Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

A Report of the Institute of Food Technologists
for the Food and Drug Administration
of the U.S. Department of Health and Human Services

submitted March 29, 2000
revised June 2, 2000

IFT/FDA Contract No. 223-98-2333
Task Order 1

This document contains the Kinetics of Microbial Inactivation for Alternative Food Processing Technologies report, revised June 2, 2000, as published in the Journal of Food Science, Volume 65, Issue Supplement s8, in November 2000.

Table of Contents

Preface

ON SEPT. 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) for 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 #1: How to Quantify the Destruction Kinetics of Alternative Processing Technologies. The Background and Scope of Work that FDA provided to IFT are included. In October 1998, IFT assembled a Scientific and Technical Panel and three subpanels: Electromagnetic Processes, Electrothermal Processes, and Physical Processes. Each panel was comprised of experts in food microbiology and food engineering: specifically, experts in the alternative technologies under review. The panel and subpanels met in person and via conference calls throughout 1999 and early 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 Panel and Subpanels, 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 in the hopes that the report makes a modest contribution to the understanding of the many exciting, emerging, alternative technologies that have potential for enhancing the safety and quality of food.

Science Advisory Board

Institute of Food Technologists

Scientific and Technical Panel on Alternative Processing Technologies

Electromagnetic Processes Subpanel

Electrothermal Processes Subpanel

Ashim K. Datta, Ph.D.
Cornell University

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

Dennis R. Heldman, Ph.D.
Heldman and Associates

Sudhir K. Sastry, Ph.D.
Ohio State University

Physical Processes Subpanel

Daniel F. Farkas, Ph.D.
Oregon State University

Dallas G. Hoover, Ph.D.
University of Delaware

Jozef L. Kokini, Ph.D.
Rutgers, The State University of New Jersey

Reviewers

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V. M. Balasubramaniam, Ph.D.
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Martin Cole, Ph.D.
Food Science Australia

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Systems Technology Analysis Inc.

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Cornell University

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University of Wyoming

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State University of New York at Buffalo

Charles E. Sizer, Ph.D.
Illinois Institute of Technology

John N. Sofos, Ph.D.
Colorado State University

Randy William Worobo, Ph.D.
Cornell University

Ahmed E. Yousef, Ph.D.
Ohio State University

Additional Acknowledgements

R. C. Swamy Anantheswaran
The Pennsylvania State University

Hisayoshi Akiyama
Otsuka Chemical Co., Ltd.

Mario Bassani
Micromac

Juan Jose Fernandez-Molina
Washington State University

Susan Fredenberg
Cornell University

Phil Hartman
FPE Inc.

Theodore P. Labuza, Ph.D.
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Rosetta Newsome, Ph.D.
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McGill University

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Juming Tang
Washington State University

Irwin Taub
U.S. Army Natick Soldier Center

Rudy Tops
Tops Foods

Randy Worobo, Ph.D.
Cornell University

Tom Yang
U.S. Army Natick Soldier Center

Food and Drug Administration

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Contract Technical Officer
Division of HACCP Programs

John W. Larkin, Ph.D.
Food Process Hazard Analysis Branch

Stephen Spinak
Division of HACCP Programs

Ed Arnold
Contracting Officer

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Information Specialist

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Administrative Assistant

Background

PROVIDED BY THE FOOD AND DRUG ADMINISTRATION TO THE INSTITUTE OF FOOD TECHNOLOGISTS

THERMAL TREATMENT OF FOOD PRODUCTS TO RENDER THEM free of pathogenic microorganisms has been practiced for more than five thousand years. However, a method by which to quantify the microbial destruction that takes place during a thermal treatment has only been understood for the last 75 years. To determine the amount of microbial destruction that a thermal treatment delivers to a process requires both an understanding of the amount of heat delivered to every portion of the food product and the destruction kinetics of the microorganisms of interest.

The amount of heat delivered by a food process is dependent on the way in which the product is heated and on its physical nature. Process dependent factors can include: processing equipment design, type of heating media, container, or food size and shape, product composition and viscosity. The thermal destruction kinetics of microorganisms or their ability to be killed within the food matrix is likewise dependent on a number of factors. These factors may include: pH of the product, levels and types of preservatives, water activity, the previous growth conditions of the microorganisms of concern, product composition, and competitive microorganisms. Heat transfer mechanics can be used to develop mathematical relationships between the rate at which a food is heated and the temperature of the coldest portion of the food. Models have been developed for a large portion of the different types of food processing systems currently used. However, not all food processing systems are easily modeled. In similar fashion, mathematical relationships have been developed to describe the kinetics of thermal destruction of microorganisms. Thermal destruction of microorganisms tends to follow first order rate reaction kinetics and have traditionally been described by the rate, at a specific temperature, required to reduce a population of organisms by 90%. This value is referred to as the D value, or decimal reduction time value. The change in D value with temperature also follows a first order relationship. The temperature increase required to reduce a microorganism's D value by 90% is referred to as the z value. For thermal processes, understanding a microorganism's D and z values allows a processor to measure the amount of microbial destruction delivered by the process. Other processing treatments (i.e., high pressure processing, pulsed electric field, chemical treatments, irradiation, and pulsed light) may require other processing or constitutive parameters in order to be able to establish the amount of destruction that takes place during a process. For example, chemical sterilization processes (i.e., hydrogen peroxide and ethylene oxide) require a measurement of time, temperature and chemical concentration. Often chemical concentration is monitored and held constant at or above a known critical limit. For high pressure processing, time, temperature, and pressure determine the rate of microbial destruction. Some researchers have proposed using a z_p to describe the effect a change in pressure has on the process.

For a number of thermal processing systems, the total number of microorganisms destroyed by the process can be estimated by incorporating the destruction rate kinetics of the microorganism of concern into the heat transfer model for that system. In cases where the temperature of a product cannot be accurately modeled, actual time-temperature measurements can be used to establish the amount of microbial destruction that takes place during a process. In the event that temperature can be neither modeled nor physically measured, microbial destruction of a process can be physically measured by inoculating a portion of the product with a known amount of an indicator organism and then measuring the number of organisms that remain in the food portion after the process. This procedure is often referred to as a biological challenge, or an inoculated pack test.

When performing a biological challenge test the indicator organism needs to be chosen carefully. For transient heat conduction processes (i.e., temperature of the cold spot changes with time) the change in destruction rate with temperature (z-value) must be the same as that of the pathogen of concern. An indicator organism with a differing z value from the pathogen of concern can be used if the z value is conservative; however, calculation errors can easily be overlooked and thus, this is not a recommended procedure. For transient heat conduction processes, the amount of destruction that is measured for a specific indicator organism cannot be mathematically adjusted for a differing z-value of the pathogen of concern without the actual time-temperature profile of the process, that being measured by the biological challenge study. Besides an appropriate z-value, the destruction rate of the indicator organism needs to be characterized for the substrate being processed. For a biological challenge test an accurate understanding of the indicator organism's D and z-values within the food matrix must be known. Additionally, this information needs to be current. Kinetic parameters for crops of biological indicator organisms can change with storage.

When an indicator organism is used for validation tests where the target process is a specific number of log reductions in the organism, how the indicator organism is grown may be important. Destruction kinetics can change with growth conditions. For example, for chemical (hydrogen peroxide) sterilization validation tests of aseptic equipment, the indicator organism is typically *Bacillus subtilis A* with a known resistance to hydrogen peroxide.

The destruction of the organism during the process is then measured to validate the process. If indicator organisms with inappropriate chemical resistance to hydrogen peroxide are used during the validation test, the system will have been underchallenged.

For milk products and low-acid canned foods, the food industry has agreed on the organisms of public health concern when thermally processing. When alternative processing technologies are used to destroy microorganisms, these same organisms may no longer be the appropriate organism(s) of concern. New alternative processing technologies may involve different mechanisms for microbial destruction, which may mean that traditional thermal destruction kinetics may not be useful in characterizing a new technology system.

Current Policy

The thermal treatment delivered to refrigerated bovine milk (pasteurized milk) and its products is based on a D_{140F} and a 12 log reduction of *Coxiella burnetti*. The U.S. Code of the Federal Regulations (CFR) for milk (21 CFR 131.3 and 21 CFR 1240.61) stipulate the minimum time and temperatures required when

pasteurizing milk and its products. This regulation defines both the meaning of pasteurization and ultra-pasteurization for milk products, as an equivalent time and temperature treatment. A processor must process milk at these minimums, or higher, in order to sell them in interstate commerce. Since 1924 the FDA, in cooperation with those involved with milk manufacturing, state and local regulators, and educational and research institutions, has developed the Grade A Pasteurized Milk Ordinance (PMO). The Grade A PMO is-a recommended ordinance for adoption by States, counties, and municipalities in order to encourage a uniform milk sanitation program within the U.S. The ordinance covers the complete production of milk and milk products, from the farm to consumer purchase. The PMO uses the same definitions for pasteurization as are defined in the CFR.

In Part II, Section 1(S) of the PMO and in 21 CFR 1240.61 the definition for pasteurization includes a provision for treatments other than time-temperature treatments. In order for a processor to be able to use anything other than a time-temperature thermal treatment on milk products, the FDA must approve the alternative process as equally effective.

