouse bioassay	no toxin	1 out of 5 fail at 6 mo

Challenge studies—"Potentially Hazardous Foods" (continued from previous page)

Product	Micro-organism	Rep	Inoculation			Incubation		Enumeration methods	Pass/fail criteria	Other
			Method	Level	End-point	T	t			
Process cheese spread (53)	C. botulinum (mixed strains)	—	added uniformly to cheese	1000 spores/g	—	45, 55, 65, 86 °F	25 wk	mouse bioassay	no toxin	1 out of 4 fail at 65 °F/12 wk
Cheese blend (pizza) (54)	S. aureus (3) B. cereus (3)		added & mixed	630/g 850/g	3,700/g No toxin 10/g No toxin	80 °F	10 h	FDA's BAM	no hazardous level/no toxin	also used pathogen model 4.0

Pass/fail criteria—"Potentially Hazardous Foods" (also continued on following pages)

Product	Ingredients	pH	aw	Processing	Packaging	Distrib. T	other	Storage T	Shelf-life	Criteria	Pass/Fail
Margarine (1)	Fat (80%), water & milk protein (19%), salt (.9%) K-sorbate (.1%)	4.8	—	—	Wrapper	—	—	Ambient	3 mo	pH, salt, sorbic acid	Pass
Pure Oil and Fats (2)	—	—	—	—	—	—	—	—	—	no growth/ no data provided	Pass
20 Salad Dressings (3)	—	< 4.6	—	—	—	—	—	Ambient	—	pH	Pass
Italian Salad Dressing (4 - Paper)	Cheese, parsley, eggs, oregano	3.5 to 3.6	—	—	—	—	—	Ambient	—	Salmonella challenge	Pass
67 Salad Dressings (5A)	Eggs, soybean oil, buttermilk, tomato paste, onion, garlic, spices, lemon juice, vinegar (2.5 to 5.4% salt), K-sorbate	3.5 to 4.2	—	Cold blended/ filled	Plastic or glass bottle or jar	Ambient	—	Ambient	7 to 9 mo	No Salmonella-challenge/ 72 h	Pass after 24 h, 48h, 72 h
Sweet Sour (6)	Water (49%), sugar (39%), Acetic acid (1.66%), sorbic acid (.06%), salt (1.47%)	4.7	< .93	—	—	—	—	Ambient	6 mo	pH, aw, acetic and sorbic acid	Pass
Chinese Style (7)	Water (50%), sugar (24.6%), acetic acid (1%), sorbic acid (.05%), salt (12.67%)	4.5	—	—	—	—	—	Ambient	6 mo	pH, salt, acetic and sorbic acid	Pass
B-B-Q Style (8)	Water (57%), sugar (20%), acetic acid (.99%), sorbic acid (.07%), salt (9.5%)	4.2	—	—	—	—	—	Ambient	6 mo	pH, salt, acetic and sorbic acid	Pass
3 Barbecue Sauces (5B) 72 h	—	—	—	Cold blended/	Plastic or glass bottle filled	Ambient or jar	—	Ambient	7 to 9 mo	No Salmonella-challenge/	Pass after 24 h, 48h, 72 h
Curry (9)	Water (51%), sugar (16.6%), acetic acid (1.5%), sorbic acid (.06%), salt (10%)	4	—	—	—	—	—	Ambient	6 mo	pH, salt, acetic and sorbic acid	Pass
4 Sauces (10)	—	> 4.6	—	—	—	—	—	Chilled	—	pH	Fail
17 Sauces (11)	—	< 4.6	—	—	—	—	—	Ambient	—	pH	Pass
Garlic Bread Sauce (12)	—	4.83	0.43	—	—	—	—	Ambient	—	pH/aw combination	Pass
Pumpkin Pie (13)	—	5.1	—	Baking	Cool, then in box	—	—	Ambient	3 to 4 d	pH, Na benzoate Na propionate, challenge	Pass

Pass/fail criteria—"Potentially Hazardous Foods" (continued from previous page)

Product	Ingredients	pH	a_w	Processing	Packaging	Distrib. T	Storage T	Shelf-life	Criteria	Pass/ Fail
Pumpkin pie (14)	—	—	—	Baking	—	—	—	—	Challenge	Pass
Lemon-meringue Pie (15)	Filling: water, sugar, modified food starch, corn syrup solids, margarine, lemon juice solids, high fructose corn syrup, sodium citrate, agar agar, K-sorbate, natural flavor, locust bean gum, artificial color (FD & C Yellow no. 5	Mer: 4.2 Int: 3.5 Fill: 3.4	0.94 0.95 0.45	Crust Baking: 400 °F, add meringue, bake 85 °F, 10 to 12 min	Cool, then in box	—	Ambient	3 d	*E. coli* (−), *Salmonella* (−), pH, challenge	Pass (?) but process control
Hush puppy mix (16)	Enriched white corn meal, enriched bleached wheat flour, dehydrated onion, sugar, defatted soy flour, leavening, salt, whey, soy protein concentrate, egg	6.8	0.98	—	—	—	Ambient	4 h	Onions, challenge	Pass
*Baked cream cheese/ fruit-filled Danish (17)	Flour, shortening, water, whole egg, shortening, bakers yeast, crème custard flavor, high fructose corn syrup, salt, baking powder, mono- and diclycerides, spice, sodium stearoyl lactylate, vanilla, lemon juice flav., imit. cream cheese (skim milk, cream, cheese cultures, enzymes, K-sorbate); fruit filling (cherries, apples, or blueberries, sugar, water, mod. corn starch, sodium citrate, citric acid, sodium benzoate, K-sorbate, salt, erythorbic acid, gellan gum, locust bean gum, carrageenan, color: Red 40, Blue 1	Cr: 6.9, Ch: 4.3, Fruit: 3.5	0.9, 0.97, 0.95	Baked in store	Poly bag; in box	Raw; frozen	Ambient	12-18 h, post-baked	Challenge w/S. aureus, L. monocyto-genes, Salmon-ella, E. coli	Pass
*Cream-filled eclaires (18)	Pastries: water, eggs, flour, hydrog. veg. oil, baking powder, sodium acid pyrophosphate, baking soda, corn starch, monocalcium phosphate, salt, malted barley; filling: water, sugar, mod. corn starch, dextrose, veg. oil, cottonseed, mono and diglycerides, salt, carrageenan, glucono delta lactone, sodium benzoate and K-sorbate (0.02%), polysorbate 60, soy lecithin, nat. & artific. flav. colored w/Yellow	Shell 7.2 Fill 5.1 to 5.8	0.87, .96 to .98	Cream 190 °F, cooled to 41 °F in 4 h; shell > 200 °F, cooled to ambient	In bulk	0 or below	Refrigerated	3 d at < 41 °F, 180 d frozen	Challenge with S. aureus, L. monocytogenes, Salmonella: lag phase or no toxin for 1.5% shelf-life	Fail
Chocolate pie and donut filling (19)	Water, sugar, corn starch, cocoa, egg, hydrogenated shortening, potassium sorbate (.49%), salt, sodium propionate (.02%), phosphoric acid, flavors	5.5 to 6.2	0.97	Heated to 190 °F, hot filled at 160 °F	HDPE pails w/lids	< 45 °F	Ambient	6 mo closed, open?	Challenge w/S. aureus, L. mono., C. sporogenes, B. cereus, Sal-monella: lag phase or no toxin for 1.5 % shelf-life	Pass
Plain foccacia (20)	Oil, romano cheese, garlic powder, oregano	5.5 to 5.3	.97 to .95	Baked	—	—	Ambient	3 d	S. aureus, Salmonella, E. coli (−) for 6 d	Pass
Pepper foccacia (21)	Roasted sliced red peppers, oil, romano cheese, garlic powder, oregano	3.9 to 4.1	0.99	Baked	—	—	Ambient	3 d	S. aureus, Salmonella, E. coli (−) for 6 d	Pass
Tomato foccacia (22)	Plum tomatoes, oil, romano cheese, garlic powder, oregano	4.1 to 4.3	0.99	Baked	—	—	Ambient	3 d	S. aureus, Salmonella, E. coli (−) for 6 d	Pass

Pass/fail criteria—"Potentially Hazardous Foods" (continued from previous page)

Product	Ingredients	pH	a_w	Processing	Packaging	Distrib. T	Storage T	Shelf-life	Criteria	Pass/ Fail
Asiago potato cheese bread (23)	No data	5.9 to 5.84	0.88	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–) for 6 d	Pass
Garlic Foccaccia (24)	No data	5.7	0.88	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–), L. mono for 6 d	Pass
South of the border foccaccia (25)	No data	5.4 to 5.5	.87 to .88	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–), L. mono for 6 d	Pass
Italiano foccaccia (26)	No data	5.6	0.87	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–), L. mono for 6 d	Pass
Jalepeño/cheddar foccaccia (27)	—	5.6 to 5.7	0.86 to 0.87	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–), L. mono for 6 d	Pass
Vegetable medley foccaccia (28)	—	5.9 to 5.8	0.88	Aseptic baking	Aseptic packaging	—	Ambient	3 d	S. aureus, Salmonella, E. coli (–), L. mono for 6 d	Pass
Meats (29)	No data	—	—	—	—	—	Chilled	—	Possible abuse during handling	Failed
Fermented sausage (30)	—	< 5.1	—	Acidulated or fermented, then cooked	Packed	—	Ambient, chilled for quality	—	pH, challenge L. mono, Salmonella, S. aureus, C. bot., % brinelt = 6%	Pass
Fettuccine Alfredo w/chicken (31)	—	5.8	0.98	Heated to 205 °F, filled and sealed at 176. Cooled to 40 °F in 2 h	MAP in plastic tray	—	40 °F	60 d	C. botulinum challenge, no toxin for 5 d at 70 °F or 60 d at 54 °F	Pass
Canned salads (32) (Ham, Chicken, Tuna)	—	< 5	0.96	Pasteurize in container	Canned	—	80 °F	—	C. botulinum challenge (data not included)	Pass
Only general (33)										
No such products (34)										
Chopped Lettuce (35)	—	—	—	—	—	—	Ambient	< 8 h	Challenge w/ Salmonella & L. mono	Pass (?)
Chopped Onions (36)	—	—	—	—	—	—	Ambient	<8 h	Challenge w/ Salmonella & L. mono	Pass (?)

Pass/fail criteria—"Potentially Hazardous Foods" (continued from previous page)

Product	Ingredients	pH	a_w	Processing	Packaging	Distrib. T	Storage T	Shelf-life	Criteria	Pass/Fail
Fresh Tomatoes (37)	—	—	—	—	—	Ambient	<4 h		Challenge with Salmonella	Pass (?)
Poultry/bacon/cheese-filled bread (38)	Turkey, bacon, process cheese, pastry covering, salt, spices, glycerol	5.6 to 5.8	.88 to .98	Baked to 165 to 170 °F internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w <.93, Fail a_w .98
Chicken/cheese-filled bread (39)	Chicken, process cheese, spices, salt, glycerol, pastry covering	5.8 to 6.0	.88 to .98	Baked to 165 to 170 °F internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w<.93 Fail a_w .98
Cheese-filled bread (40)	Cheese, tomato sauce, spices, glycerol, salt, pastry covering	5.0 to 5.1	.94 to .98	Baked to 165 to 170 °F internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w<.98
Ham/cheese-filled bread (41)	Cured ham, process cheese, spices, glycerol, salt, pastry covering	5.4 to 5.6	.89 to .98	Baked to 165 to 170 °F internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w<.93 Fail a_w .98
Ground beef/process cheese-filled bread (42)	Ground beef (pre-cooked), process cheese, spices, glycerol, salt, pastry covering	5.5 to 5.8	.89 to .98	Baked to 165 to 170 °F internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w<.93 Fail a_w .98
Cheese-filled bread (43)	Process cheese, pastry covering, salt, glycerol	5.6 to 5.7	.90 to .97	Baked to 165 to 170 °F, internal	MAP-100% N2 barrier pouch	40 to 45 °F	Ambient?	Refrigerated: 90 d	C. botulinum challenge, no toxin	Pass a_w<.93 Fail a_w .98
Process cheese (44) (Monterey Jack or Swiss)	Swiss or Monterey Jack cheese, milkfat, water, citrate and phosphate emulsifiers, salt (1.9 to 2.5%), sorbic acid (2000 ppm max), color	5.7 to 6.0	.94 to .95	160 °F for 30 s, hot filled	slices or bulk	Refrigerate	Refrigerate	180 to 210 d, if refrigerated	Challenge with L. mono, S. aureus, Salmonella, E. coli O157:H7, no growth at 86 °F	Swiss: pass 96 h
										Monterey: pass at 24 h, failed at 48, 72, 96 h
Process cheese (44C) (American)	American cheese, milkfat, water, citrate and phosphate emulsifiers, salt (2.3-2.6), sorbic acid (2000 ppm max), color	5.5 to 5.8	.94 to .95	160 °F for 30 s, hot filled	slices or bulk	Refrigerate	Refrigerate	180 to 210 d, if refrigerated	Challenge with L. mono, S. aureus, Salmonella, E. coli O157:H7, no growth at 86 °F	no data included
Cottage cheese hot pack (45)	Cultured pasteurized milk, Grade A skim milk, milk and cream, water, whey; <1 food starch, salt, natural flavors, enzymes, gums and Vitamin A	4.8 to 5.4	N/A	Past., ferm., hot filled	Thermoformed, cups, lid	35 to 45 °F	Ambient?	60 d	C. botulinum challenge, no toxin	Pass
									S. aureus challenge, no toxin during process	Pass
Cheeses (46)	No data	> 4.6	—	—	—	—	Chilled, unless past		Possible abuse during handling	Failed
18 Process cheeses slices (47) (Cream)	Cheese, cream, milkfat, water, citrate and phosphate emulsifiers, salt (1.9-2.86%), whey, nonfat dry milk, color, sorbic acid (2000 ppm)	5.5 to 6.1	0.9	160 °F for 30 s, hot filled into slices	Slices	Refrigerate	Refrigerate	180 to 210 d, if refrigerated	Challenge with C. bot. for 4, 8 wk; no toxin at 86 °F	Depends on formulation, salt