The regulations for low-acid canned foods (21 CFR 108, 113 and 114) require a scheduled thermal treatment sufficient to render the food product commercially sterile at normal storage, temperatures. Commercially sterile is defined in *21* CFR 113.3 as a treatment necessary to render the food product free of viable microorganisms having public health significance, as well as microorganisms of nonhealth significance, capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution.@ For a process that deviates from the scheduled process the food product must be shown to be free of only microorganisms of public health significance (i.e., *Clostridium botulinum* spores). The low-acid canned food industry has traditionally used a 12 log (i.e., a 12 D treatment) reduction in *Clostridium botulinum* spores as a target thermal treatment necessary to render the food product free of any potential public health hazard. The thermal treatment necessary to render a food product commercially sterile is typically more than that required to deliver a 12 log reduction in *Clostridium botulinum.* It is the processor's responsibility to develop the time-temperature treatment necessary to produce a commercially sterile low-acid canned food product.

Scope of Work

AS ASSIGNED BY THE FOOD AND DRUG ADMINISTRATION TO THE INSTITUTE OF FOOD TECHNOLOGISTS

THE INSTITUTE OF FOOD TECHNOLOGISTS 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. What alternative processing technologies might be used to produce food products free from any public health hazard and what are their critical control points? IFT shall provide information about the different types of alternative processing technologies that might be used for both pasteurization and sterilization type processing. These technologies would include but are not limited to: high pressure processing, pulsed electric field, pulsed x-ray or ultraviolet light, ohmic heating, inductive heating, pulsed light, combined ultraviolet light, and low-concentration hydrogen peroxide, submegahertz ultrasound, filtration, oscillating magnetic fields and any other technology which may serve as an alternative to traditional thermal processes. IFT shall do an in-depth review of how these alternative technologies work and what critical control points are important to each of them. IFT shall provide definitions of the process technology. For example, is there a standard definition for "pasteurization" that can be used for each alternative processing technology, or should new terms be developed (i.e., cold pasteurization for high pressure processing)?

2. IFT shall do an in-depth review on which organism(s) of public health concern is the most resistant to the process(es). The agency understands that the mechanism of microbial destruction for an alternative processing technology may not follow that of traditional thermal processing. Thus, the organism of public health significance that is the most resistant for each of the alternative processing technologies may be different from that established for traditional thermal processing. The agency seeks guidance as to how to determine the most resistant organism of concern and its variation in resistance that might occur within nature. This might involve a number of factors, which may include but are not limited to: growth phase and growth conditions of organism, processing substrate or food matrix, the pathogenic organisms associated with specific foods, processing conditions, storage conditions and potential storage abuse.

3. IFT shall review various options on how to quantify the lethal efficacy (destruction kinetics) of the process. The agency is interested in how to determine the effectiveness of an alternative processing technology. What type of method should be used to express the destruction rate of an alternative technology process, mathematical model and/or a biological challenge test and why? For thermal treatments the amount of lethal treatment that is delivered by a process can be determined from an understanding of the time-temperature history of the process and the D and z value of the organism of concern. If there is a mathematical relationship for the microbial destruction rate of the alternative processing technology, what are the parameters to the model and how is the model used? The agency seeks guidance on how to develop a model for alternative process technologies where mathematical models do not exist.

4. IFT shall review which indicator organism(s) can be used to validate the alternative technology process(es). A properly designed process requires that the process be validated. If there is sufficient understanding as to the kinetics associated with the process, can physical measurements of the process delivery be used to validate the process and how could this be accomplished? Indicator organisms will also be used to validate mathematical relationships that have been proposed for a process. What are appropriate indicator organism(s) for each of the mathematical relationships proposed for a process or how to go about selecting one.

5. IFT shall review how process deviations are to be handled. For all processes, there will be times when the process, as validated, was not delivered to the product. For each alternative processing technology, IFT shall provide a sample process deviation, examine and review it, and state possible way(s) as to how FDA might determine the severity of the deviation with regards to public health.

Executive Summary

THIS REPORT EVALUATES THE SCIENTIFIC INFORMATION AVAILable on a variety of alternative food processing technologies. The purpose of the report is to help the Food and Drug Administration evaluate each technology's effectiveness in reducing and inactivating pathogens of public health concern. Where information is too limited for a thorough evaluation and conclusion, research needs are identified.

The report begins with a discussion of overarching principles that apply to all of the technologies, specifically focusing on kinetic parameters and pathogens of public health concern. Kinetic parameters and models are used to compare the rates of microbial inactivation for each technology. Limitations of the parameters are discussed at length. Pathogens of concern for all the technologies are also addressed.

The report then provides a detailed review and analysis of the alternative technologies. For each technology, FDA asked the panel to define the technologies, identify pathogens of public health concern most resistant to the technology, describe the mechanisms of pathogen inactivation and their kinetics, identify ways to validate the effectiveness of microbial inactivation, identify critical process factors, and describe process deviations and ways to handle them. The panel also provides a description of synergistic effects between technologies, where available, and articulates research needs for each technology.

Overarching Principles

Kinetics

Kinetic parameters and models are used for the development of food preservation processes to ensure safety. They also permit comparison of different process technologies on reduction of microbial populations. The parameters, with their recognized limitations, are used to analyze and report the reduction of a microbial population as a function of process parameters and include empirical coefficients experimentally determined from microbial reduction kinetics. The models and kinetic parameters are used to present and compare microbial inactivation data from thermal, pressure, and electromagnetic processes. The parameters (D-value and z(T), z(P), z(*E*), E, k, K and V) have been calculated from data previously reported, using the models for thermal, pressure, and pulse electric field (PEF) technologies. The thermal parameters apply to microwave energy and electrical resistance (ohmic) processes, as well as any other technology where temperature is the primary factor. The parameters for pressure or PEF treatments should apply to any process where pressure or electricity is the primary critical factor in reducing microbial populations. Given the scarcity of data, these are estimated parameters and there is an imminent need for more research in this area. The quantity of data for several of the other technologies, describing the influence of the treatment on reduction of microbial populations, is insufficient for a comparison.

The basic model assumes a linear first-order relationship between microbial population and time. There are considerable discussions about the appropriateness of using a first-order model to describe the reduction in microbial population for all preservation technologies, but without strong evidence to support alternative needs, first-order kinetics were used.

Kinetic parameters for microbial populations exposed to thermal treatments have been assembled over a significant period of time. Published literature has included kinetic parameters needed to respond to most process, product, and microbial situations. Thermal parameters provide a sound basis for development of processes for the microwave energy and electrical resistance (ohmic) technologies.

There are limitations to interpreting these parameters. Care should be taken when the parameters are used to develop processes, to compare the resistance of different microbial populations, or to identify appropriate microorganisms.

Data used to determine the D-value and/or k for pressure treatment of microbial populations appear useful. Identifying the key pathogens of concern and their surrogates continue to be an ongoing challenge. Limitations of these data are primarily associated with temperature control or temperature changes during the pressure treatments. Evidence suggesting a synergistic impact of pressure and temperature on microbial populations is too limited for use. Much of the data were collected at a single pressure. Only 4 studies have used 3 to 5 pressure levels, while controlling all other factors affecting the parameters.

Data available on the influence of PEF on microbial populations have many limitations. The kinetic parameters are based on 2 points on the survivor curve. No single report has measured the inactivation of microbial populations at several levels of electric field strength, leading to the quantification of the PEF coefficient, nor has the synergistic influence of temperature been quantified.

Electrothermal alternative technologies utilize the well-established thermal kinetic parameters for thermal inactivation of vegetative cells of *Salmonella, Escherichia coli, Yersinia enterocolitica, Vibrio* spp., *Aeromonas hydrophila, Campylobacter jejuni, Listeria monocytogenes* and *Staphylococcus aureus.* In general, the thermal resistance constants z(T) for the vegetative microorganisms fall between 4 and 7.7 °C. The largest D-value (smallest k-value) reported at 110 °C for toxin-producing, sporeforming microorganisms is 12.42 min (0.185/min) for *Clostridium botulinum* proteolytic Type B spores at 110 °C in pureed peas.

An independent additional inactivation mechanism due to the electric current during ohmic heating may occur, but at this time evidence is not sufficient to consider the use of alternate kinetic parameters for development of ohmic heating processes. The nonthermal effects of microwave processes on microbial inactivation have not been confirmed and appear insufficient in magnitude to be considered during development of processes. For processes involving the use of pressure for reduction of microbial populations, the F-value is the time the product needs to be exposed to the specified pressure and other conditions (that is, temperature) to accomplish the recommended amount of inactivation.

The combined influence of pressure and temperature on inactivation kinetics has been investigated on only a limited basis. Pressure appears to significantly inactivate *S. aureus.* However, in comparable experiments, inactivation rates of selected strains of various *Listeria* spp. with, for example, D-values ranging from 1.48 min (k = 1.556/min) at 350 MPa to 15 min (k = 0.154/min) at

400 MPa were lower than the ones for *S. aureus*. These data were measured at ambient temperatures (20 to 25 °C).

Comprehensive data on inactivation rates of *Clostridium sporogenes* spores show the influence of pressure on inactivation rate, z(P), to be 725 MPa at 93 °C, 962 MPa at 100 °C and 752 MPa at 108 °C. Data for *C. botulinum* Type E Alaska and Type E Beluga indicate that their D-values were in the same range as *C. sporogenes*. The D-values for *C. botulinum* Type A 62-A are generally higher than the values for *C. sporogenes*, even when considering the influence of temperature and pressure. In another study, high-pressure resistance was reported for *L. monocytogenes* and *S. aureus*. The most pressure-resistant pathogenic vegetative cell populations appear to be those of *E. coli* O157:H7 with a D-value of 6 min (k = 0.384/min) at 600 MPa, and *S. aureus* with a D-value of 7.14 min (k = 0.323/min) at 600 MPa. The most resistant pressure spores appear to be *C. sporogenes* with a D-value of 16.772 min (k = 0.138/min) at 600Mpa (T = 90 °C) and *C. botulinum* Type A 62-A with a D-value of 6.7 min (k = 0.344/min) at 827 MPa (T = 75 °C). The pressure coefficient z(P) of 1524 MPa for *C. botulinum* Type A 62-A constitutes an additional indication of the pressure resistance of the spore populations. A recent report shows little if any inactivation after 30 min of *C. botulinum* 17B and Cap 9B exposure to 827 MPa at 75 °C.

Adequate inactivation data for estimating the kinetic parameters for microbial populations exposed to PEF are scarce but in a form that fits the basic model. Even with major limitations, the models could be used to establish process time (F) in the short term, but a great effort would be needed to evaluate the outcome.

Parameters based on 2-point curves allowed direct comparisons of the effectiveness of PEF in reducing different microbial populations and the influence of the media on microbial inactivation. The D-values for *Bacillus cereus* spores are higher than for other microbial populations at the same field strength and temperature. The survivor data for PEF are too limited for definite conclusions. For instance, data based on the same field strength and temperature are lacking. In addition, only a few of the published reports provide information on the threshold field strengths needed to initiate inactivation.

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 (usually targeting spores of *Clostridium botulinum*) capable of germinating and growing in the food under normal storage conditions.

Efficacy of any preservation technology 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 sublethal injury. Each influences the resistance independently of the apparent inactivation capacity of that particular process.

Extreme environments may select for forms resistant to severe conditions leading to a microbial population of greater resistance. An example of this is the higher heat resistance of acid- or salt-adapted, heat-shocked or starved *E. coli* O157:H7 cells. The questions relative to process design and verification are: (1) are the microorganisms and food environments likely to result in stress induction? (2) would stress induced resistance possibly occur? and (3) if it did, would it significantly impact the inactivation?

Pathogens of Public Health Concern

The following bacteria are known to be responsible for causing foodborne disease: *A. hydrophila, B. cereus, C. jejuni, C. botulinum, Clostridium perfringens*, pathogenic *E. coli, L. monocytogenes, Salmonella* serovars, *Shigella* spp, *S. aureus, Vibrio* spp., and *Y. enterocolitica*. The primary virus of concern that is carried by foods is Hepatitis A. *Cryptosporidium* and *Cyclospora* are protozoa of concern mainly because they produce resistant cysts. When exploring the new preservation technologies, their preservation level should be compared to that of classical pasteurization or commercial sterilization technologies.

Establishment of traditional thermal processes for foods has been based on 2 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. Validity of the established process is often confirmed using an inoculated test pack study tested under actual plant conditions using surrogate microorganisms as biological indicators that can mimic the pathogen. Thus, the 2 factors described above, which are well established for thermal processes, should be used for establishing and validating scheduled electrothermal processes.

For other preservation processes not based on heat inactivation, key pathogens of concern and nonpathogenic surrogates need to be identified and their significance evaluated. Surrogates are selected from the population of well-known organisms that have well-defined characteristics and a long history of being nonpathogenic. Surrogates need to be nonpathogenic organisms and not susceptible to injury, with nonreversible thermal or other inactivation characteristics that can be used to predict those of the target organism. The durability to food and processing parameters should be similar to the target organism. Population of surrogates should be constant and have stable thermal and growth characteristics from batch to batch. Enumeration of surrogates should be rapid and with inexpensive detection systems that easily differentiate them from natural flora. Genetic stability of surrogates is desirable to obtain reproducible results. It is recommended also 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 (e.g. thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity, etc.) should be avoided.

Microwave And Radio Frequency Processing

MICROWAVE AND RADIO FREQUENCY HEATING REFERS TO THE use of electromagnetic waves of certain frequencies to generate heat in a material through 2 mechanisms—dielectric and ionic. Microwave and radio frequency heating for pasteurization and sterilization are preferred to conventional heating because they require less time to come up to the desired process temperature, particularly for solid and semisolid foods. Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 y, but commercial radio frequency heating systems for the purpose of food pasteurization or sterilization are not known to be in use.

For a microwave sterilization process, unlike conventional heating, the design of the equipment can dramatically influence the critical process parameter—the location and temperature of the coldest point. This uncertainty makes it more difficult to make general conclusions about processes, process deviations and how to handle deviations.

Many techniques have been tried to improve the uniformity of heating. The critical process factor when combining conventional heating and microwave or any other novel processes would most likely remain the temperature of the food at the cold point, primarily due to the complexity of the energy absorption and heat transfer processes.

Since the thermal effect is presumably the sole lethal mechanism, time-temperature history at the coldest location will determine the safety of the process and is a function of the composition, shape and size of the food, the microwave frequency and the applicator (oven) design. Time is also a factor in the sense that, as the food heats up, its microwave absorption properties can change significantly and the location of cold points can shift.

Ohmic and Inductive Heating

OHMIC HEATING (SOMETIMES ALSO REFERRED TO AS JOULE heating, electrical resistance heating, direct electrical resistance heating, electroheating and electroconductive 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.

A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation and extraction. The principal advantage claimed for ohmic heating is its ability to heat materials rapidly and uniformly, including products containing particulates. The principal mechanisms of microbial inactivation in ohmic heating are thermal. While some evidence exists for nonthermal effects of ohmic heating, for most ohmic processes which rely on heat, it may be unnecessary for processors to claim this effect in their process filings.

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 0 °C to above 100 °C. Commercial exposure times can range from a millisecond pulse to over 20 min. Chemical changes in the food generally will be a function of the process temperature and treatment time.

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 3 °C per 100 MPa. The temperature of a homogenous food will increase uniformly due to compression. 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 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 45 to 50 °C appear to increase the rate of inactivation of food pathogens and spoilage microbes. Temperatures ranging from 90–110 °C in conjunction with pressures of 500–700 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), product initial temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity and concurrent processing aids. Other processing factors present in the process line before or after the pressure treatment were not included.

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. For vegetative bacteria, nonpathogenic *L. 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.

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 2 electrodes. PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s. Energy loss due to heating of foods is minimized, reducing the detrimental changes of the sensory and physical properties of foods.

Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature and the design of electrodes that minimize the effect of electrolysis.

Although different laboratory- and pilot-scale treatment chambers have been designed and used for PEF treatment of foods, only 2 industrial-scale PEF systems are available. The systems (including treatment chambers and power supply equipments) need to be scaled up to commercial systems.

To date, PEF has been applied mainly to improve the quality 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 liquid food in both static and flow treatment modes is a limitation.

Several theories have been proposed to explain microbial inactivation by PEF. The most studied are electrical breakdown and electroporation.

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.

Although PEF has potential as a technology for food preservation, existing PEF systems and experimental conditions are diverse, and conclusions about the effects of critical process factors on pathogens of concern and kinetics of inactivation need to be further studied.

High Voltage Arc Discharge

ARC DISCHARGE IS AN EARLY APPLICATION OF ELECTRICITY TO pasteurize fluids by applying rapid discharge voltages through an electrode gap below the surface of aqueous suspensions of microorganisms. A multitude of physical effects (intense wave) and chemical compounds (electrolysis) are generated, inactivating the microorganisms. The use of arc discharge for liquid foods may be unsuitable largely because electrolysis and the formation of highly reactive chemicals occur during the discharge. More recent designs may show some promise for use in food preservation, although the reported results should be confirmed by independent researchers.

Pulsed Light Technology

PULSED LIGHT IS A METHOD OF FOOD PRESERVATION THAT involves the use of intense and short-duration pulses of broad spectrum "white light" (ultraviolet to the near infrared region). For most applications, a few flashes applied in a fraction of

a second provide a high level of microbial inactivation.

This technology is applicable mainly in sterilizing or reducing the microbial population on packaging or food surfaces. Extensive independent research on the inactivation kinetics under a full spectrum of representative variables of food systems and surfaces is needed.

Oscillating Magnetic Fields

STATIC (SMF) AND OSCILLATING (OMF) MAGNETIC FIELDS HAVE been explored for their potential to inactivate microorganisms. For static magnetic fields, the magnetic field intensity is constant with time, while an oscillating magnetic field is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. OMF applied in the form of pulses reverses the charge for each pulse. The intensity of each pulse decreases with time to about 10% of the initial intensity. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperature of 0 to 50 °C for a total exposure time ranging from 25 ms to 100 ms.

The effects of magnetic fields on microbial populations have produced controversial results. Consistent results concerning the efficacy of this method are needed before considering this technology for food preservation purposes.

Ultraviolet Light

THERE IS A PARTICULAR INTEREST IN USING ULTRAVIOLET (UV) light to treat fruit juices, specially apple juice and cider. Other applications include disinfection of water supplies and food contact surfaces. Ultraviolet processing involves the use of radiation from the ultraviolet region of the electromagnetic spectrum. The germicidal properties of UV irradiation (UVC 200–280 nm) are due to DNA mutations induced by DNA absorption of the UV light. This mechanism of inactivation results in a sigmoidal curve of microbial population reduction.

To achieve microbial inactivation, the UV radiant exposure must be at least 400 J/m^2 in all parts of the product. Critical factors include the transmissivity of the product, the geometric configuration of the reactor, the power, wavelength and physical arrangement of the UV source(s), the product flow profile and the radiation path length. UV may be used in combination with other alternative process technologies, including various powerful oxidizing agents such as ozone and hydrogen peroxide, among others.

Ultrasound

ULTRASOUND IS ENERGY GENERATED BY SOUND WAVES OF 20,000 or more vibrations per second. Although ultrasound technology has a wide range of current and future applications in the food industry, including inactivation of microorganisms and enzymes, presently, most developments for food applications are nonmicrobial.