Pass/fail criteria—"Potentially Hazardous Foods" (continued from previous page)

Product	Ingredients	pH	a_w	Processing	Packaging	Distrib. T	Storage T	Shelf-life	Criteria	Pass/ Fail
Processed cheese (48)									C. botulinum challenge	?
Sliced cheeses (paper-49)	—	—	—	Processed	—	—	30 °C	96 h	Challenge w/ S. aureus, L. mono, Salmonella, E. coli O157:H7	Pass
14 Process cheeses (50) (Sauce)	Corn syrup solids, milk protein concentrate, cheese, salt (1 to 1.4%), phosphate emulsifiers, alginate, tapioca, butter, cheese powder, yeast exract, color, water, lactic acid (.1%), sorbic acid (0.05%)	5.7 to 6.2	0.98	160 °F for 30 s, hot filled	Jars	Refrigerate	Refrigerate	5 to 7 mo	Challenge with C. botulinum, no toxin at 86 °F, 45, 55, 65	Fail (no data)
Process cheese sauce with spices (51)	Cheddar cheese, soybean oil, whey, corn syrup solids, maltodextrin, phosphate emulsifiers, salt (1.82%), lactic acid, Worcestershire sauce, mustard flour, sodium alginate, color	5.71	52.50%	160 °F for 30 s, hot filled	Pouches	Refrigerate	Refrigerate	10 mo	Challenge with C. botulinum, no toxin at 86 °F	Pass
Process cheese spread (52)	Skim cheese, corn syrup solids, maltodextrin, nonfat dry milk, carrageenan, salt (1.4 to 2.2%), sorbic acid (.1%), phosphate emulsifiers	5.8 to 6.0	.94 to .95	160 °F for 30 s, hot filled	Loaves	Refrigerate	Refrigerate	10 mo	Challenge with C. botulinum, no toxin at 86 °F	Depends on formulation
Process cheese spread (53)	Cheese, butter, skim milk powder, whey, rennet casein, phosphate emulsifiers, salt (1.5 to 1.8%), lactic acid, water	5.5 to 5.6	53 to 62%	140 °F for 12 s, hot filled	Tubes	Refrigerate	Refrigerate	6 mo	Challenge with C. botulinum no toxin at 45, 55, 65, 86 F	Depends on formulation
Cheese blend (pizza) (54)	Sodium chloride 1.81%, nitrite level < 1ppm	5.56	0.978	Baked	—	—	Ambient	Use period (10 h)	Hazardous level S.aureus or B. cereus, no-toxin	Pass

* Model used unknown

Appendix C:
Scientific Data Used to Develop Framework

1. Determination of pH and water activity limits for TCS foods

To determine pH and water activity limits for TCS foods, as they are presented in the framework (see Chapter 8) the panel used data from the literature and from the results of the survey (see appendix B). Data for products with identified preservative systems were not included to assure that conservative data were used to determine limits. Professional judgment of the panel was used to omit unrealistic data. For example, studies using artificially high inoculum levels in laboratory media with no competitive microflora, using mild humectants (for example, glycerol) or acidulants (for example, HCl) were not used if results varied significantly with substantial data in food systems.

While numerous studies have been done on growth of foodborne pathogens, many studies do not report the pH and water activity of the growth medium. As a result, only limited data are available to study the interaction of pH and a_w on the growth of foodborne pathogens. The panel strongly encourages researchers to include pH and water activity data in scientific publications to assist the incorporation of data into analyses such as the one being performed here.

1.1. Spores

Data on water activity and pH interaction effects on growth or toxin production of foodborne pathogenic sporeformers are illustrated below. The lines indicate the parameter limits that the panel considered to develop the framework.

For foods that are treated to inactivate vegetative foodborne pathogens, the panel concluded that the following parameters effectively control the growth of sporeforming foodborne pathogens:
- pH = 4.6, or
- a_w = 0.92, or
- pH = 5.6 and a_w = 0.95

The panel believes that these parameters are conservative since numerous studies demonstrated lack of growth and/or toxin production above these levels. Products that fall in the non-TCS area should be considered in a "safe-harbor" that does not require time/temperature control. Products that fall in the potential TCS region may be stable depending on shelf life expectations, presence of preservatives, temperature, and other factors affecting growth (see Chapter 3). Challenge studies may be performed for foods in the high pH and a_w ranges and/or for those foods with extended shelf life expectations.

An equation to fit the data could also be used to identify pH and a_w combinations that would inhibit sporeforming pathogen growth; however, panel members believe that this would be more difficult to implement and/or communicate to non-technical users of the information.

1.2. Vegetative cells

Literature data on interaction of pH and water activity on control of vegetative pathogens is more limited than that for sporeformers. Published studies and modeling programs generally use broth media or foods with high a_w that are not near the minimum for growth. Studies conducted for short shelf life products are relevant to foodservice operators who are primarily interested in the

Figure 1—Spore growth or toxin production

Figure 2—Cell growth or toxin production

potential for pathogen growth in hours or a few days; however, these studies may not be applicable to extended shelf life products.

The following data were considered in developing the framework for vegetative pathogen control, in addition to the minimum pH and a_w values for vegetative pathogen growth (see Chapter 3). However, the panel believes that intended shelf life must be considered in addition to pH and a_w in determining the need for time/temperature control. The lines indicate the parameter limits that the panel considered to develop the framework.

References

Asplund K, Nurmi E. 1991. The growth of salmonella in tomatoes. Int J Food Microbiol 13:177-82.

Baird-Parker TC. 1990. The staphylococci—an introduction. J Appl Bacteriol Symposium Suppl:1S-8S. (cited in ICMSF 1996a).

Barbut S, Tanaka N, Maurer AJ. 1986. Effects of varying levels of chloride salts on *Clostridium botulinum* toxin production in turkey frankfurters. J Food Sci 51:1129-31. (cited in ICMSF 1996a).

Bartsch AG, Walker HW. 1982. Effect of temperature, solute and pH on the tolerance of *Clostridium perfringens* to reduce water activities. J Food Sci 47:1754-5.

Chung KC, Goepfert JM. 1970. Growth of *Salmonella* at low pH. J Food Sci 35:326-8.

Clavero MRS, Beuchat LR. 1996. Survival of *Escherichia coli* 0157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. Appl Environ Microbiol 62:2735-40.

Clavero MRS, Brackett RE, Beuchat LR, Doyle MP. 2000. Influence of water activity and storage conditions on survival and growth of proteolytic *Clostridium botulinum* in peanut spread. Food Microbiol 17:53-61.

Dodds KL. 1989. Combined effect of water activity and pH on inhibition of toxin production by *Clostridium botulinum* in cooked, vacuum-packed potatoes. Appl Environ Microbiol 55:656-60.

Glass KA, Doyle MP. 1991. Relationship between water activity of fresh pasta and toxin production by proteolytic *Clostridium botulinum*. J Food Prot 54:162-5.

Hall P. 2001. *Clostridium botulinum* toxin production in various foods. Personal communication.

Hauschild AHW, Hilscheimer R. 1979. Effect of salt content and pH on toxigenesis by Clostridium botulinum in caviar [proteolytic strains only]. J Food Prot 43:245-8. (cited in ICMSF 1996a).

[ICMSF] International Commission on Microbiological Specification for Foods. 1996. Microorganisms in foods. Volume 5, Characteristics of microbial pathogens. London: Blackie Academic & Professional. 513 p.

Ikawa JY. 1991. Clostridium botulinum growth and toxigenesis in shelf stable noodles. J Food Sci 56:264-5.

Ito KA, Chen JK, Lerke PA, Seeger ML, Unverferth JA. 1976. Effect of acid and salt concentrations in fresh-pack pickles on the growth of *Clostridium botulinum* spores. Appl Environ Microbiol 32:121-4. (cited in ICMSF 1996a).

Jakobsen M, Trolle G. 1979. The effect of water activity on growth of clostridia. Nord Vet-Med 31:206-13.

Kang CK, Woodburn M, Pagenkopf A, Cheney R. 1969. Growth, sporulation and germination of *Clostridium perfringens* in media of controlled water activity. Appl Microbiol 18:789-805. (cited in ICMSF 1996a).

Li K-Y, Torres JA. 1993. Water activity relationships for selected mesophiles and psychrotrophs at refrigeration temperatures. J Food Prot 56:612-5. (cited in ICMSF 1996a).

McClure PJ, Roberts TA, Otto Oguru P. 1989. Comparison of the effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plats and in liquid medium. Lett Appl Microbiol 9:95-9. (cited in ICMSF 1996a).

Miller AJ. 1992. Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. J Food Prot 55:414-18. (cited in ICMSF 1996a).

Notermans S, Heuvelman CJ. 1983. Combined effect of water activity, pH and suboptimal temperature on growth and enterotoxin production of *Staphylococcus aureus*. J Food Sci 48:1832-5, 40.

Petran RL, Aottola EA. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. J Food Sci 54(2):45-60. (cited in ICMSF 1996a).

Raevuori M, Genigeorgis C. 1975. Effect of pH and sodium chloride on growth of Bacillus cereus in laboratory media and certain foods [rice and meat data only]. Appl Microbiol 29:68-73.

Razavilar V, Genigeorgis C. 1998. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. Int J Food Microbiol 40:149-57.

Simpson MV, Smith JP, Dodds KL, Ramaswamy HS, Blanchfield B, Simpson BK. 1995. Challenge studies with *Clostridium botulinum* in a sous vide spaghetti and meat sauce product. J Food Prot 58:229-34.

Swanson KMJ. 2001. *Clostridium botulinum* toxin production in various foods. Personal communication.

Tienungoon S, Ratkowsky DA, McMeekin TA, Ross T. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. Appl Environ Microbiol 66:4979-87.

[USDA] U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Laboratory. 2001. USDA Pathogen Modeling Program Version 5.1.

Industry Protocol for Establishing the Shelf Stability of Pumpkin Pie

Introduction

This protocol provides a process that a manufacturer may use to demonstrate the shelf stability of a pumpkin pie product as per the Food Code sections 1-210.10B(61)(a) and (61)(c)(v).

"(61)(a) 'Potentially hazardous food' means a food that is natural or synthetic and that requires temperature control because it is in a form capable of supporting:

(i) the rapid and progressive growth of infectious or toxigenic microorganisms;

(ii) the growth and toxin production of *Clostridium botulinum*. . ."

The Food Code goes on to state that a 'potentially hazardous food' does not include:

(61)(c)(v). A food for which laboratory evidence demonstrates that the rapid and progressive growth of infectious or toxigenic microorganisms can not occur, . . . and that may contain a preservative, other barrier to the growth of microorganisms, or a combination of barriers that inhibit the growth of microorganisms;

Note: The above definition is exerpted from The Model Food Code section 1-201.10B(61). The complete definition as it appears in the Food Code is in Appendix 3 of this document.

It is the responsibility of the manufacturer to produce and distribute a safe food product. Product and process validation are complex issues with no single method that will work in all cases. Therefore, this protocol is a guide and does not replace good science or good judgment applied by the manufacturer to their product and process. Furthermore, this protocol does not limit the ability of the manufacturer to develop additional data beyond those described in this protocol to demonstrate the safety of their food product. Pumpkin pie products that fail to meet the criteria of this protocol must be refrigerated during distribution and retail display to maintain food safety unless shelf stability has been established through an equivalent science-based method of process validation.

Scope

This protocol applies only to pumpkin pies intended for distribution and display at retail at ambient temperatures without refrigeration. Any manufacturer of shelf stable pumpkin pie products can use the criteria of this protocol. A 'manufacturer' is defined as any establishment that bakes a pumpkin pie that is distributed or displayed at retail without refrigeration.

Objective

The objective of this protocol is to define the product and process criteria that a manufacturer may use to establish that their pumpkin pie product meets the requirements of the Model Food Code sections 1-210.10B(61)(a) and (61)(c)(v) and is therefore safe for distribution and retail display without refrigeration. See Appendix 2 for the basis of how this protocol builds on the Model Food Code.

Criteria 1: Compliance with Good Manufacturing Practices (GMPs)

Requirement

The manufacturer must maintain and demonstrate compliance with all applicable GMP requirements in the manufacture of the pumpkin pie product as identified in 21 CFR:

Part 110.10 Personnel
(a) Disease control
(b) Cleanliness
(c) Education and training
(d) Supervision
Part 110.20 Plant and grounds
(a) Grounds
(b) Plant construction and design
Part 110.35 Sanitary operations
(a) General maintenance
(b) Substances used in cleaning and sanitizing; storage of toxic materials.
(c) Pest control
(d) Sanitation of food-contact surfaces
(e) Storage and handling of cleaned portable equipment and utensils
Part 110.37 Sanitary facilities and controls
(a) Water supply
(b) Plumbing
(c) Sewage disposal
(d) Toilet facilities
(e) Hand-washing facilities
(f) Rubbish and offal disposal
Part 110.40 Equipment and utensils
Appendix D
Part 110.80 Processes and controls
Part 110.110 Natural and unavoidable defects in food for human use that present no health hazard.

Validation

The manufacturer is responsible for maintaining and demonstrating via inspection their compliance with GMP requirements.

Rationale

Complying with GMPs is a required step in developing a safe

and sanitary production process that is capable of manufacturing a wholesome food product. Compliance to GMPs is the foundation upon which the other criteria of this protocol are built and without which this protocol cannot be successful.

Criteria 2: Internal Bake Temperature

Requirement

The pumpkin pie product must be baked to achieve an internal temperature of at least 180° F (82° C) at the coolest point in the product.

Validation

Perform a process validation study to identify variation in internal product temperature after baking due to location in the oven and to identify the coolest point in the product. Repeat this process validation study when significant changes are made to the product or baking process. Use this process study to establish a routine temperature validation test and perform that test periodically on product immediately after bake to validate that the internal temperature reaches at least 180° F (82° C) at the coolest point. Demonstrate compliance through periodic temperature measurements taken and documented during processing or through effective alternative means defined by the manufacturer.

Rationale

Exposing a food to a temperature of 165° F (74° C) for 15 seconds results in an effective destruction of vegetative microorganisms (See 1999 Model Food Code Section 3-401.11(A)(3)). The requirement of 180° F (82° C) provides a 15 degree safety buffer to assure that an adequate kill of vegetative microorganisms is achieved. Pumpkin pies are relatively dense and uniform in structure resulting in a slow but uniform heat penetration. The performance of a process validation study assures that the manufacturer understands the baking characteristics of the oven and the product and has established a routine test that assures that adequate heat penetration has been achieved in all parts of the pie.

Criteria 3: Cooling and Packaging Baked Product

Requirement

The baked product must be cooled to ambient temperature with adherence to GMPs and maintenance of hygienic personnel practices in order to minimize post-bake contamination. Pies must be adequately cooled before packaging to minimize moisture condensation on the inside of the package.

Finished product must be packaged within 4 hours of leaving the oven in a clean, protective package. The package must fully enclose and protect the finished product from environmental contamination and inadvertent human contact during distribution and display. The package must contain air and must not be vacuum-packed or flushed with non-oxygen containing gases.

Validation

Perform the following:
● Confirm that the package completely encloses the product to protect it against contamination during distribution and display.
● Confirm that the package is sealed to prevent unintentional opening during distribution and display.
● Confirm that the product is packaged and sealed within 4 hours of leaving the oven.

Rationale

When the product exits the oven it is essentially free of vegetative microorganisms. Assure the safety of the product by controlling and minimizing the introduction of spoilage bacteria onto the product during cooling and packaging. The package protects the product from contamination during distribution and display. The package must contain air and must not be vacuum-packed or flushed with non-oxygen containing gases.

Table 1

Microbiological tests of product within 24 hours of packaging	Test method reference*
Aerobic plate count	AOAC/BAM
Coagulase positive *Staphylococcus aureus*	AOAC/BAM
Coliforms	AOAC/BAM
Salmonella	AOAC/BAM
Oxidation reduction potential	Test filling as is

*or equivalent standardized method

Table 2

Microbiological tests of product at the end of product use life [stored at 90 °F (32 °C)]	Test method reference*
Aerobic plate count	AOAC/BAM
Coagulase positive *Staphylococcus aureus*	AOAC/BAM
Coliforms	AOAC/BAM
Salmonella	AOAC/BAM

*or equivalent standardized method

Table 3—Finished Product Microbiological Test Limits

Microbiological test	Microbial limits For intact product < 24 h after packaging	Microbial limits for intact product at the end of product use life [stored at 90 °F (32 °C)]
Aerobic plate count (APC)	1000 cfu per gram	100,000 cfu per gram
Coagulase positive *S. aureus*	<10 per gram	<10 per gram
Coliforms	<10 per gram	<10 per gram
Salmonella	Negative per 125 grams	Negative per 125 gram
Oxidation reduction potential	>100 mv	NA

tive microorganisms. Assure the safety of the product by controlling and minimizing the introduction of spoilage bacteria onto the product during cooling and packaging. The package protects the product from contamination during distribution and display. The result is a wholesome, safe product delivered to the consumer.

Criteria 4: Microbial Analysis for Process Validation

Requirement

Microbial testing is performed using standardized methods at the beginning and end of the intended product use life to validate that the manufacturing process is capable of producing a wholesome pumpkin pie product that is microbiologically stable for distribution and display without refrigeration.

Perform the following microbiological analyses shown in Table 1 on representative samples of intact, finished, packaged product within 24 hours of being packaged.

Store additional samples of intact, finished, packaged product at 90 °F (32 °C) for the duration of the product's intended use life. See Appendix 1 for the definition of product use life.

Set up the microbiological analyses shown in Table 2 on representative samples of intact, finished, packaged product after storage at 90 °F (32 °C) for the duration of the product's intended use life.

The acceptance limits for the microbiological testing of the fin-

ished product at the beginning and end of product use life are as follows in Table 3.

The pumpkin pie product must be shown to not support the growth or toxin production of *Clostridium botulinum*. This is done by measuring the Oxidation Reduction Potential of the pie filling after baking and achieving an acceptably high positive value.

Validation

Microbiological testing of the finished pumpkin pie product must be performed initially to validate the process and repeated if any significant changes are made in the ingredients, process, packaging, distribution or display of the product. Results of these tests must be documented.

The packaged pumpkin pie product must be stored after baking under ambient atmospheric conditions with no modified atmosphere or vacuum packing. The normal ambient oxygen concentration and the resulting oxidation-reduction potential in the pie are inhibitory to the growth and toxin production of the anaerobic bacterium *Clostridium botulinum*.

Rationale

The microbial testing performed as part of this protocol will enumerate the total level of microorganisms, and selective pathogenic species, if present in a finished pumpkin pie product at the beginning and the end of the product use life.

The thermal treatment during the baking process effectively kills vegetative bacterial cells and would destroy the toxin from *Clostridium botulinum* if it were present. Since the finished pumpkin pie is cooled, packaged and stored at normal ambient atmospheric conditions, the oxygen concentration (O_2), which is measured by the Oxidation Reduction Potential test, creates an environment that is inhibitory to the growth and toxin production of *Clostridium botulinum*.

These analyses, when combined with the other criteria of this protocol, demonstrate the adequacy of the manufacturing process to produce a wholesome finished product that will be microbiologically stable during the product's intended use life.

- **At the beginning of product life**: Low microbial counts at the beginning of product life confirm preparation under hygienic conditions. An Oxidation Reduction Potential of at least 100 mv indicates that the product will not support the growth of anaerobic bacteria.
- **At the end of product life**: Moderate APC counts (< 2 log increase) at the end of product life indicate that there are not unsafe levels of microbial growth. High microbial counts at the end of product life indicate that the product is not stable. The limits shown in Table 3 are conservative from food safety and epidemiological perspectives and are based on industry experience.
- **90 °F (32 °C) Storage temperature**: Storing the product for its intended use life at 90 °F (32 °C) provides an additional safety factor to assure that the product will be safe and wholesome. 90 °F (32 °C) is a conservatively high temperature that takes into account potential distribution, shelf display and home storage temperatures.

Criteria 5: Finished Product Shelf Life Labeling

Requirement

Manufacturers and/or retailers must label their pumpkin pies intended for distribution or display at retail without refrigeration with either "sell by . . . " or "use by . . . " dating to inform consumers of the intended use life of the product. Manufacturers and/or retailers are encouraged to label a "use by . . . " date.

The purpose of "sell by . . . " or "use by . . . " dating is to inform consumers of the intended use life of the product and to ensure proper rotation and disposal of past age product.

The manufacturer determines the intended product use life based on product stability studies that take into account the microbial stability of the product (Criteria 4 of this protocol) as well as other factors such as flavor and texture. The manufacture may establish an intended product use life that is shorter than indicated by microbial testing due to sensory factors such as taste and texture.

Manufacturers must label their pumpkin pie products that meet the criteria of this protocol with the instructional phrase "**Refrigerate after opening**" to advise consumers to refrigerate the pie after opening to protect against unintentional contamination of the product. The statement must be printed on a prominent display panel.

Manufacturers must also provide a mark on the package to inform regulators and retail store personnel that the product meets the criteria of this protocol and is safe for distribution and retail display at room temperature without refrigeration. The required mark is "**RT**", which stands for 'Room Temperature'. This symbol must be printed on the package label immediately after the 'sell by' or 'use by' date, in the same size type.

Validation

Confirm the presence of "use by . . . " or "sell by . . ." dating and the instructional phrase on the package.

Rationale

"Use by. . ." or "sell by. . ." dating is marked on the package to communicate to the consumer the fact that shelf stable pumpkin pies have a limited and short intended use life based on product stability testing.

The instruction to the consumer to "refrigerate after opening" provides protection from unintentional contamination of the product during handling in the home. "Refrigerate after opening" is a technically sound and due diligence approach to assist the consumer in maintaining and consuming a safe and wholesome product within the intended product use life.

The symbol "RT" on the package immediately following the "sell by" or "use by" date, informs the regulator and store personnel that the product meets the criteria of this protocol and is safe for room temperature display without refrigeration.

Appendix 1 – Intended Product Use Life

Define product use life as:

- if "**use by . . .** " dating is provided on the retail package, the intended product use life is the length of time from when the product is placed into the final retail package until midnight on the date printed on the retail package. For an extended production run, use the length of time from the earliest start time until midnight of the date printed on the retail package.
- if "**sell by . . .** " dating is provided on the retail package, the product use life must be at least as long as the following calculation: multiply by 1.3 the length of time from when the product is placed into the final retail package until midnight on the date printed on the package. For an extended production run, use the length of time from the earliest start time.

The factor of 1.3 represents a reasonable length of time beyond the "sell by . . . " date for consumption of the product. The National Institute of Standards and Technology (NIST) Handbook 130, considers "a reasonable period for consumption to be one third of the appropriate total shelf life of the perishable food."

Appendix 2 – Basis for Establishing the Shelf Stability of Pumpkin Pie Products Under the Model Food Code

The Industry Protocol for Establishing the Shelf Stability of

Pumpkin Pie provides a methodology for establishing that specific pumpkin pie products can be safely held by food establishments at room temperature and require no refrigeration prior to purchase by consumers under the Model Food Code (MFC).