Data on inactivation of food microorganisms by ultrasound in the food industry are scarce, and most applications use combinations with other preservation methods. The bactericidal effect of ultrasound is attributed to intracellular cavitation, that is, micro-mechanical shocks that disrupt cellular structural and functional components up to the point of cell lysis. The heterogeneous and protective nature of food with the inclusion of particulates and other interfering substances severely curtails the singular use of ultrasound as a preservation method. Although these limitations make the current probability of commercial development low, combination of ultrasound with other preservation processes (e.g. heat and mild pressure) appears to have the greatest potential for industrial applications.

Critical processing factors are assumed to be the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food and the temperature of treatment.

Pulsed X-rays

A NUMBER OF STUDIES HAVE COMPARED THE EFFECTS OF ELECtron beam, gamma rays and X-rays, but comparison between these technologies is inconclusive due to differences in the doses applied. Electrons have a limited penetration depth of about 5 cm in food, while X-rays have significantly higher penetration depths (60–400 cm) depending upon the energy used.

Pulsed X-ray is a new alternative technology that utilizes a solid state-opening switch to generate electron beam X-ray pulses of high intensity (opening times from 30 ns down to a few nanoseconds; repetition rates up to 1000 pulses/s in burst mode operation). The specific effect of pulsed in contrast to non-pulsed X-rays has yet to be investigated.

The practical application of food irradiation by X-rays in conjunction with existing food processing equipment is further facilitated by: (1) the possibility of controlling the direction of the electrically produced radiation; (2) the possibility of shaping the geometry of the radiation field to accommodate different package sizes; and (3) its high reproducibility and versatility.

Potentially, the negative effects of irradiation on the food quality can be reduced.

Research Needs

THIS IS A SUMMARY OF RESEARCH NEEDS APPLICABLE TO ALL OR most of the technologies. See the chapters on each technology for additional research needs, as well as the complete list of research needs at the end of the full report.

- Evaluate the adequacy of the linear first-order survivor curve model. Although there is evidence of various types of deviations from this historical model, a universally accepted alternative has not evolved. Future research on an appropriate model(s) would be beneficial to all preservation technologies.
- Establish experimental protocol for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to various alternative technologies, especially pulsed electric fields, pulsed light, oscillating magnetic fields and X-rays. For example, PEF studies should incorporate multiple levels of electric field intensity, as well as test the potential for synergy with temperature.
- Identify differences of inactivation action/mechanism(s) among alternative technologies. For example, pulsed light and ultraviolet light, ohmic and microwave, PEF and thermal, etc.
- Determine the synergism or antagonism of one alternative process used with another and their combined effect on microbial inactivation efficiency.
- Determine potential formation of unpalatable and toxic by-products due to processing.
- Develop methods for measuring and monitoring temperatures or other treatment actions within individual, large, solid particulates.
- Identify new or changing critical process factors and their effect on microbial inactivation.
- Investigate the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) and/or activation volumes (V) are quantified. Synergistic effects among pressure, temperature and other variables also should be evaluated.

Introduction

KINETIC PARAMETERS AND MODELS ARE USED FOR THE DEVELopment of food preservation processes to ensure safety. They also provide the tools to compare the impact of different process technologies on reduction of microbial populations. The parameters used to analyze and report the reduction of a microbial population as a function of process parameters include empirical coefficients experimentally determined from microbial reduction kinetics, as well as constants from expressions based on chemical reaction kinetics. The models and kinetic parameters are used to present and compare microbial inactivation data from thermal, pressure, and electromagnetic processes.

Kinetic parameters may be used for development of processes and the comparison of parameters obtained for various microorganisms, including a discussion on the limitations of the parameters. They also can help identify research needs with recommendations on experimental approaches to be considered in the future.

The parameters (D-value and z(T), z(P), z(*E*), E, k, K, and V) have been calculated from data previously reported and using the models for thermal, pressure, and PEF technologies. The thermal treatment apply to microwave energy and electrical resistance (ohmic) processes, as well as any other technology where temperature is the primary factor. The parameters for pressure or PEF treatments should apply to any process where pressure or electricity is the primary critical factor in reducing microbial populations. Given the scarcity of data, these are estimated parameters and there is an imminent need for more research in this area. The quantity of data for several other technologies, describing the influence of the treatment on reduction of microbial populations is insufficient for a comparison.

The basic model assumes a linear first-order relationship between microbial population and time. There are considerable discussion about the appropriateness of using a first-order model to describe the reduction in microbial population for all preservation technologies but without strong evidence to support alternative needs, first order kinetics were used.

There are limitations to interpreting these parameters. Care should be taken when they are intended to be used as tools in the develop of processes, comparison of the resistance of different microbial populations, or identification of appropriate microorganisms.

Kinetic parameters for microbial populations exposed to thermal treatments have been assembled over a significant period of time. Published literature has included kinetic parameters needed to respond to most process, product, and microbial situations. Parameters provide a sound basis for development of processes for the microwave energy and electrical resistance (ohmic) technologies. Electrothermal treatments lack conclusive evidence on the existence of nonthermal effects influencing the reduction in microbial populations.

Data used to determine the D-value and/or k for pressure treatment of microbial populations appear to be adequate. Limitations of these data are primarily associated with temperature control or temperature changes during the pressure treatments. Evidence suggesting a synergistic impact of pressure and temperature on microbial populations is too limited for use.

The most serious deficiency is that parameters were collected at a single pressure. Only three studies have used three to five pressure levels, while controlling all other factors affecting the parameters.

Data available on the influence of PEF on microbial populations have many limitations. The kinetic parameters are based on two points on the survivor curve. No single report has measured the inactivation of microbial populations at several levels of electric field strength, leading to the quantification of the PEF coefficient. No published report evaluates the potential for a synergistic influence of electric field strength and temperature. There are only two reports with kinetic parameters based on equation (11) and (12) and they do not include any of the microorganisms of food safety concern.

Literature provides an impressive array of kinetic parameters to be used in the development of thermal processes. Promising alternative thermal processes to reduce pathogenic microbial populations are the use of microwave energy and electrical resistance (ohmic), which are included in this report.

Kinetic parameters for thermal inactivation of vegetative cells of *Salmonella, Escherichia coli, Yersinia enterocolitica, Vibrio, Aeromonas hydrophila, Campylobacter jejuni, Listeria monocytogenes,* and *Staphylococcus aureus* are well established.

In general, the thermal resistance constants z(T) for the vegetative microorganisms fall in the range between 4 and 7.7 °C.

The largest D-value (smallest k-value) reported at 110 °C for toxin-producing sporeforming microorganisms is 12.42 min (0.185/min) for *Clostridium botulinum* proteolytic Type B spores at 110 °C in pureed peas.

An independent and additional inactivation mechanism due to the electric current during the ohmic heating may occur. However, the overall influence of these nonthermal effects is not sufficient to consider the use of alternate kinetic parameters for development of ohmic heating processes. A two-stage process involving ohmic heating, interrupted by a 20 min incubation, resulted in enhanced inactivation of *B. subtilis* spores.

The nonthermal effects of microwave processes on microbial inactivation have not been confirmed and appear to be of insufficient magnitude to be considered during development of processes.

For processes involving the use of pressure for reduction of microbial populations, the F-value is the time the product needs to be exposed to the specified pressure and other conditions (i.e., temperature) to accomplish the recommended amount of inactivation.

The combined influence of pressure and temperature on inactivation kinetics has been investigated on only a limited basis.

Pressure appears to have a significant influence on inactivation rates for populations of *S. aureus.* In general, inactivation rates of various *Listeria* seem lower than the ones for *S. aureus,* with D-values ranging from 1.48 min (k = 1.556/min) at 350 MPa to 15 min (k = 0.154/min) at 400 MPa.

Comprehensive data on inactivation rates of *Clostridium sporogenes* spores show the influence of pressure on inactivation rate, z(P), to be 725 MPa at 93 °C, 962 MPa at 100 °C, and 752 MPa at 108 °C.

Recent inactivation data for *C. botulinum* Type E Alaska and Type E Beluga indicate that their D-values were in the same range as for *C. sporogenes.* The D-values for *C. botulinum* Type A 62-A are generally higher than the values for *C. sporogenes,* even when considering the influence of temperature and pressure.

An in-depth investigation of pressure inactivation of *S. cerevisiae* reported for apple juice and orange juice a z(P) of 115 MPa and 117 MPa, respectively. These values are much lower than those reported for *C. sporogenes* and *C. botulinum.*

In summary, the most pressure-resistant pathogenic vegetative cell populations appear to be those of *E. coli* O157:H7 with a D-value of 6 min (k = 0.384/min) at 600 MPa, and *S. aureus* with a D-value of 7.14 min (k = 0.323/min) at 600 MPa. The most resistant pressure spores appear to be *C. sporogenes* with a D-value of 16.772 min (k = 0.138/min) at 600Mpa (T = 90 °C) and *C. botulinum* Type A 62-A with a D-value of 6.7 min (k = 0.344/min) at 827 MPa (T = 75 °C). The pressure coefficient z(P) of 1524 MPa for *C. botulinum* Type A 62-A constitutes an additional indication of the pressure resistance of the spore populations.

Currently, the majority of the kinetic parameters for the PEF technologies are in a form that fits the basic model. Even with the limitations mentioned above, the use of the parameters and model to establish process time (F) would seem appropriate in the short term. Models provide desirable alternatives, but a great effort would be needed to evaluate them.

Adequate inactivation data for estimating the kinetic parameters for microbial populations exposed to PEF are scarce.

The D-values (k-values) have been determined from measurements of microbial population reduction after one exposure time to a given electrical field strength. Parameters are presented in this report to allow for more direct comparisons of the effectiveness of PEF in reducing different microbial populations, as well as to note the influence of the media on microbial inactivation. There is a great need to better understand survivor curve shapes for microbial populations exposed to pulsed electric fields. This suggests a significant advantage for PEF, when compared to the other technologies, assuming first order kinetics in the range of 6 to 12Ds. This assumption may not be valid because inactivation of more than 99.9% of a cell population is frequently difficult to achieve.