1. General Scope of Application of the Model Food Code

Pursuant to section 311(a) of the Public Health Service Act, which provides for Federal assistance to states with respect to the "prevention and suppression of communicable diseases," FDA maintains the MFC to assist states in establishing effective programs to prevent foodborne illness. 42 U.S.C. 243;21 C.F.R. 5.10(a). Specifically, the MFC is intended "to assist food control jurisdictions at all levels of government by providing them with a scientifically sound technical and legal basis for regulating the retail segment of the food industry." Model Food Code 1999 at ii. Accordingly, the MFC does not itself constitute a federal law or regulation, but rather FDA recommendations to the States concerning the safe handling of food by food establishments at the retail level. The Model Food Code and related interpretive guidance documents serve to inform the development of regulatory requirements in the States.

Refrigeration of Foods Held for Sale by Food Establishments

The MFC provides for refrigeration of foods held for sale in food establishments when foods are deemed to be "potentially hazardous." Cognizant of the complexities of food matrices and food handling and their relationships to food safety, the MFC does not attempt to define the category by describing foods that are "potentially hazardous." Rather, the MFC takes a factually based approach which focuses specifically on whether the particular "form" of any food held for sale in a food establishment, in fact, requires temperature control for that food to be safely delivered to into the consumer's hands.

Specifically, section 1-210.10B(61) of the MFC provides that "potentially hazardous food" is a "food that is natural or synthetic and that requires temperature control because it is in a form capable of supporting . . . the rapid and progressive growth of infectious or toxigenic microorganisms; [or] . . . the growth and toxin production of *Clostridium botulinum* . . ." Model Food Code 1999 at 1-210.10B(61)(a). For heat-treated foods of plant or animal origin, the definition specifically excludes those forms that "are modified in a way that results in mixtures that do not support [such] growth" of unsafe microorganisms. Id. At 1-210.10B(61)(b). The definition further provides a selected list of criteria that may be used to establish that particular forms of food are not "potentially hazardous" and require no refrigeration in food establishments to be delivered safely into the hands of consumers. Section 1-210.10B(61)(c)(v) specifically provides that "laboratory evidence" may be used to demonstrate that unsafe microbial growth "can not occur" in the particular food because of barriers including the use of preservatives, or "combination[s] of barriers that inhibit the growth of microorganisms." Other listed criteria specifically recognize that such "barriers" to unsafe microbial growth may not only be chemical (e.g., preservatives), but may include physical barriers to environmental contamination. See Model Food Code 1999 at 1-210.10B(61)(c)(i) and (iv)(excluding certain packaged food, and shell eggs based on the natural barrier provided by the intact shell). This provision excludes from the "potentially hazardous food" category, and thus the need for refrigeration by food establishments, specific forms of food presented for consumer sale for which effective chemical and physical barriers to unsafe microbial growth have been erected, as demonstrated by laboratory evidence.

In contrast, a wholly separate basis for exclusion from the "potentially hazardous food" definition applies when chemical and physical barriers cannot be demonstrated to block unsafe microbial growth during the product shelf life. Section 1-210.10(B)(61)(c)(vi) provides that a food that contains unsafe microorganisms may nonetheless be excluded from the "potentially hazardous food" category when evidence shows that food "does not support the growth of microorganisms . . . at a level sufficient to cause illness." Such a showing might be made through evidence showing that unsafe microorganisms do not grow to unsafe levels during the product shelf life. Section 1-210.10B(61)(c)(vi) may provide a separate potential basis for establishing the shelf stability of pumpkin pie products that do not conform with the Industry Protocol.

This Protocol specifies manufacturing, heat-treatment, and post-bake handling practices that have been established scientifically to render pumpkin pie safe to be held by food establishments for sale to consumers without refrigeration during the shelf life of the product. The Industry Protocol also specifies laboratory methods and procedures that should be used by manufacturers under section 1-210.10B(61)(c)(v) to develop laboratory evidence demonstrating that "the rapid and progressive growth of infectious or toxigenic microorganisms . . . or *C. botulinum* can not occur" in pumpkin pie products manufactured, distributed, and sold to consumers in conformance with this Protocol. Model Food Code 1999 at 1-210.10B(61)(c)(v). The Industry Protocol constitutes a basis for establishing that particular pumpkin pie products are excluded from the definition of "potentially hazardous food," and can be held by food establishments without refrigeration during the shelf life of the product under the MFC.

Appendix 3—The Model Food Code definition of "Potentially Hazardous Food"

(61) Potentially Hazardous Food.

(a) **"Potentially hazardous food"** means a FOOD that is natural or synthetic and that requires temperature control because it is in a form capable of supporting:

(i) The rapid and progressive growth of infectious or toxigenic microorganisms;

(ii) The growth and toxin production of *Clostridium botulinum*; or

(iii) In raw shell eggs, the growth of **Salmonella** Enteritidis.

(b) **"Potentially hazardous food"** includes an animal FOOD (a FOOD of animal origin) that is raw or heat-treated; a FOOD of plant origin that is heat-treated or consists of raw seed sprouts; cut melons; and garlic-in-oil mixtures that are not modified in a way that results in mixtures that do not support growth as specified under Subparagraph (a) of this definition.

(c) ***"Potentially hazardous food"*** *does not include:*

(i) An air-cooled hard-boiled egg with shell intact;

(ii) A FOOD with an aw value of 0.85 or less;

(iii) A FOOD with a pH level of 4.6 or below when measured at 24° C (75° F);

(iv) A FOOD, in an unopened HERMETICALLY SEALED CONTAINER, that is commercially processed to achieve and maintain commercial sterility under conditions of nonrefrigerated storage and distribution;

(v) A FOOD for which laboratory evidence demonstrates that the rapid and *progressive growth of infectious or toxigenic microorganisms or the growth of **S.** Enteritidis in eggs or **C. botulinum** can not occur, such as a FOOD that has an a_w and a pH that are above the levels specified under Subparagraphs (c)(ii) and (iii) of this definition and that may contain a preservative, other barrier to the growth of microorganisms, or a combination of barriers that inhibit the growth of microorganisms; or*

(vi) A FOOD that does not support the growth of microorganisms as specified under Subparagraph (a) of this definition even though the FOOD may contain an infectious or toxigenic microorganism or chemical or physical contaminant at a level sufficient to cause illness.

Non-potentially hazardous foods

**NSF International Standard/
American National Standard**

NSF®

NSF International, an independent, not-for-profit, non-governmental organization, is dedicated to being the leading global supplier of public health and safety-based risk management services serving the interests of all stakeholders.

NSF International
American National Standard
for Non-Potentially Hazardous Foods

Non-potentially
hazardous foods

Standards Developer
NSF International

Adopted November 8, 2000
NSF International Board of Directors

Designated as an ANSI Standard
November 8, 2000
American National Standards Institute

Prepared by
Non-Potentially Hazardous Foods and
Task Group for Microbiological Challenge Testing

Recommended for adoption by
The NSF Council of Public Health Consultants

Adopted by
The NSF Board of Directors
November 2000

Disclaimers[1]

NSF, in performing its functions in accordance with its objectives, does not assume or undertake to discharge any responsibility of the manufacturer or any other party. The opinions and findings of NSF represent its professional judgment. NSF shall not be responsible to anyone for the use of or reliance upon this Standard by anyone. NSF shall not incur any obligation or liability for damages, including consequential damages, arising out of or in connection with the use, interpretation of, or reliance upon this Standard.

NSF Standards provide basic criteria to protect the public health.

Participation in NSF Standards development activities by regulatory agency representatives (federal, local, state) shall not constitute their agency's endorsement of NSF or any of its Standards.

Preference is given to the use of performance criteria measurable by examination or testing in NSF Standards development when such performance criteria may reasonably be used in lieu of product design and information.

The illustrations, if provided, are intended to assist in understanding their adjacent standard requirements. However, the illustrations may not include *all* requirements for a specific method.

Unless otherwise referenced, the annexes are not considered an integral part of NSF Standards. The annexes are provided as general guidelines to the manufacturer, regulatory agency, user, laboratory, or certifying organization.

---

[1] The information contained in this Disclaimer is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this Disclaimer may contain material that has not been subjected to public review of a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

This page is intentionally blank.

Contents

This page is intentionally blank.

Foreword[2]

The purpose of NSF/ANSI 75 is to serve as a communication tool between manufacturers of product, retailers, and public health officials. This Standard provides test methods and evaluation criteria to allow for the determination that a food product meets FDA Food Code criteria for a "non-potentially hazardous food" and does not require refrigeration for safety. The Standard is intended to provide the mechanism for laboratory evidence to demonstrate that rapid and progressive growth of infectious or toxigenic microorganisms cannot occur. The Standard does not provide a means or methodology for determining whether a food has been adulterated. In fact, a food may be unsafe due to adulteration and still meet the criteria to be considered non-potentially hazardous per the definition in the FDA Food Code.

This Standard applies only to those items outlined in the scope. The scope of NSF/ANSI 75 has been carefully defined to include only a subset of non-potentially hazardous products, for which laboratory demonstration that the rapid and progressive growth of infectious or toxigenic microorganisms cannot occur, is routinely requested by retailers, regulators, and manufacturers.

The Standard also includes a list of products that have been specifically excluded from the scope. Some of these products are excluded because retailers and public health officials have not questioned whether these products should be refrigerated, such as white bread; bagels; donuts; muffins; individually preportioned, pre-wrapped snack cakes; and fruit filled pastries. Other products, such as those using modified atmosphere packaging, are excluded because they have been made shelf stable by their packaging and not by their formulation only. This Standard does not address specialty-packaging techniques.

Suggestions for improvement of this Standard are welcome. Comments should be sent to Chair, Joint Committee on Non-potentially Hazardous Foods, c/o NSF International, Standards Department, P.O. Box 130140, Ann Arbor, Michigan, 48113-0140, USA.

---

[2] The information contained in this Foreword is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this Foreword may contain material that has not been subjected to public review of a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

This page is intentionally blank.

NSF/ANSI Standard
for Non-Potentially Hazardous Foods —

Non-potentially hazardous foods

1 General

1.1 Purpose

This Standard will provide test methods and evaluation criteria to allow for the determination that a product does not require storage in a refrigerator for safety. This Standard is intended to provide the mechanism for laboratory evidence to demonstrate that the rapid and progressive growth of infectious or toxigenic microorganisms can not occur. This Standard does not provide a means or methodology for determining whether a food product has been adulterated. This Standard is intended only to be applied to the items indicated in the scope. It is not implied that products excluded from the scope of this Standard are innately safe.

1.2 Scope

This Standard contains requirements for food products that:

– are intended to be held without temperature controls during transportation, holding, display, sale, or use; and

– are considered to be potentially hazardous; and

– are rendered non-potentially hazardous by formulation or through a manufacturing process or both; and

– are included in one of the following categories:

I	Specialty breads or pastries containing fresh, canned, frozen, or rehydrated vegetables or soft cheeses added prior to baking.
II	Bakery products including specialty breads or pastries filled or topped with cream, creme, custard, or cheese after baking.
III	Products filled prior to baking such as pumpkin, sweet potato, custard or meringue pies.
IV	Toppings, glazes, icings, or fillings stored without temperature control prior to use in other products.

The scope of this Standard also includes food products which meet the above requirements and are a component of a food product, are processed into a finished fully assembled form for sale or use by a food establishment, or have been processed into an unfinished, fully assembled form and are intended to be finished at a food establishment for sale or use by the food establishment.

1.3 Exclusions from the scope

This Standard does not apply to food products specified by the manufacturer for storage without temperature control for less than 24 hours or for 31 days or more. This Standard does not apply to meat, poultry, or seafood products or products which are a mixture of garlic with butter, margarine or oil.

The following bakery products are excluded from the scope of this Standard:

– bakery products containing only dehydrated vegetables;

– bakery products containing fruit fillings, such as fruit pies and pastries;

– individually pre-portioned and wrapped, fruit-filled, creme-filled, and/or frosted cake products;

– traditional breads and pastries including white bread, bagels, donuts, or muffins;

– fruit fillings such as fruit pie filling; and

– food products which are rendered non-potentially hazardous by special packaging (for example modified atmosphere packaging).

2   Normative references

The following document contains provisions that, through reference in this text, constitute provisions of this Standard. At the time this Standard was written, the edition indicated was valid. All documents are subject to revision, and parties are encouraged to investigate the possibility of applying the recent edition of the document indicated below.

AOAC International[3], Food and Drug Administration, *Bacteriological Analytical Manual*, eighth edition (1995)

AOAC International[3], *Official Methods of Analysis*, sixteenth edition (1995)

APHA[4], *Compendium of Methods for the Microbiological Examination of Foods*, third edition, 1992

FDA[5], *Food Code 1999 Recommendations of the United States Public Health Service Food and Drug Administration*

---

[3] AOAC International, 481 N. Frederick Avenue, Suite 500, Gaithersburg, MD 20877

[4] American Public Health Association (APHA), 1015 Fifteenth Street NW, Washington, DC 20005

[5] U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Washington, DC 20204

3   Definitions

Terms used in this Standard that have special technical meaning are defined here.

3.1 challenge organism: A pathogenic organism which is intentionally inoculated into food for the purpose of testing whether the food will support growth of the organism within a specified time frame when stored without temperature control.

3.2 component: A food consisting of one or more ingredients which is intended to be used as part of a food product, e.g., a topping or filling for a pie.

3.3 consumer: A person who takes possession of a food product and is not functioning in the capacity of an operator of a food establishment or food processing plant.

3.4 finished product: A fully prepared, ready-to-eat food product.

3.5 food: Any raw, cooked, or processed edible substance, beverage, or ingredient intended for human consumption.

3.6 food establishment: An operation that stores, prepares, packages, serves, vends, sells, or otherwise provides food for human consumption.

3.7 fully assembled food product: A product in which all ingredients and components are combined by the manufacturer.

3.8 homogeneous product: A product having a uniform texture and content.

3.9 interface: A point at which two or more distinct components or ingredients meet.

3.10   lot: A quantity of product made with the same ingredients utilizing the same equipment in a continuous fashion at a specified manufacturing time.

3.11   manufacturer: The commercial operation that produces or manufactures the product.

3.12   master batch: A quantity of product which has been mixed with the inoculum to achieve the desired level of inoculation of challenge organisms, which will then be divided into individual samples for challenge testing.

3.13   organoleptic: Related to sensory evaluation including appearance, texture, aroma, and/or taste as related to product quality.

3.14   pH: The negative logarithm of the hydrogen ion concentration.

3.15   potentially hazardous food:

a)   A food that is natural or synthetic and that requires temperature control because it is in a form capable of supporting: the rapid and progressive growth of infectious or toxigenic microorganisms; the growth and toxin production of *Clostridium botulinum*; or in raw shell eggs, the growth of *Salmonella enteritidis.*

b)   Potentially hazardous food includes an animal food (a food of animal origin) that is raw or heat-treated, a food of plant origin that is heat treated or consists of raw seed sprouts; cut melons; and garlic and oil mixtures that are not acidified or otherwise modified at a food processing plant in a way that results in mixtures that do not support growth as specified in (a).

c)   Potentially hazardous food does not include:

–   an air-cooled hard-boiled egg with shell intact;

–   a food with a water activity (a_w) value of 0.85 or less;

–   a food with a pH level of 4.6 or less when measured at 24 °C (75 °F);

–   a food, in an unopened hermetically sealed container, that is commercially processed to achieve and maintain commercial sterility under conditions of nonrefrigerated storage and distribution;

–   a food for which laboratory evidence demonstrates that the rapid and progressive growth of infectious or toxigenic microorganisms or the growth of *S. enteritidis* in eggs or *C. botulinum* cannot occur, as defined previously in this section and that may contain a preservative, other barrier to the growth of microorganisms, or a combination of barriers that inhibit the growth of microorganisms; or

–   a food that does not support the growth of microorganisms as specified under part (a) of this definition even though the food may contain an infectious or toxigenic microorganism or chemical or physical contaminant at a sufficient level to cause illness.

3.16   special packaging: Packaging and related processing that may be used to render a product non-potentially hazardous.

3.17   temperature control: Maintaining a food product at a temperature of 60 °C (140 °F) or more or a temperature of 5 °C (41 °F) or less.

3.18   unfinished product: A product that requires further preparation before it is consumed.

3.19   water activity (a_w): A measure of the free moisture in a food equal to the ratio of the water vapor pressure of a substance to the vapor pressure of pure water at the same temperature.

4   Labeling and literature requirements

If a product is intended to be finished by a food establishment before being sold or used as a non-potentially hazardous food product, the manufacturer shall provide the food establishment with written instructions for handling and preparing the product to its finished form and for labeling the product as having met this Standard.

5   Product requirement

A non-potentially hazardous food product shall:

–   have a pH of 4.6 or less as demonstrated in accordance with 6.1; or

–   have a water activity of 0.85 or less as demonstrated in accordance with 6.2; or

–   not support the rapid and progressive growth of infectious and toxigenic microorganisms as demonstrated in accordance with 6.3.

6   Test methods

Each component of products in Categories I, II, and III shall be tested to determine the pH in accordance with 6.1. Each component shall be tested to determine the water activity in accordance with 6.2. If each component has a pH of 4.6 or less or each component has a water activity of 0.85 or less, then no further testing is required. If the product does not meet the acceptance criteria in 6.1.3 or 6.2.3, the product shall be tested in accordance with 6.3. The temperature during testing shall be 24 ± 2 °C (75 ± 3 °F). The relative humidity during testing shall be 45%-70%.

Products in Category IV shall be tested to determine the pH in accordance with 6.1. Products in Category IV shall be tested to determine the water activity in accordance with 6.2. If the product has a pH of 4.6 or less or a water activity of 0.85 or less, then no further testing is required. If the product does not meet the acceptance criteria in 6.1.3 or 6.2.3, the product shall be tested in accordance with 6.3. The temperature during testing shall be 24 ± 2 °C (75 ± 3 °F). The relative humidity during testing shall be 45%-70%.

The products shall be tested in accordance with figure 1. If pH is the only factor rendering the product non-potentially hazardous, the product shall be tested in accordance with 6.1. If water activity is the only factor rendering the product non-potentially hazardous, it shall be tested in accordance with 6.2. If other factors, including a combination of reduced pH and water activity, render the product non-potentially hazardous, it shall be tested in accordance with 6.3. Annex A is informative and provides a flowchart of the test methods.

6.1 pH testing

6.1.1   Product requirement

If pH is the sole factor to render the product non-potentially hazardous, the pH of the food product shall be 4.6 or less.

6.1.2   Test method

If the product is in an unfinished form, the product shall be prepared according to the manufacturer's instructions except that the product shall be baked/cooked for 100% of the manufacturer's lowest recommended time and at the manufacturer's lowest recommended temperature.

The pH of three representative product samples shall be measured. If the product is non-homogeneous, the pH of three samples of each component shall be measured.

The pH shall be measured in accordance with the methods in sections 8.6 and 8.7 of the *Compendium of Methods for the Microbiological Examination of Foods* at 24 ± 2 °C (75 ± 3 °F) using an instrument with an accuracy of ± 0.01 pH unit or better.

6.1.3   Acceptance criteria

The pH of each sample of each component measured shall be 4.6 or less.

6.2   Water activity testing

6.2.1   Product requirement

If water activity is the sole factor to render the product non-potentially hazardous, the water activity of the food product shall be 0.85 or less.

6.2.2   Test method

If the product is in an unfinished form, the product shall be prepared according to the manufacturer's instructions except that the product shall be baked/cooked for 100% of the manufacturer's lowest recommended time at the manufacturer's lowest recommended temperature.

The water activity of three representative product samples shall be measured. If the product is non-homogeneous, the water activity of three samples of each component shall be measured.

The water activity shall be measured in accordance with the methods in sections 8.1 - 8.5 of the *Compendium of Methods for the Microbiological Examination of Foods* at 24 ± 2 °C (75 ± 3 °F) using an instrument capable of achieving a standard deviation of ± 0.005 or less.

6.2.3   Acceptance criteria

The water activity of each sample of each component measured shall be 0.85 or less.

4

6.3 Microbiological challenge test

6.3.1   Test duration and time points

This test shall be conducted to evaluate if a food product is capable of supporting the rapid and progressive growth of a composite of 5 strains of challenge organisms inoculated into the product. Prior to testing, the pH and water activity of the product shall be measured in accordance with 6.1 and 6.2.

The challenge organisms for a particular product shall be determined in accordance with table 1. The product shall be evaluated for 1.3 times the length of time specified by the manufacturer that the product in its finished form may be stored outside of temperature control without special packaging (any partial day shall be rounded up to a full day). The time points shall be determined in accordance with table 2. The t_o organism counts shall be determined two hours after inoculation.

6.3.2   Preparation of inocula

A separate inoculum shall be prepared for each genus of challenge organisms. The inoculum for each organism shall contain a mixture of the five strains specified in table 3. All test strains shall be obtained directly from the ATCC or relevant source and revived, if necessary, according to the instructions provided with the culture. Cultures shall be maintained according to standard laboratory practices for culture maintenance.

As required, the inocula for *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *and Staphylococcus aureus* shall be prepared in accordance with 6.3.2.1. The inoculum for *Bacillus cereus* shall be prepared in accordance with 6.3.2.2. The inoculum for *Clostridium perfringens* shall be prepared in accordance with 6.3.2.3.

6.3.2.1 Preparation of the inoculum for *Salmonella* spp., *Listeria monocytogenes, Escherichia coli,* or *Staphylococcus aureus*

A cell suspension shall be prepared for each strain in the inoculum. Each cell suspension shall be prepared by the following method: a pure culture of the strain shall be inoculated into one or more tubes of Trypticase Soy Broth. The broth shall be incubated for 24 ± 2 h at 35 ± 2 °C (95 ± 3 °F). The cell suspensions from each strain shall be mixed to prepare an inoculum, which contains an approximately equal number of cells of each

strain. As necessary, the suspensions may be diluted in Butterfield's Phosphate Buffer dilution water prior to determining the concentration of the inoculum. The concentration of the inoculum shall be standardized using the spread plating method.

6.3.2.2 Preparation of the inoculum for *Bacillus cereus*

A suspension containing both vegetative cells and spore forms shall be prepared for each strain in the inoculum. Each suspension shall be prepared by the following method: a pure culture of the strain shall be inoculated into one or more tubes of Trypticase Soy Broth. The broth shall be incubated for 24 ± 2 h at 35 ± 2 °C (95 ± 3 °F). After incubation, the broth shall be used to inoculate the surface of Tryptic Soy Agar (TSA). The agar shall be incubated for 24 ± 2 h at 35 ± 2 °C (99 ± 3 °F). The suspensions from each strain shall be mixed to prepare an inoculum, which contains an approximately equal number of cells of each strain. As necessary, the suspensions may be diluted in Butterfield's Phosphate Buffer dilution water prior to determining the concentration of the inoculum. The concentration of the inoculum shall be standardized using the spread plating method.

6.3.2.3 Preparation of the inoculum for *Clostridium perfringens*

A suspension containing both vegetative cells and spore forms shall be prepared for each strain in the inoculum. Each suspension shall be prepared by the following method: A pure culture of the strain shall be inoculated into one or more tubes of Fluid Thioglycollate Broth. The broth shall be incubated for 48 to 96 h at 35 ± 2 °C (95 ± 3 °F). At 48 h, 72 h and 96 h, microscopic examination of the broth shall be performed to determine whether free spores or cells with prespores are present. When the broth contains 10%-50% free spores and cells with prespores, incubation is complete. The suspensions from each strain shall be mixed to prepare an inoculum, which contains an approximately equal number of cells of each strain. As necessary, the suspensions may be diluted in Butterfield's Phosphate Buffer dilution water prior to determining the concentration of the inoculum. The concentration of the inoculum shall be standardized using the spread plating method.

6.3.3   Preparation and inoculation of samples

If the product is in an unfinished form, the product shall be prepared according to the manufacturer's instructions except that the product shall be baked/cooked for 100% of the manufacturer's lowest recommended time at the manufacturer's lowest recommended temperature.

When necessary, the product shall be brought to a temperature of $24 \pm 2\,°C$ ($75° \pm 3\,°F$) before samples are prepared.

6.3.3.1 Control samples

At a minimum, two control samples shall be prepared to evaluate changes in the pH, water activity, aerobic plate count, and yeast and mold count of the product during the test period.

To prepare control samples for products in Categories I, II, and III, the sample shall be sliced into a sufficient number of slices to evaluate the sample for the presence of the challenge organisms occurring naturally and to evaluate the sample for the above parameters at the first time point (Day 0) and at the last time point. The sample slices shall be repackaged according to the procedures in 6.3.3.2.4.1.