The D-values for *B. cereus* spores are higher than for other microbial populations at the same field strength and temperature.

In summary, the survivor data for microbial populations exposed to PEF are too limited to be used in reaching definite conclusions about the magnitude of the kinetic parameters. Data are not adequate to calculate parameters to compare the relative resistance of various microbial populations to PEF. For instance, data based on the same field strength and temperature are lacking. In addition, only a few of the published reports provide information on the threshold field strengths needed to initiate inactivation.

The following is a list of research areas that need further investigation:

- Evaluation of the adequacy of a linear first-order survivor curve. Although there is evidence of various types of deviation from the historical model, a universally accepted alternative has not evolved. Future research on an appropriate model would be beneficial to all preservation technologies.
- Investigation on the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) and/or activation volumes (V) are quantified. These investigations should also evaluate synergistic effects between pressure and temperature.
- Research on developing an experimental protocol for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to PEF. These studies should incorporate multiple levels of electric field intensity, as well as the potential for synergy with temperature.
- Further research on the PEF microbial inactivation models presented as equation (11) or (12). The investigations need to provide reliable kinetic parameters for these models and for the microbial population of interest in food safety.

Efficacy of a preservation technology 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 sublethal injury. Each influences the resistance independently of the apparent inactivation capacity of that particular process.

Among the food microbial hazards, bacteria are generally the most resistant microorganisms of concern and therefore should be the primary targets in most preservation. However, in designing processes to inactivate all pathogens, it is also advisable to consider the resistance properties of other microorganisms such as yeasts, molds, parasites, and protozoa that may persist in or grow in foods.

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 all microbial life (usually targeting spores of *Clostridium botulinum*) capable of germinating and growing in the food under normal storage conditions.

Differences in resistance of microorganisms may be found not only between genera and species but also between strains of the same species. Another factor that can affect resistance of bacteria to preservation processes is stage of growth. Extreme environments may result in the selection of mutants resistant to severe conditions suggesting that bacterial stress may induce hypermutability. Leading to a microbial population of greater resistance. Higher heat resistance of acid- or salt-adapted, heat-shocked, or starved *E. coli* O157:H7 cells. Sublethal stresses to ethanol, acid, hydrogen peroxide, heat, or salt had variable effects on subsequent exposure of *L. monocytogenes* to lethal levels of the same stressors. The questions relative to process design and verification are: (1) are the microorganisms and food environments likely to be of the type involved in stress induction, (2) would stress induced resistance possibly occur at any point in the food processing operation, and (3) if it did, would it significantly impact the inactivation process leading to possible underprocessing. Inactivation often results in a continuum of effects with some degree of injury. Injured cells can be easily underestimated, resulting in misleading conclusions about the efficiency of the method. The choice of "best" method to enumerate the test organism will largely depend on the experimental variables.

The following bacteria are known to be responsible for causing foodborne disease: *Aeromonas, Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens,* pathogenic *Escherichia coli, Listeria monocytogenes, Salmonella, Shigella, Staphylococcus aureus, Vibrio* species, and *Yersinia enterocolitica.* The primary virus of concern that is carried by foods is Hepatitis A. *Cryptosporidium* and *Cyclospora* are protozoa of concern mainly because they produce resistant cysts. When exploring the new preservation technologies, their preservation level should be compared to that of classical pasteurization or commercial sterilization technologies.

Bacteria of the genus *Salmonella* is one of the most well-known and frequently encountered pathogen in foods. Approximately 2,200 serovars of *Salmonella enterica* subsp. *enterica* exist.

Inadequate heating of foods of animal origin or cross contamination are the primary vehicles for salmonellosis outbreaks.

The maximum growth temperature for *Salmonella* is 49.5 °C. The microorganism is considered to be sensitive to heat and is killed easily by pasteurization of milk equivalent to 71.7 °C for 15 s.

The two primary foodborne pathogens that cause microorganisms for shigellosis, are *Shigella flexneri* and *Shigella sonnei.* The microorganism is not particularly heat resistant.

Five to six types of diarrheagenic *Escherichia coli* are known today, including enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroadherent, and enteroaggregative. Enterohemorrhagic *Escherichia coli* (EHEC) causes a sequela called hemolytic uremic syndrome. Foods implicated in outbreaks of EHEC include ground beef, roast beef, alfalfa sprouts, raw milk, apple cider, meat sandwiches, mayonnaise, lettuce, and dry salami. The heat resistance of *Escherichia coli* is equivalent to or slightly lower than *Salmonella.*

Yersinia enterocolitica is a pathogen that causes a foodborne infection with an onset time of 3–7 days. *Yersinia, enterocolitica,*

can grow at refrigeration temperatures and has very low heat resistance in milk.

Vibrio cholerae, Vibrio parahaemolyticus, and *Vibrio vulnificus* are found in the marine environments. Heating above 60 °C should easily inactivate this heat-sensitive organism (Table 6).

Aeromonas hydrophila. Infections among healthy people are generally self-limiting whereas children are at the greatest risk. *Aeromonas hydrophila* is eliminated by mild heat treatments.

Campylobacter jejuni is the leading cause of foodborne illness in humans in the United States. It causes a diarrheal infection but can also have a more severe sequela known as Guillain-Barre Syndrome and is extremely sensitive to heat and would be inactivated at temperatures as low as 55 °C.

Listeria monocytogenes is the cause of a foodborne illness known as listeriosis. It often attacks persons with suppressed immune systems, including pregnant females, neonates, the elderly, and those persons immunosuppressed by medications. *Listeria monocytogenes* is truly ubiquitous and can survive for long periods of time under extreme and adverse conditions. Heating equivalent to milk pasteurization heating to 68.3 °C or above should inactivate the microorganism in milk or other foods.

Staphylococcus aureus can produce a toxin that, if ingested, will produce mild to severe symptoms of nausea, cramps, vomiting, diarrhea, and prostration in 2–7 h, lasting 1 to 2 days. The toxin produced is resistant to heating. The cells are not heat resistant.

Bacillus cereus is a sporeforming organism that produces two types of illness: the diarrheal syndrome, which develops within 20 h following ingestion, or the emetic response, which occurs 1 to 5 h after ingestion. It produces spores, and is considered resistant to at least pasteurization conditions. Spores of *Bacillus cereus* have D_{95C} values ranging from 1.5 to 36.2 min.

Clostridium perfringens food poisoning is the result of an enterotoxin action and generally the result of poor refrigeration and inadequate reheating of cooked foods. Ingestion of large numbers of organisms is necessary both for the microorganism to survive passage through the stomach and to initiate growth and toxin production in the intestines

Clostridium botulinum produces a potent neurotoxin that may be toxic to both humans and animals. Fruits and vegetables can carry heat-resistant Type A, B, and F *C. botulinum* spores that are a major concern in low-acid canned foods. Type E spores also can be found in fish and seafood products. The heat $D_{110\,°C}$-values of *botulinum* Types A and proteolytic B spores generally range from 0.6 to 3 min at (Tables 14 and 15). *Clostridium botulinum* Type E spores are much less resistant.

The protozoa, *Cryptosporidium parvum* and *Cyclospora cayetanensis,* are not able to replicate in foods, but they do produce cysts that can remain infectious in foods for extended periods of time. Thermal resistance values include $D_{60\,°C}$ of 20 sec for *Cryptosporidium* oocysts in distilled water and $D_{71.7\,°C}$ of <1sec in milk. Heat resistance of *Cyclospora* may be similar to that of *Cryptosporidium.*

Enteric viruses that can cause foodborne infection from unpasteurized foods include Hepatitis A virus, Rotavirus, and Norwalk virus. Shellfish and unprocessed fruits are the most vulnerable to viral contamination because they do not receive a heat pasteurization step.

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, generally defined by a heat transfer rate. Validity of the established process is often confirmed using an inoculated test pack study tested under actual plant conditions Surrogate organisms are often utilized to validate the process as biological indicators that can mimic the pathogen.

One of the challenges in using new processing technologies for food preservation and pathogen inactivation is to determine if traditional methodologies can be used to establish and validate the new process. Thus, the two factors described above, which are well established for thermal processes, should be used for establishing and validating scheduled electrothermal processes. For other preservation processes not based on heat inactivation, nonpathogenic surrogates still need to be identified and their significance evaluated. Research needs to be done in the area.

Surrogates are selected from the population of well-known organisms that have well-defined characteristics and a long history of being nonpathogenic. The following microbial characteristics are desirable:

- Nonpathogenic.
- Thermal or other inactivation characteristics that can be used to predict those of the target organism.
- Durability to food and processing parameters similar to target organism (e.g., pH stability, refrigeration stability, oxygen tolerance, etc.).
- Stable thermal and growth characteristics that are similar from batch to batch.
- Easily prepared into high-density population forms.
- Once prepared, population is constant until utilized.
- Easily enumerated using rapid and inexpensive detection systems.
- Easily differentiated from other natural flora.
- Follows inactivation kinetics in a manner similar to pathogens receiving the same inactivation treatment.
- Genetically stable so results can be reproduced independently of laboratory or time of experiment.
- Will not establish itself as a "spoilage" organism on equipment or in the production area.
- Not susceptible to injury or reversible inactivation properties.