To prepare each control sample for products in Category IV, a sufficient amount of product to evaluate the sample for the presence of the challenge organisms occurring naturally, and to evaluate the sample for the above parameters at the first time point (Day 0) and at the last time point, shall be aseptically weighed into a sterile container or stomacher bag. The two control samples shall be incubated at $24 \pm 2\,°C$ ($75 \pm 3\,°F$).

6.3.3.2 Test samples

Test samples shall be prepared to evaluate the ability of the product to support the rapid and progressive growth of a composite of 5 strains of challenge organisms inoculated into the product.

6.3.3.2.1   Number of test samples for t_0 time point

Separate test samples shall be prepared for each composite of challenge organisms. The t_0 time point shall have 5 samples prepared according to the procedures in 6.3.3.2.4 and 6.3.3.2.5. The formula shall be as follows:

5 test samples	x	3 different lots	x	15 samples needed per product at t_0

6.3.3.2.2 Test samples other than t_0 for products in categories I, II, and III

Separate test samples shall be prepared for each composite of challenge organisms. For each time point, samples shall be prepared and inoculated in accordance with the procedures in 6.3.3.2.4. For products large enough to provide two samples, one product will be needed for each time point. For example, a cake may contain two slices. Each slice is one sample. For products too small to provide two samples, multiple products may be used. During the storage test, each product will be stored in accordance with the procedures in 6.3.3.2.4.1. To determine the total number of products needed per composite of challenge organisms, refer to table 2 to determine the number of time points and complete the formula:

number of products to provide 2 samples	x	3 different lots	x	number of time points per table 2	=	number of products needed

6.3.3.2.3 Test samples other than t_0 for products in category IV

For each time point, samples shall be prepared and inoculated in accordance with the procedures in 6.3.3.2.5. To determine the number of samples needed per composite of challenge organisms, refer to table 2 to determine the number of time points and complete the formula:

2 test samples	x	3 different lots	x	number of time points per table 2	=	number of samples needed

Samples shall then be incubated for the specified time points. Following removal at each time point, each sample shall be analyzed according to the procedures in 6.3.4.2.

6.3.3.2.4   Inoculation of products in categories I, II, and III

Each product shall be removed from the package and divided with a sterile knife into uniform slices. The weight of each slice shall be taken and the average weight shall be determined in order to calculate the amount of inoculum required to achieve a final level of 10,000 cfu/gm. Each component shall be inoculated at the product slice by micropipettor with a fraction of the total inoculum volume. For example, if a product has four com-

ponents, each component shall be inoculated with ¼ of the total inoculum. Annex A is informative and provides illustrations of inoculation techniques.

6.3.3.2.4.1 Following inoculation, the slices of the product shall be reassembled into the original shape of the product and repackaged to resemble the original packaging per the manufacturer's recommendation. The product will be stored in the repackaged state during the storage time until it is evaluated. Manufacturers may need to provide additional packaging materials.

6.3.3.2.5   Inoculation of products in category IV

For each composite of challenge organisms, a master batch for each lot of product shall be prepared to include sufficient product to include all time points for testing of the product according to table 2. The master batch shall be thoroughly mixed in a stomacher with the inoculum prior to dispensing into 25 gram samples. The mixing time shall vary in accordance with the texture of the product. Care must be taken to produce an adequate distribution of organisms in the product. Product may require extended mixing. However, separation of individual components or excessive heating of components shall not occur. From this master batch, a sufficient amount of 25 gram samples shall be dispensed into a sterile container or stomacher bag to complete the time point storage study. The inoculum value for the master batch shall allow for a final inoculum value for each 25 gram sample to be 2×10^3 - 5×10^4 cfu/g. Samples of each product shall then be incubated for the specified time points.

Following removal at each time point, each sample shall be analyzed according to procedures in 6.3.4.2.

6.3.3.3 Incubation of test samples

The test samples shall be incubated at $24 \pm 2\ ^\circ$C ($75 \pm 3\ ^\circ$F).

6.3.4   Evaluation at each time point

6.3.4.1 Control samples

On Day 0 (the day of inoculation), the control samples shall be evaluated for the presence of naturally occurring challenge organisms in accordance with the methods outlined in 6.3.4.2.

On Day 0 and at the last time point, the pH, temperature, water activity, aerobic plate count, and yeast and mold count of at least two control samples shall be measured. The pH and water activity shall be evaluated in accordance with 6.1 and 6.2. To determine the aerobic plate count and yeast and mold count of each sample, a representative 25-gram portion shall be aseptically weighed into a sterile container or stomacher bag. 225 ml of sterile Butterfield's Phosphate Buffer shall be added to the 25-gram sample and the mixture shall be blended for two minutes. Tenfold dilutions of the homogenate shall be prepared in Butterfield's Phosphate Buffer. To determine the aerobic plate count, the dilutions shall be pour plated on plate count agar. To determine the yeast and mold count, the dilutions shall be pour plated on plate count agar with 100 ug/ml chloramphenicol.

6.3.4.2 Test samples

The number of cfu/gram of each challenge organism shall be determined for the 2 test samples per lot inoculated with the organism at each time point except for the t_o time point at which 5 samples shall be examined. Where there are applicable enumeration methods by AOAC, they shall be used. Duplicate plate counts shall be performed.

> NOTE – Guidance on enumeration may also be found in *Compendium of Methods for the Microbiological Examination of Foods*, third edition (see 2).

The following methods shall be used to determine the challenge organism counts:

–   ***Bacillus cereus*** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The mixture shall be blended for two minutes using a stomacher. Tenfold dilutions of the homogenate shall be made in Butterfield's Phosphate Buffer. The dilutions shall be spread plated on Mannitol Yolk Polymyxin Agar (MYP) and incubated 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

–   ***Escherichia coli*** **O157:H7** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The mixture shall be blended for two minutes using a stomacher. Tenfold dilutions of the homogenate shall be made in Butterfield's Phosphate Buffer. The dilutions shall be spread plated on

Sorbitol-MacConkey Agar and incubated 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

–   ***Listeria monocytogenes*** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The mixture shall be blended for two minutes using a stomacher. Tenfold dilutions of the homogenate shall be made in Butterfield's Phosphate Buffer. The dilutions shall be spread plated on Oxford Agar and incubated 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

–   ***Salmonella* spp** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The mixture shall be blended for two minutes using a stomacher. Tenfold dilutions of the homogenate shall be made in Butterfield's Phosphate Buffer. The dilutions shall be spread plated on Xylose-Lysine-Desoxycholate (XLD) Agar and incubated 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

–   ***Staphylococcus aureus*** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The mixture shall be blended for two minutes using a stomacher. Tenfold dilutions of the homogenate shall be made in Butterfield's Phosphate Buffer. The dilutions shall be spread plated on Baird Parker Agar and incubated 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

–   ***Clostridium perfringens*** A 1:10 dilution of the sample shall be prepared in Butterfield's Phosphate Buffer. The dilution shall be mixed thoroughly by gentle shaking to avoid aeration as much as possible. The dilutions shall be spread plated on Tryptose-Sulfite-Cycloserine (TSC) Agar. The plates shall be incubated anaerobically in a jar at 35 °C (95 °F) for 20-24 hours. Representative colonies shall be confirmed by appropriate methods to ensure that the test organisms are being recovered.

> NOTE – An alternate medium may be used when the medium is specified in the AOAC method. Duplicate plate counts shall be performed.

6.3.5   Acceptance Criteria

The average count of *Salmonella spp., Listeria monocytogenes, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, or Escherichia* coli 0157:H7 shall not increase more than 1 log for two consecutive time points or more than 1 log by the last time point compared to the average count on Day 0. The average count shall be the geometric mean value of two samples per lot for each time point. The geometric mean shall be derived from reducing each value to its logarithm, adding these values and dividing by the number of determinations to obtain the log average. The antilog of the log average then gives a real number which is the best estimate of the population.

The challenge test will be discontinued if the visual or odor criteria for quality of the product is not met.
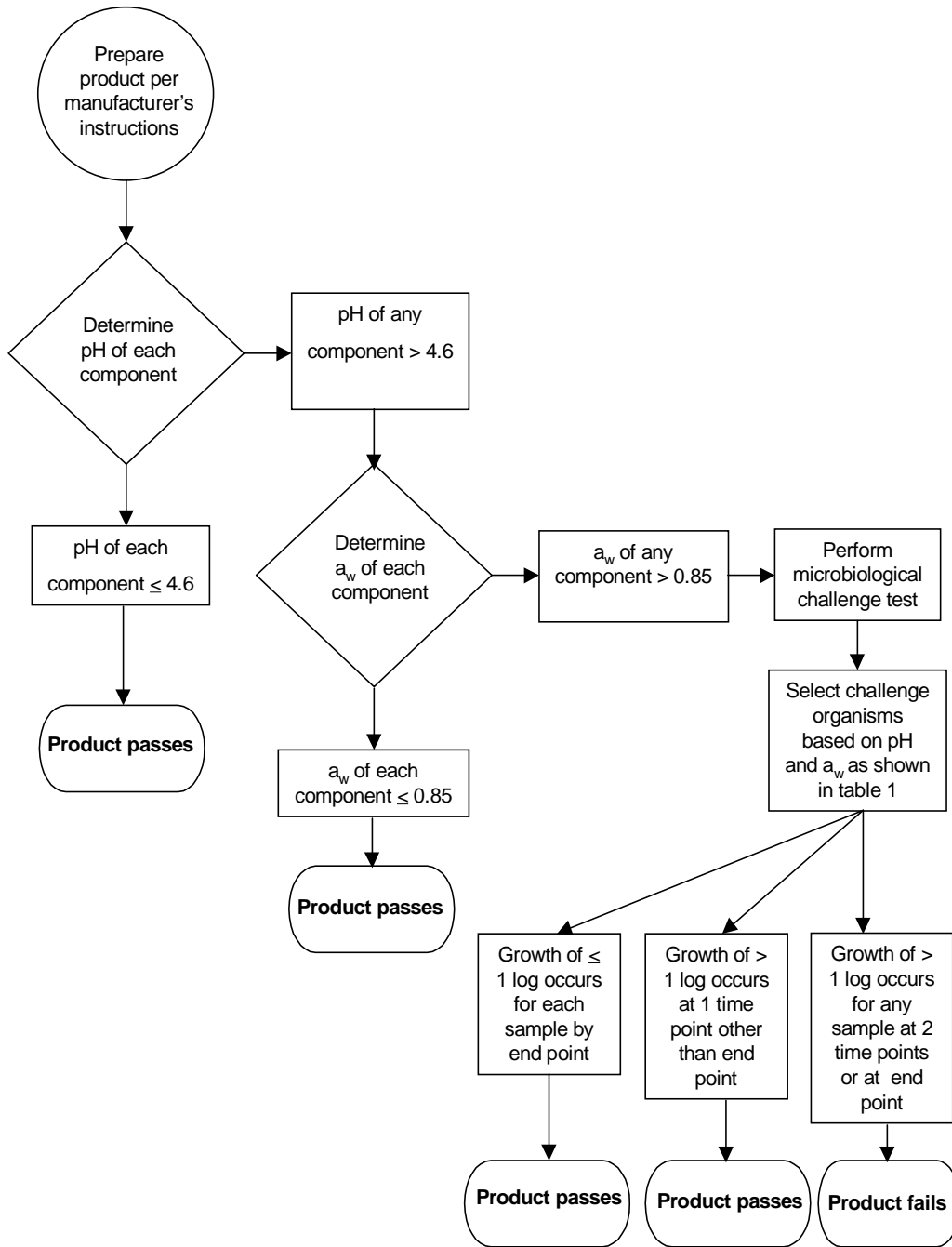
Prepare product per manufacturer's instructions

Determine pH of each component

pH of any component > 4.6

pH of each component $\leq$ 4.6

Product passes

Determine a_w of each component

a_w of any component > 0.85

Perform microbiological challenge test

Select challenge organisms based on pH and a_w as shown in table 1

a_w of each component $\leq$ 0.85

Product passes

Growth of $\leq$ 1 log occurs for each sample by end point

Growth of > 1 log occurs at 1 time point other than end point

Growth of > 1 log occurs for any sample at 2 time points or at end point

Product passes

Product passes

Product fails

Figure 1– Test method

Table 1 - Organism selection for microbiological challenge testing

Minimum pH of product components	Minimum a_W of product components	Challenge organism
> 4.6	> 0.85	*Staphylococcus aureus*
> 5.0	> 0.93	*Bacillus cereus*
> 4.6	> 0.94	*Salmonella spp.*
> 4.6	> 0.95	*Escherichia coli* O157:H7
> 4.6	> 0.92	*Listeria monocytogenes*
> 5.5	> 0.93	*Clostridium perfringens*

Reference: *Microorganisms in Food 5*, Chapman and Hall, 1996 for pH and water activity requirements of organisms.