The use of surrogate organisms to determine and validate processes for alternative processes should consider

- Keep the approach as easy, accurate, and simple as possible.
- Design the process so that the surrogate exhibits a predictable time-temperature process character profile that correlates to that of the target pathogen.
- Be attentive to the introduction of system modifications or variables, which could alter the properties of the food leading to inaccurate results (e.g. thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity, etc.).
- Validate the susceptibility or tolerance of a surrogate, if it is known.

Status of the Report on Technologies[1,2,3]

FDA Questions	Alternative Processing Technologies	
	Ohmic Heating	**Microwave and Radio Frequency**
Process Description	Well described	Well described
Mechanism Of Inactivation	Well described	Well described
Critical Process Factors And Quantification	Well described Hard to predict cold zones	Well described Hard to predict cold zones
Process Deviations	As in conventional thermal processing	As in conventional thermal processing
Organisms Of Concern	As in conventional thermal processing	As in conventional thermal processing
Indicator Organisms	As in conventional thermal processing	As in conventional thermal processing
Main Research Need	Prediction of cold zones	Prediction of cold zones and uniformity of heating

[1] Not enough information was available on pulsed X-rays processing to be presented in this table.
[2] UV not presented in this table because only recent studies were discussed, not a comprehensive review.
[3] Not enough information was available on inductive heating processing to be presented in this table.

Status of the Report on Technologies

FDA Questions	Alternative Processing Technologies			
	High Voltage Arc Discharge	**PEF**	**Pulsed Light**	**OMF**
Process Description	Well described	Well described	Well described	Well described
Mechanism Of Inactivation	Well described	Well described	Described	Not identified
Critical Process Factors and Quantification	Not identified	Described Kinetic models proposed, need validation	Not well defined	Not well defined
Process Deviations	Not identified[1]	Identified	Not identified[1]	Not identified[1]
Pathogens Of Concern	Not identified	Not identified	Not identified	Not identified
Indicators[2]	Not identified	Not identified	Not identified	Not identified
Main Research Need	Independently conducted research	Treatment measurement and kinetic models validation	Independently conducted research	Consistent microbial effects

[1] Lack of critical process factors quantification does not permit suggested responses to process deviations.
[2] Must identify pathogens of concern before indicators are finalized.

Status of the Report on Technologies

FDA Questions	Alternative Processing Technologies	
	Ultrasound	**High Pressure**
Process Description	Well described	Well described
Mechanism Of Inactivation	Described	Well described
Critical Process Factors and Quantification	Suggested	Well described Proposed models
Process Deviations	Not identified[1]	Well described
Pathogens Of Concern	Not identified	Identified
Indicators	Not identified[2]	Suggested
Main Research Need	Multiple in combination with other technologies	Validation of kinetic models Influence of synergistic processing conditions

[1] Lack of critical process factors quantification does not permit suggested responses to process deviations.
[2] Must identify pathogens of concern before indicators are finalized.

Overarching Principles: Kinetics and Pathogens of Concern for All Technologies

1. Kinetic Parameters for Inactivation of Microbial Populations

1.1. Models and Parameters

Kinetic parameters and models are used for the development of food preservation processes to ensure safety. They also provide the tools to compare the impact of different process technologies on reduction of microbial populations. The parameters used to analyze and report the reduction of a microbial population as a function of process parameters include empirical coefficients experimentally determined from microbial reduction kinetics, as well as constants from expressions based on chemical reaction kinetics. The purpose of this section is to present the models and kinetic parameters used to present and compare microbial inactivation data from thermal, pressure and electromagnetic processes.

1.1.1. Rate constants

The traditional approach to describing changes in microbial populations as a function of time has used the survivor curve equation:

$$\log [N / N_0] = -t / D \quad (1)$$

where:

N = microbial population at any time, t

N_0 = initial microbial population

D = decimal reduction time, or time required for a 1-log cycle reduction in the microbial population.

The corresponding model from chemical reaction kinetics is the first-order kinetic model:

$$dN / dt = -kN \quad (2)$$

where:

k = reaction rate constant (first-order), or the slope of the natural logarithm of survivors in contrast to time for the microbial population.

Equation (2) can be integrated to obtain a more familiar expression for the reduction of microbial populations:

$$\ln [N / N_0] = -k t \quad (3)$$

By comparing Eq. (1) and (3), the relationship between the decimal reduction time and the first-order reaction rate constant is:

$$k = 2.303 / D \quad (4)$$

The primary parameters (D-value or k) would describe the microbial population reduction at a constant and defined temperature, pressure and/or electric field. The inherent assumption in the use of these models (and the corresponding parameters) is that the reduction in microbial population is described by the first-order reaction model. Alternative models are being developed to explain microbial inactivation kinetics when the linearity of the data is questionable (Peleg and Cole 1998; Anderson 1996). If there is evidence of a different reaction model, different parameters need to be identified and used for process development and prediction purposes.

Only a limited amount of the published data on microbial inactivation has been analyzed using the reaction rate model to quantify first-order rate constants (k). On the other hand, most published data on changes of food quality attributes have been presented as reaction rate constants (k). As indicated by the relationship between D-value and k, published data can be easily transformed.

1.1.2. Temperature coefficients

Traditionally, the influence of temperature on microbial population inactivation rates has been expressed in terms of the thermal resistance constant (z-value) using the following model:

$$\log [D / D_R] = -(T - T_R) / z \quad (5)$$

The thermal resistance constant z(T) is the temperature increase needed to accomplish a 1-log cycle reduction in the D–value. The reference decimal reduction time (D_R) is the magnitude at a reference temperature (T_R) within the range of temperatures used to generate experimental data. Microbial populations with higher resistance to temperature change are described by larger z(T). The most evident examples are the larger z(T) for spores compared to the ones for vegetative cells.

An alternative model for describing the influence of temperature on microbial population reduction rates is the Arrhenius equation. The model illustrates the influence of temperature on the reaction rate constant (k), as follows:

$$k = k_0 \exp [-E / R T_A] \quad (6)$$

where:

k_0 = Arrhenius Constant

E = Activation Energy Constant

T_A = Absolute Temperature

R = Universal Gas Constant

Based on the Arrhenius model (Eq. 6), the slope of ln (k) in contrast to $1/T_A$ plot determines the temperature coefficient E (activation energy constant). The activation energy constant describes the influence of temperature on the magnitude of the first-order reaction rate constant (k).

When the thermal resistance model and the Arrhenius model are applied to microbial population reduction rate data over the same temperature range, a relationship between the 2 coefficients [z(T) and E] is evident. By comparison of Eq. (5) and (6), the following relationship can be obtained:

$$E = 2.303 R T_A^2 / z \quad (7)$$

The temperature used in Eq. (7) should be selected as a midpoint in the range of temperatures used to generate the original experimental data. Equation (7) does suggest that the relationship between the 2 temperature coefficients [E and z(T)] de-

pends on temperature. The magnitudes of the 2 coefficients, however, are significantly different, and any influence of temperature is negligible as long as the temperature reference is within the range used for data collection. The use of the coefficients [z(T) or E] should be limited to the range of temperatures used to obtain experimental D-values. The z(T) should only be used with a defined reference temperature as emphasized by Datta (1992).

The use of the first-order models and the corresponding models for temperature influence must be applied within the limits of data used to generate the parameters within the expressions. The estimation of the kinetic parameters from the appropriate model requires careful attention to statistical limits created by the experimental data. Several authors, including Arabshahi and Lund (1985) and van Boekel (1996), have demonstrated the influence of statistical parameters on the use of the prediction models.

1.1.3. Pressure coefficients

There are only limited references to parameters used to describe the influence of pressure on the rate of microbial population reduction. Zook and others (1999) have used a parameter similar to the thermal resistance constant z(T), based on the following model:

$$\log[D / D_R] = -(P - P_R) / z \quad (8)$$

where D_R = decimal reduction time at a reference pressure (P_R).

In this report, the pressure coefficient will be defined as z(P) = the pressure increase required to accomplish a 1-log cycle reduction in the decimal reduction time (D-value).

In order for pressure resistance constant z(P) to be meaningful, it is important to include a minimum of 3 D-values in the analysis of data. All D-values must be obtained at the same temperature and above the threshold pressure needed for the target microbial inactivation. The threshold pressure (or critical pressure) is the pressure below which microbial inactivation does not occur.

An alternate model to describe the influence of pressure on microbial inactivation rates is based on the Eyring equation, as proposed by Weemaes and others (1999). The model describes the reaction rate constants (k) as follows:

$$\ln (k) = \ln(k_R) - [V(P - P_R)/RT_A] \quad (9)$$

where:

k_R = reaction rate constant at reference pressure (P_R)
V = activation volume constant
P = pressure
T_A = absolute temperature

The activation volume constant (V) is the pressure coefficient obtained from the slope of the ln (k) in contrast to (P – P_R) plot. The magnitude of V increases as the slope of the plot increases. When the rate of microbial inactivation increases significantly with small changes in pressure, the magnitude of the V will be larger. Alternatively, smaller values of V describe microbial populations with inactivation rates that would change less when pressure changes. As suggested when describing z(P) values, it is important for all reaction rate constants (k) used in the analysis to be measured at the same temperature. For the activation volume constant (V) to be useful and meaningful, the k constant should be measured at pressures above the threshold pressure needed to inactivate the target microbial population.

1.1.4. Electric field coefficients

As in the case of pressure processes, when microbial populations are exposed to pulsed electric fields (PEF), the electric field intensity applied should be above the threshold electric field intensity, the critical electric field intensity for the target microorganism. A model similar to those for temperature and pressure can be used to describe the influence of electric field intensity on the rate of microbial population reduction. The proposed model would be:

$$\log [D / D_R] = -(E - E_R) / z \quad (10)$$

where:

D_R = decimal reduction time at a reference electric field intensity (E_R).