Table 2 – Time points for testing

Test duration	Time points
24 h - 5 d	1-5 time points, every 24 h starting with point 0
6 - 14 d	5 time points, every 1 - 3 d starting with point 0
15 - 21 d	6 time points, every 3 - 4 d starting with point 0
22 - 31 d	7-10 time points, every 3 d starting with point 0
32 - 40 d	8-10 time points, every 4 d starting with point 0

Table 3 – Required strains of each challenge organism

| Challenge organism	Required strains
Bacillus cereus	ATCC 33018
	ATCC 49063
	ATCC 49064
	ATCC 95992
	SLRCC 1361
Escherichia coli O157:H7	ATCC 43895
	ATCC 35150
	ATCC 43890
	ATCC 43894
	ATCC 43888
Listeria monocytogenes	ATCC 51414
	ATCC 51775
	ATCC 51779
	SLRCC 525
	SLRCC 518
Salmonella spp.	SLRCC 1468
	SLRCC 143
	SLRCC 1434
	SLRCC 539
	SLRCC 1443
Staphylococcus aureus	ATCC 51740
	ATCC 13565
	ATCC 27664
	ATCC 13567
	ATCC 51811
Clostridium perfingens	ATCC 8679 (SC9)
	SLRCC 1154
	SLRCC 1155
	SLRCC 1156
	SLRCC 1157

ATCC denotes American Type Culture Collection. SLRCC denotes Silliker Laboratories Research Culture Collection.

NOTE – If more appropriate isolates are identified for particular applications, they will be added to the Standard.

This page is intentionally blank.

Annex A[6]

Cross-section of a Chocolate Meringue Pie

Meringue

Filling & Meringue Interface

Filling

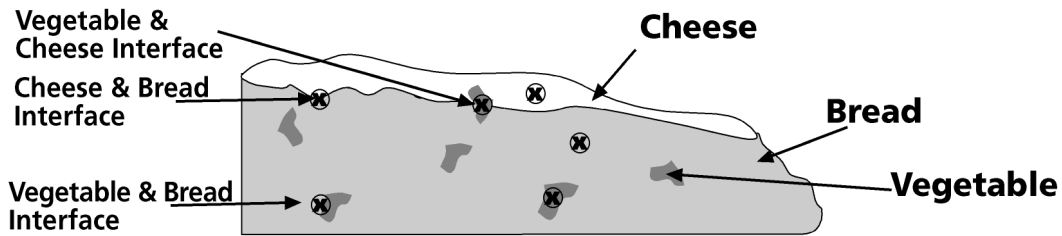**Filling & Crust Interface**
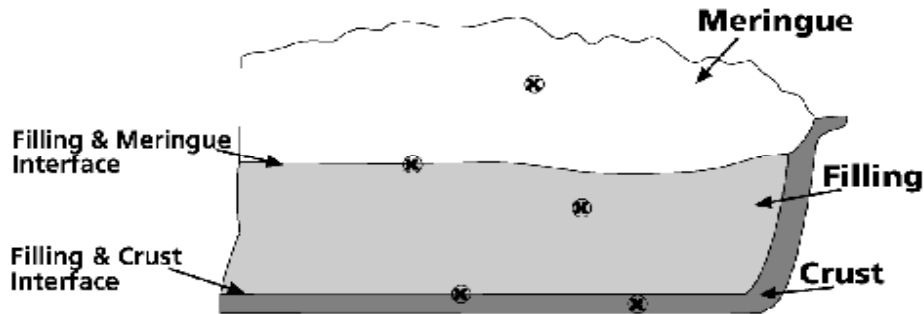
Crust

⊗ Point of Inoculation

Points of Inoculation
1. Meringue
2. Filling & Meringue Interface
3. Filling
4. Filling & Crust Interface
5. Crust

Cross-section of Vegetable and Cheese Bread

Vegetable & Cheese Interface

Cheese

Cheese & Bread Interface

Bread

Vegetable & Bread Interface

Vegetable

⊗ Point of Inoculation

Points of Inoculation
1. Bread
2. Bread & Cheese Interface
3. Cheese
4. Cheese & Vegetable Interface
5. Vegetable
6. Bread & Vegetable Interface

---

[6] The information contained in this Annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this Annex may contain material that has not been subjected to public review of a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

This page is intentionally blank.

Standards and Criteria[7]

The following standards and criteria established and adopted by NSF as minimum voluntary consensus standards are used internationally:

2   Food equipment
3   Commercial warewashing equipment
4   Commercial cooking, rethermalization, and powered hot food holding and transport equipment
5   Water heaters, hot water supply boilers, and heat recovery equipment
6   Dispensing freezers
7   Commercial refrigerators and freezers
8   Commercial powered food preparation equipment
12   Automatic ice making equipment
13   Refuse processors and processing systems
14   Plastics piping system components and related materials
18   Manual food and beverage dispensing equipment
20   Commercial bulk milk dispensing equipment
21   Thermoplastic refuse containers
24   Plumbing system components for manufactured homes and recreational vehicles
25   Vending machines for food and beverages
29   Detergent and chemical feeders for commercial spray-type dishwashing machines
35   High pressure decorative laminates (HPDL) for surfacing food service equipment
36   Dinnerware
37   Air curtains for entranceways in food and food service establishments
40   Residential wastewater treatment systems
41   Non-liquid saturated treatment systems
42   Drinking water treatment units – Aesthetic effects
44   Residential cation exchange water softeners
46   Evaluation of components and devices used in wastewater treatment systems
49   Class II (laminar flow) biohazard cabinetry
50   Circulation system components and related materials for swimming pools, spas/hot tubs
51   Food equipment materials
52   Supplemental flooring
53   Drinking water treatment units – Health effects
55   Ultraviolet microbiological water treatment systems
58   Reverse osmosis drinking water treatment systems
59   Mobile food carts
60   Drinking water treatment chemicals – Health effects
61   Drinking water system components – Health effects
62   Drinking water distillation systems
75   Non-potentially hazardous foods
116   Non-food compounds used in food processing facilities – Food grade lubricants (draft standard for trial use)
173   Dietary supplements (draft standard for trial use)
184   Residential dishwashers
14159   Safety of machinery – Hygiene requirements for the design of machinery
14159-1   Hygiene requirements for the design of meat and poultry processing equipment
C-2 Special equipment and/or devices

---

[7] The information contained in this Standards and Criteria page is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this Standards and Criteria page may contain material that has not been subjected to public review of a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

SPES HOMINUM

THE HOPE OF MANKIND *rests in the*
ability of man to define and seek out
the environment which will permit him
to live with fellow creatures of the
earth, in health, in peace, and in
mutual respect.

[ABA] American Bakers Association. 2000 Jan 18. Industry protocol for establishing the shelf stability of pumpkin pie [final version plus executive summary]. Washington (DC): ABA. 18 p. Available from: lsanders@americanbakers.org.

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

[ANZFA] Australian New Zealand Food Authority. 2001a. The Food Standards Code,Vol. 2, Standard 3.2.2; Food Safety Practices and General Requirements (Australia only). http://www.anzfa.gov.au/foodstandards/foodstandardscodecontents/standard32.cfm. Accessed 2001 Oct 9.

[ANZFA] Australian New Zealand Food Authority. 2001b. Food safety: the priority classification system for food business. Lkd. http://www.anzfa.gov.au/mediareleasespublications/publications/index.cfm. Accessed 2001 Nov 20.

Asplund K, Nurmi E. 1991. The growth of salmonella in tomatoes. Int J Food Microbiol 13:177-82.

Baird-Parker TC. 1990. The staphylococci—an introduction. J Appl Bacteriol Symposium Suppl:1S-8S. (cited in ICMSF 1996a).

Banwart GJ. 1979. Basic Food Microbiology. Westport, Conn.: AVI. Chapter 4, Factors that affect microbial growth in food; p 115 (table 4.6).

Barbut S, Tanaka N, Maurer AJ. 1986. Effects of varying levels of chloride salts on Clostridium botulinum toxin production in turkey frankfurters. J Food Sci 51:1129-31. (cited in ICMSF 1996a).

Barkai-Golan R. 1992. Suppression of postharvest pathogens of fresh fruits and vegetables by ionizing radiation. In: Rosenthal, editor. Electromagnetic Radiations in Food Science, I. Berlin: Springer-Verlag. p 155-94.

Bartsch AG, Walker HW. 1982. Effect of temperature, solute and pH on the tolerance of Clostridium perfringens to reduce water activities. J Food Sci 47:1754-5.

Bartz JA, Showalter RK. 1981. Infiltration of tomatoes by aqueous bacterial suspensions. Phytopathology 71(5):515-8.

[CAST] Council for Agricultural Science and Technology. 1996. Radiation pasteurization of food. Issue paper no. 7. Ames (IA): CAST. p 10.

Castro AJ, Barbosa-Canovas GV, Swanson BG. 1993. Microbial inactivation of foods by pulsed electric fields. J Food Process Pres 17:47-73.

[CFIS] Canadian Food Inspection System, Canadian Food Inspection System Implementation Group. 2001a. Food retail and food services regulation. Lkd. Canadian Food Inspection System Model Regulations and Codes web page at http://www.cfis.agr.ca/english/regcode/frr_frme.htm. Accessed 2001 Oct 10.

[CFIS] Canadian Food Inspection System, Canadian Food Inspection System Implementation Group. 2001b. Food retail and food services code. Lkd. Canadian Food Inspection System Model Regulations and Codes web page at http://www.cfis.agr.ca/english/refcode/frfsc_frme.htm. Accessed 2001 Oct. 10.

Chung KC, Goepfert JM. 1970. Growth of Salmonella at low pH. J Food Sci 35:326-8.

Clavero MRS, Beuchat LR. 1996. Survival of Escherichia coli 0157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. Appl Environ Microbiol 62:2735-40.

Clavero MRS, Brackett RE, Beuchat LR, Doyle MP. 2000. Influence of water activity and storage conditions on survival and growth of proteolytic Clostridium botulinum in peanut spread. Food Microbiol 17:53-61.

Clay CE, Board RG. 1991. Growth of Salmonella enteritidis in artificially contaminated hens' shell eggs. Epidemiol Infect 106:271-81.

Curiale MS. 1991. Shelf-life evaluation analysis. Dairy Food Environ Sanit 11(4):364-9.

Davidson PM, Branen AL, editors. 1993. Antimicrobials in foods. 2nd ed. New York: Marcel Dekker. 647 p. (Food Science, 10).

Del Rosario BA, Beuchat LR. 1995. Survival and growth of enterohemorrhagic Escherichia coli 0157:H7 in cantaloupe and watermelon. J Food Prot 58:105-7.

Diehl JF. 1995. Safety of irradiated foods. 2nd revised ed. New York: Marcel Dekker, Inc.

Dodds KL. 1989. Combined effect of water activity and pH on inhibition of toxin production by Clostridium botulinum in cooked, vacuum-packed potatoes. Appl Environ Microbiol 55:656-60.

Doyle MP, Beuchat LR, Montville TJ, editors. 2001. Food microbiology: fundamentals and frontiers. 2nd ed. Washington (DC): American Society for Microbiology.

Farber JM. 1991. Microbiological aspects of modified atmosphere packaging technology—A review. J Food Prot 54:58-70.

Farber JM, Gendel SM, Tyler KD, Boerlin P, Landry WL, Fritschel SJ, Barrett TJ. 2001. Chapter 11: Molecular typing and differentiation. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington (DC): American Public Health Assoc.

Farkas J. 1997. Physical methods of food preservation. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington (DC): ASM Pr. p 497-519.

[FDA] Food and Drug Administration. 1999. Food Code: 1999 recommendations of the United States Public Health Service, Food and Drug Administration. Springfield (VA): U.S. Dept. of Commerce, Technology Administration, National Technical Information Service. Report nr PB99-115925. Chapter 1, Part 1-201.10(B)(61). p 12. Available from: http://www.foodsafety.gov/%7Edms/foodcode.html.

[FDA] Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. The "Bad Bug Book" [Foodborne pathogenic microorganisms and natural toxins handbook]. http://www.cfsan.fda.gov/~mow/intro.html. Accessed 2001 Dec 10.