The electric field coefficient in this model is defined as:

z(E) = the increase in electric field intensity (E) required to reduce the decimal reduction time (D) by 1-log cycle at a specific temperature and pressure.

All D-values used in this type of analysis should be acquired at the same temperature and pressure. A minimum of 3 D-values should be obtained for the data analysis.

An alternative model for describing the influence of electric field intensity on the survival of a microbial population was proposed by Peleg (1995). The model is based on the Fermi equation and can be expressed as:

$$N / N_0 = 1/\{1 + \exp[(E - E_d) / K]\} \quad (11)$$

where:

E_d = the electric field intensity when microbial population has been reduced by 50%.

K = a coefficient with magnitude based on the slope of the survivor curve obtained at several levels of electric field intensity.

This model has been applied to survivor data for several different microbial populations to generate typical magnitudes of the coefficient (K) (Peleg 1995). Larger magnitudes of the coefficient would suggest a higher resistance to changes in electric field intensity.

A similar model has been proposed and used by Hulsheger and others (1981) and applied by Jeyamkondan and others (1999). The model describes the survivor number as a function of electric-field strength and treatment time:

$$N / N_o = \{ t / t_c \}^{[- (E - Ec) / K]} \quad (12)$$

where:

t = treatment time

t_c = critical treatment time or treatment time below which no inactivation of microorganisms occurs

E_c = critical electric field strength or electric field strength below which no inactivation of target microorganism occurs

K = specific rate constant

The model proposed by Hulsheger and others (1981) is similar to Eq. (11), but accounts for exposure time at a given electric field intensity. The coefficient (K) has a similar relation to electric field intensity as in Eq. (11) and the relative magnitudes should be interpreted in the same manner.

1.2. Kinetic Parameters for Inactivation of Microbial Pathogens

The purpose of this section is to provide an overview and discussion on the kinetic data of microbial population inactivation. This section addresses the use of kinetic parameters for development of processes and the comparison of parameters obtained for various microorganisms, including a discussion on the limitations of the parameters. Finally, the research needs will be addressed, with specific attention to recommendations on experi-

mental approaches to be considered in the future.

Kinetic parameters describing the inactivation of microbial pathogens are presented in Tables 1A, B and C and are a summary of parameters presented in other sections of this report. The intent of the summary is to provide an overview and a comparison of the kinetic parameter magnitudes for the various microorganisms for each process technology. The parameters defined in Section 1.1. (D-value and z(T), z(P), z(*E*), E, k, K and V) have been calculated from data previously reported and using the models in Section 1.1 for thermal, pressure and PEF technologies. The parameters for thermal treatment also apply to microwave energy and electrical resistance (ohmic) processes, as well as any other technology where temperature is the primary factor in reduction of the microbial population. Likewise, the parameters for pressure or PEF treatments should apply to any process where pressure or electricity is the primary critical factor in reducing microbial populations. It must be noted that, given the scarcity of data, these are estimated parameters and there is an imminent need for more research in this area. Although this report contains references to several other technologies, the quantity of data describing the influence of the treatment on reduction of microbial populations is insufficient at this time.

Like in most of the published literature, in this report data have been analyzed assuming that the reduction in microbial populations follows a linear first-order model, with the exception of the PEF parameters that will be discussed in Section 1.2.2.4. The potential of nonlinear inactivation data or the use of alternative models cannot be ignored. Because there is currently insufficient information on alternative models to allow the type of comparisons being considered in this portion of the report, these issues will be discussed when describing the specific technologies.

The use of consistent parameters for all preservation technologies should improve the efficiency of future investigations and encourage uniformity in the methodologies for establishment of minimum process requirements.

1.2.1. Process development

The parameters presented in Tables 1A, 1B and 1C parallel the traditional parameters used for development of thermal preservation processes. The basic model for process development is based on the survivor curve Eq. (1) or (2):

$$F = - D \log [N_0 / N] = D \log [N / N_0] \quad (13)$$

or:

$$F = - \ln [N_0 / N] / k = \ln [N / N_0] / k \quad (14)$$

where F is the total time required to reduce the microbial population by a specified magnitude needed to ensure product safety, under the conditions defined by D-value or k. The basic model assumes a linear first-order relationship between microbial population and time. Currently, there is a lack of historical evidence to support alternative models; however, there is considerable discussion about the appropriateness of using a first-order model to describe the reduction in microbial population for all preservation technologies. For example, models for PEF technology as presented in Eq. (11) and (12) should continue to be evaluated, but at this time, input parameters for these models are limited.

1.2.2. Inactivation data and parameters

1.2.2.1. Limitations of the calculated parameters

A few limitations need to be considered when interpreting the parameters presented in Tables 1A, B and C. Care should be taken when they are intended to be used as tools to develop processes, to compare the resistance of different microbial populations, or to identify appropriate surrogate microorganisms.

As illustrated in this report, the kinetic parameters for microbial populations exposed to thermal treatments have been assembled over a significant period of time. Over time, the published literature has included kinetic parameters needed to respond to most process, product and microbial situations. The parameters provide a sound basis to develop processes for the microwave energy and electrical resistance (ohmic) technologies. In addition, the available parameters provide a sound basis to compare different microbial populations and the influence of different product environments on the parameter magnitudes. The key issue for these electrothermal treatments is the lack of conclusive evidence on the existence of nonthermal effects influencing the reduction in microbial populations. It is believed, however, that those effects would add an extra factor of safety to the preservation process (see Microwave and Ohmic and Inductive Heating chapters).

In general, the data used to determine the D-values (and k-values) for pressure processes appear to be adequate. The limitations to these data are primarily associated with temperature control during pressure treatments. In addition, when temperature changes have been reported, the influence on the kinetic parameters has not been analyzed. The evidence suggesting a synergistic impact of pressure and temperature is too limited for use in process evaluation.

The most serious deficiency in pressure process kinetics is that most of the parameters (D and k) have been measured at a single pressure. Only 4 studies (Rovere and others 1996; Kalchayanand and others 1998; Zook and others 1999; Reddy and others 1999) have used 3 to 5 pressure levels, while controlling all other factors influencing the parameters. The results from these studies are adequate to evaluate the pressure coefficient [z(P)] and/or activation volume [V]. With exception of the 4 publications cited above, the estimated parameters are limited by the number of pressure magnitudes used, the lack of temperature control and the lack of multiple data for the same microorganism and/or product/substrate. By overcoming these limitations, parameters from future investigations will meet the needs of process development and product/microorganism comparisons.

The data available on the influence of PEF on microbial populations have many limitations. As will be indicated during the discussion of parameters in Table 1C, the kinetic parameters (D-value or k) are based on 2 points on the survivor curve, the initial population and the final population. It should be recognized that the values of parameters in Table 1C were not based on linear regression analysis. In addition, temperature controls and collection of multiple data points at the same temperature level are lacking.

At this time, no single report has measured the inactivation of microbial populations at several levels of electric field strength, leading to the quantification of the PEF coefficient z(*E*) . Although 3 such coefficients are presented in Table 1C, these coefficients have been estimated based on kinetic parameters reported in separate investigations and must be used with these limitations in mind. There are no published reports that evaluate the potential for a synergistic influence of electric field strength and temperature. There are only 2 reports with kinetic parameters based on Eq. (11) and (12) and these reports provide limited parameters on microorganisms of food safety concern. They do not include any of the microorganisms of food safety concern.

1.2.2.2. Thermal processes

The literature provides an impressive array of kinetic parameters to be used in the development of thermal processes. In addition to data and parameters on inactivation of microbial populations, Table 1A includes additional information on the medium

used and specific experimental conditions (that is, temperature) when available. The time parameters are the decimal reduction time (D-value) and the corresponding rate constant (k). The temperature coefficients include the thermal resistance constant [z(T)] and the activation energy constant (E).

The kinetic parameters calculated for the thermal inactivation of microbial pathogens in Tables 1A, B and C should be considered when using any process technology where temperature is the primary mode of microbial inactivation. The most promising alternative thermal processes to reduce pathogenic microbial populations are microwave energy and electrical resistance (ohmic), which are included in this report. As suggested in the chapters on microwave and ohmic and inductive heating, it is assumed that the direct influence of microwave energy or electrical resistance on microorganisms is negligible. Therefore, thermal kinetic parameters should be considered for the above-mentioned electrothermal processes (ohmic, inductive and microwave heating).

Kinetic parameters for vegetative cells of *Salmonella* serovars, pathogenic *Escherichia coli, Yersinia enterocolitica,* pathogenic *Vibrio* spp., *Aeromonas hydrophila, Campylobacter jejuni, Listeria monocytogenes* and *Staphylococcus aureus* are presented in Table 1A. In general, the D-values are relatively small and the k-values are relatively large for the vegetative microorganisms normally targeted in pasteurization or other mild thermal processes. Other than the abnormally high D-values (low k-values) for *Salmonella* pathogens in milk chocolate, *Salmonella Typhimurium* and *L. monocyctogenes* are the most thermally resistant vegetative microorganisms. The largest D-value for Salmonella Typhimurium is 18.3 min (k= 0.126/min) at 55 °C. For *L. monocyctogenes*, the largest D-value is 16.7 min (k = 0.14/min) at 60 °C. The largest D-values for *E. coli* are 6.6 min (55 °C) for O111:B4 and 6.4 min (57 °C) for O157:H7. Based on limited data for O157:H7 in ground beef, a z(T) of 5.3 °C has been estimated. Other significant magnitudes for D-values include 6.6 min for *A. hydrophila* at 48 °C and 16.7 min for *L. monocytogenes* in cured ground beef at 60 °C. A z(T) of 5.56 °C for *L. monocytogenes* in milk has been estimated, based on published data.