[FDA] U.S. Food and Drug Administration. 1986 May 9. Retail food protection program information manual, part 6—Inspection, chapter 01—code interpretations, section 04 - interpretations by code section. Washington (DC): FDA, Center for Food Safety and Applied Nutrition, Retail Food Protection Branch. Table 6, p 11-12.

[FDA/USDA] Food and Drug Administration/Center for Food Safety and Applied Nutrition, U.S. Dept. of Agriculture/Food Safety and Inspection Service. 2001. Draft assessment of the relative risk to public health from foodborne Listeria monocytogenes among selected categories of ready-to-eat foods. http://www.foodsafety.gov/~dms/lmrisk.html. Accessed 2001 Dec 13.

Ferreira MASS, Lund BM. 1987. The influence of pH and temperature on initiation of growth of Salmonella spp. Lett Appl Microbiol 5:67-70.

Glass KA, Doyle MP. 1991. Relationship between water activity of fresh pasta and toxin production by proteolytic Clostridium botulinum. J Food Prot 54:162-5.

Golden DA, Rhodehamel EJ, Kautter DA. 1993. Growth of Salmonella spp. in cantaloupe, watermelon, and honeydew melons. J Food Prot 56:194-6.

Hall P. 2001. Clostridium botulinum toxin production in various foods. Personal communication.

Hauschild AHW, Hilscheimer R. 1979. Effect of salt content and pH on toxigenesis by Clostridium botulinum in caviar [proteolytic strains only]. J Food Prot 43:245-8. (cited in ICMSF 1996a).

[HC] Health Canada, Health Protection Branch. 1992 Mar 1. Guidelines for production, distribution, retailing and use of refrigerated prepackaged foods with extended shelf life [Guideline No. 7].

Huss HH, Schaeffer I, Rye Peterson E, Cann DC. 1979. Toxin production by Clostridium botulinum type E in fresh herring in relation to the measured oxidation-reduction potential (Eh). Nord Veterinaermed 31:81-6.

[ICMSF] International Commission on Microbiological Specification for Foods. 1980. Microbial ecology of foods. Volume 1, Factors affecting life and death of microorganisms. Orlando: Academic Pr. 311 p.

[ICMSF] International Commission on Microbiological Specification for Foods. 1996. Microorganisms in foods. Volume 5, Characteristics of microbial pathogens. London: Blackie Academic & Professional. 513 p.

[ICMSF] International Commission on Microbiological Specification for Foods. 1998. Microorganisms in foods. Volume 6, Microbial ecology of food commodities. New York: Blackie Academic & Professional. 615 p.

[IFT] Institute of Food Technologists, Dept. of Science and Technology Projects. 2000. Special supplement: Kinetics of microbial inactivation for alternative food processing technologies. Barach JT, Barbosa-Canovas GV, Busta FF, Datta AK, Davidson PM, Farkas DF, Heldman DR, Hoover DG, Kokini JL, Pflug IJ, Pierson MD, Sastry SK, Schaffner DW, Zhang QH, editors. Chicago: IFT. [108] p. (Journal of Food Science; vol. 65, no.8, suppl).

[IFT] Institute of Food Technologists, Dept. of Science and Technology Projects. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Beuchat LR, Busta FF, Farber JM, Garrett EH, Harris LJ, Parish ME, Suslow TV, editors. Chicago: IFT. (Journal of Food Science Supplement). Forthcoming.

Ikawa JY. 1991. Clostridium botulinum growth and toxigenesis in shelf stable noodles. J Food Sci 56:264-5.

Ito KA, Chen JK, Lerke PA, Seeger ML, Unverferth JA. 1976. Effect of acid and salt concentrations in fresh-pack pickles on the growth of Clostridium botulinum spores. Appl Environ Microbiol 32:121-4. (cited in ICMSF 1996a).

Jakobsen M, Trolle G. 1979. The effect of water activity on growth of clostridia. Nord Vet-Med 31:206-13.

Jay JM. 1996. Modern food microbiology. 5th ed. New York: Chapman & Hall.

Jay JM. 2000. Modern food microbiology. 6th ed. Gaithersburg (MD): Aspen. p 679.

Kang CK, Woodburn M, Pagenkopf A, Cheney R. 1969. Growth, sporulation and germination of *Clostridium perfringens* in media of controlled water activity. Appl Microbiol 18:789-805. (cited in ICMSF 1996a).

Kaur P. 1986. Survival and growth of Bacillus cereus in bread. J Appl Bacteriol 60:513-6.

Leistner L. 1995. Principles and applications of hurdle technology. In: Gould GW, editor. New methods of food preservation. London: Blackie Academic & Professional. p 1-21.

Li K-Y, Torres JA. 1993. Water activity relationships for selected mesophiles and psychrotrophs at refrigeration temperatures. J Food Prot 56:612-5. (cited in ICMSF 1996a).

Lin CM, Wei CI. 1997. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. J Food Prot 60(7):858-63.

Loss CR, Hotchkiss JH. 2002. Inhibition of microbial growth by low-pressure and ambient pressure gasses. In: Juneja VK, Sofos JN, editors. Control of foodborne microorganisms. New York: Marcel Dekker. p 245-79. Forthcoming.

Luck E, Jager M. 1997. Antimicrobial food additives: characteristics, uses, effects. Springer: Berlin. 260 p.

Lund BM, Baird-Parker TC, Gould GW, editors. 2000. The microbiological safety and quality of foods. Volume 1 & 2. Gaithersburg (MD): Aspen.

Marcotte M. 1992. Irradiated strawberries enter the U.S. market. Food Technol 46(5):80-6.

McClure PJ, Roberts TA, Otto Oguru P. 1989. Comparison of the effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plats and in liquid medium. Lett Appl Microbiol 9:95-9. (cited in ICMSF 1996a).

Miller AJ. 1992. Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. J Food Prot 55:414-18. (cited in ICMSF 1996a).

Montville TJ, Matthews KR. 2001. Chapter 2: Principles which influence microbial growth, survival, and death in foods. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington (DC): ASM Pr. p 13-32.

Morris JG. 2000. The effect of redox potential. In: Lund BL, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Volume 1. Gaithersburg (MD): Aspen. p 235-50.

Mossel DAA, Thomas G. 1988. Securite microbiologique des plats prepares refrigeres: recommendations en matiere d'analyse des risques, conception et surveillance du processus de fabrication. Microbiologie—Aliements—Nutrition 6:289-309.

Mossel DAA, Corry JEL, Struijk CB, Baird RM. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley and Sons. 699 p.

Moy JH. 1983. Radurization and radication: fruits and vegetables. In: Josephson ES, Peterson MS, editors. Preservation of food by ionizing radiation. Boca Raton (FL): CRC Pr. p 83-108.

[NACMCF] National Advisory Committee on Microbiological Criteria for Foods. 1998. Hazard analysis and critical control point principles and application guidelines. J Food Prot 61:762-75.

[NIST] National Institute of Standards and Technology. 2000. Uniform laws and regulations in the areas of legal metrology and engine fuel quality [as adopted by the 84th National Conference on Weights and Measures 1999]. 2000 ed. Gaithersburg (MD): U.S. Dept. of Commerce, Technology Administration, National Institute of Standards and Technology. Uniform open dating regulation; p 117-22. (NIST Handbook 130).

Notermans S, Heuvelman CJ. 1983. Combined effect of water activity, pH and suboptimal temperature on growth and enterotoxin production of *Staphylococcus aureus*. J Food Sci 48:1832-5, 40.

Notermans S, Veld P, Wijtzes T, Mead GC. 1993. A user's guide to microbiological challenge testing for ensuring the safety and stability of food products. Food Microbiol 10(2):145-57.

[NSF] NSF International. 2000 Nov. 10. Non-potentially hazardous foods. Ann Arbor (MI): NSF International. Report nr ANSI/NSF 75-2000. 12 p.

Patterson MF, Quinn M, Simpson R, Gilmour A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. J Food Prot 58(5):524-9.

Petran RL, Aottola EA. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. J Food Sci 54(2):45-60. (cited in ICMSF 1996a).

Raevuori M, Genigeorgis C. 1975. Effect of pH and sodium chloride on growth of Bacillus cereus in laboratory media and certain foods [rice and meat data only]. Appl Microbiol 29:68-73.

Ray B. 1996. Fundamental food microbiology. Boca Raton (FL): CRC Press. 516 p.

Razavilar V, Genigeorgis C. 1998. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. Int J Food Microbiol 40:149-57.

Reitsma CJ, Henning DR. 1996. Survival of enterohemmorhagic *Escherichia coli* 0157:H7 during the manufacture and curing of cheddar cheese. J Food Prot 59(5):460-4.

Ross T, Todd E, Smith M. 2000. Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods: preliminary report for joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. Rome: Food and Agriculture Organization of the United Nations. Report nr MRA 00/02. 242 p.

Simpson MV, Smith JP, Dodds KL, Ramaswamy HS, Blanchfield B, Simpson BK. 1995. Challenge studies with *Clostridium botulinum* in a sous vide spaghetti and meat sauce product. J Food Prot 58:229-34.

Smelt JPPM, Raatjes JGM, Crowther JC, Verrips CT. 1982. Growth and toxin formation by *Clostridium botulinum* at low pH values. J Appl Bacteriol 52:75-82.

Smittle RB. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. J Food Prot 63(8):1144-53.

Sommer NF, Maxie EG. 1966. Recent research on the irradiation of fruits and vegetables. In. Food Irradiation. Vienna: International Atomic Energy Agency. p 571.

Stewart CM, Cole MB, Legan JD, Slade L, Vandeven MH, Schaffner DW. 2001. Modeling the growth boundary of *Staphylococcus aureus* for risk assessment purposes. J Food Prot 64(1):51-7.

Swanson KMJ. 2001. *Clostridium botulinum* toxin production in various foods. Personal communication.

Tanaka N, Traisman E, Plantong P, Finn L, Flom W, Meskey L, Guggisberg J. 1986. Evaluation of factors involved in antibotulinal properties of pasteurized process cheese spreads. J Food Prot 49(7):526-31.

Thayer DW, Josephson ES, Brynjolfsson A, Giddings GG. 1996. Radiation pasteurization of food. Council for Agricultural Science and Technology Issue Paper Nr. 7(Apr):1-12.

The Council of the European Communities. 1993. Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. Official Journal L 175, 19/07/1993 p. 0001-0011. Annex Chapter IX, paragraph 4. http://europa.eu.int/eur-lex/en/lif/dat/1993/en_393L0043.html. Accessed 2001 Nov 8.

Thomas P. 1986. Radiation preservation of foods of plant origin. V. Temperate fruits: pome fruits, stone fruits, and berries. Crit Rev Fd Sci Technol 24:357-400.

Tienungoon S, Ratkowsky DA, McMeekin TA, Ross T. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. Appl Environ Microbiol 66:4979-87.

[UK] United Kingdom, Ministry of Agriculture, Fisheries and Food. 1995. The Food Safety (Temperature Control) Regulations. London: HMSO.

Urbain WM. 1986. Fruits, vegetables, and nuts. In: Schweigert BS, editor. Food Irradiation. Orlando (FL): Academic Pr. p 170-216. (Food Science and Technology, A series of monographs).

[USDA] U.S. Dept. of Agriculture, Food Safety and Inspection Service. 1996 [with change 98-01 (Sept 1996 thru Feb 1998)]. Food standards and labeling policy book. Available from: Superintendent of Documents, PO Box 371954, Pittsburgh, PA 15250-7954; (202)512-1800.

[USDA] U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Laboratory. 2001. USDA Pathogen Modeling Program Version 5.1.

Vestergaard EM. 2001. Building product confidence with challenge studies. Dairy Food Environ Sanit 21(3):206-9.

Worobo RW. 2000. Efficacy of the CiderSure 3500 ultraviolet light unit in apple cider. Ithaca (NY): Cornell University, Department of Food Science and Technology. 1-6 p.

Wyatt CJ, Guy VH. 1981. Incidence and growth of *Bacillus cereus* in retail pumpkin pies. J Food Prot 44(6):422-4.

Zegota H. 1988. Suitability of Dukat strawberries for studying effects on shelf life of irradiation combined with cold storage. Z Levensm Unters Forsch 187:111-4.

Zhang QH, Qin B-L, Barbosa-Canovas GV, Swanson BG. 1995. Inactivation of E. coli for food pasteurization by high-strength pulsed electric fields. J Food Process Pres 19(2):103-18.