In general, the thermal resistance constants z(T) for the vegetative microorganisms fall in the range between 4 and 7.7 °C. This range includes a z(T) of 5.3 °C for *E. coli* O157:H7 in ground beef and of 5.56 °C for *L. monocytogenes* in milk, both estimated from limited data presented in the references cited. The larger z(T) presented include 12.4 to 25 °C for *Vibrio* species (in fish products) and 17.7 to 18.9 °C for *Salmonella* serovars (in milk chocolate). These abnormally high z(T) for vegetative microorganisms should be noted for these products and may be specifically associated with them.

S. aureus, a vegetative microorganism that produces a heat-stable toxin, has D-values similar to other vegetative populations. The z(T) of 9.5 °C is relatively high and must be considered when developing processes for situations where *S. aureus* could present a health hazard.

The largest D-value (smallest k-value) reported at 110 °C for toxin-producing, sporeforming microorganisms is 12.42 min (0.185/min) for *Clostridium botulinum* proteolytic Type B spores in pureed peas. Most other D-values are in the more typical range of 1 to 3 min for sporeforming microorganisms. Other values to be noted are the D-value of 36.2 min (k = 0.064/min) for *Bacillus cereus* spores at 95 °C and 100 min (k = 0.023/min) for *C. botulinum* nonproteolytic Type E spores at 70 °C. When expressed at 110 °C, these D-values become 1.18 min for the *B. cereus* spores and less than 1 sec for the Type E spores.

Data for *Bacillus subtilis* spores have been included in Table 1A to illustrate the influence of ohmic heating on inactivation kinetics. These data were reported by Cho and others (1999) and indicate that the reduction in D-value (higher k-value) and the increase in z(T) (lower E) when using ohmic heating are statistically significant. These results suggest an independent and additional inactivation mechanism due to the electric current during the ohmic heating. The overall influence of these nonthermal effects, however, is not sufficient to consider the use of alternate kinetic parameters for development of ohmic heating processes. These authors have demonstrated that a 2-stage process involving ohmic heating, interrupted by a 20-min incubation, resulted in enhanced inactivation of *B. subtilis* spores. This increase in inactivation has been attributed to the positive influence of electric treatment on spore germination.

Separate data for microwave heating are not included in this section. The nonthermal effects of microwave processes on microbial inactivation have not been confirmed and appear to be of insufficient magnitude to be considered during development of processes.

1.2.2.3. Pressure processes

For processes involving the use of pressure for reduction of microbial populations, the F-value is the time the product needs to be exposed to the specified pressure and other conditions (that is, temperature) to accomplish the recommended amount of inactivation. Since the application of most of the pressure technologies involves instantaneous adjustment to the process pressure, the use of the basic model is straightforward. The pressure coefficients [z(P) or V] provide users with the flexibility to select the most appropriate pressure for the specific application. For pulsed-pressure technologies, the model would need to incorporate the influence of time and incremental pressure. In this case, the estimation of kinetic parameters will require the measurement of other variables.

The kinetic parameters for inactivation of microbial populations due to pressure are presented in Table 1B. The time parameters, decimal reduction time (D-value) and first-order rate constant (k), were calculated based on the reduction in microbial population at a constant pressure. The pressure coefficients are z(P) and the activation volume constant (V), as defined in Section 1.1.3. and indicate influence of pressure on the rate of inactivation. In most references cited, there are insufficient data to estimate these coefficients. Special consideration needs to be given to the combined use of pressure and temperature. Based on the current available information, the z(P) and z(T) parameters should be adequate for process development. The combined influence of pressure and temperature on inactivation kinetics for microbial populations has been investigated, although not extensively. Published reports suggest a synergistic impact of pressure and temperature on inactivation rates, but additional investigations are needed. The independent influence of pressure on rates, as indicated by the z(P) or V parameters, needs to be clearly established. The influence of temperature can be quantified in several ways, but the optimum approach would be based on the dependence of z(P) or V on temperature. Although minimum pressure thresholds for microbial inactivation are not presented in this section, these parameters are discussed in the section on high pressure processing.

Several investigations on Salmonella indicate that decimal reduction times (D-value) range from 1.48 to 6 min (k = 0.348 to 1.556/min), with pressure having an obvious influence on the rate. Most of the studies have been conducted at ambient temperatures (20 to 25 °C). The D-values for *E. coli* are as high as 15 min (k = 0.154/min) at 300 MPa and 6 min (k = 0.384/min) at 600 MPa for O157:H7. There are insufficient data to establish the influence of pressure or temperature and therefore z(P) or z(T) were not estimated.

Pressure appears to have a significant influence on inactiva-

tion rates for populations of *S. aureus*, apparently one of the most pressure-resistant vegetative bacteria, as suggested by D-values of 7.14 min (k = 0.323/min) at 600 MPa compared to 150 min (k = 0.015) at 400 MPa. D-values reported for 500 MPa are lower than the ones for 400 MPa, but were measured in a different medium and may be influenced by temperature. However, in comparable experiments, inactivation rates of selected strains of various *Listeria* spp. with, for example, D-values ranging from 1.48 min (k = 1.556/min) at 350 MPa to 15 min (k = 0.154/min) at 400 MPa were lower than the ones for *S. aureus*. These data were measured at ambient temperatures (20 to 25 °C). Recently, D-values of over 5 min were also reported for *L. monocytogenes* at 345 MPa and 25 °C (Alpas and others 1999).

Comprehensive data on inactivation rates of *Clostridium sporogenes* spores were reported by Rovere and others (1996). These data indicate that D-values are 0.695 min (k = 3.314/min) at 800 MPa at 108 °C compared to 16.772 min (k = 0.136/min) at 600 MPa at 90 °C. The magnitudes of these D-values are similar to the D-value of 12 min at 680 MPa reported in a separate investigation (Crawford and others 1996), even though the latter was measured at ambient temperatures. From the Rovere and others (1996) data, the influence of pressure on inactivation rate, z(P), were estimated to be 725 MPa at 93 °C, 962 MPa at 100 °C and 752 MPa at 108 °C. The inconsistent influence of temperature on z(P) may be associated with the limited range of temperatures and pressures used in the experimental investigation, as well as adequacy of temperature control during data collection.

Recent inactivation data for *C. botulinum* Type E Alaska and Type E Beluga (Reddy and others 1999) indicate that their D-values were in the same range as for *C. sporogenes*. The D-values for *C. botulinum* Type E Alaska were lower in crab meat than in a buffer. The D-values for *C. botulinum* Type A 62-A are generally higher than the values for *C. sporogenes*, even when considering the influence of temperature and pressure. The pressure coefficient z(P) for the Type A 62-A data was 1524 MPa. Surprisingly, this value was much higher than the z(P) values reported for *C. sporogenes*, even though data from *C. sporogenes* were recorded at lower temperatures.

An in-depth investigation of pressure inactivation of *Saccharomyces cerevisiae* in orange and apple juice has been reported by Zook and others (1999). The calculated D-values were 10.81 min (k = 0.21/min) at 300 MPa, where temperatures have been maintained at levels between 34 and 43.4 °C. These D-values are slightly higher than the ones reported earlier by Parish and others (1998). For apple juice and orange juice z(P) were 115 MPa and 117 MPa, respectively. These values are much lower than those reported for *C. sporogenes* and *C. botulinum*. Since data for 5 different pressures have been reported by Zook and others (1999), the activation volumes (V) could be estimated to be 1.24×10^{-4} for orange juice and 1.37×10^{-4} m^3/mole for apple juice.

In summary, the most pressure-resistant pathogenic vegetative cell populations appear to be those of *E. coli* O157:H8 with a D-value of 6 min (k= 0.384/min) at 600 MPa, and *S. aureus* with a D-value of 7.14 min (k = 0.323/min) at 600 MPa. The most pressure-resistant spores appear to be *C. sporogenes* with a D-value of 16.772 min (k = 0.138/min) at 600MPa (T = 90 °C) and *C. botulinum* Type A 62-A with a D-value of 6.7 min (k = 0.344/min) at 827 MPa (T = 75 °C). The pressure coefficient z(P) of 1524 MPa at 75 °C for *C. botulinum* Type A 62-A constitutes an additional indication of the pressure resistance of the spore populations. A recent report shows little if any inactivation after 30 min of *C. botulinum* 17B and Cap 9B exposure to 827 MPa at 75 °C (Larkin and Reddy 1999).

1.2.2.4. Pulsed electric field processes

Currently, the majority of the kinetic parameters for the PEF technologies are in a form that fits the basic model [Eq. (13) or (14)]. Even with the limitations mentioned above, the use of the parameters and model to establish process time (F) would seem appropriate in the short term. Models, such as Eq. (11) or (12), provide desirable alternatives, but a great effort would be needed to evaluate them. The use of z(*E*) values provides the users with flexibility to select the optimum electric field strength for a given product and to evaluate the influence of other factors such as synergistic effects of electric field strength and temperature. Adequate inactivation data for estimating the kinetic parameters for microbial populations exposed to PEF are scarce. The information presented in Table 1C compares decimal reduction times (D-value) and first-order rate constants (k), for experiments where electrical field strength (*E*) and initial temperature were mostly available. Three different PEF coefficients have been presented: the z(*E*), the specific rate constant (K) from the Hulsheger model (Hulsheger and others 1981) and a similar constant (K) based on the analysis by Peleg (1995).

It should be noted that the D-values (k-values) have been determined from measurements of microbial population reduction after 1 exposure time to a given electrical field strength. The parameters obtained should be considered with this limitation. Furthermore, there is no evidence that the survivor curve during exposure to a pulsed electric field is described by a first-order model. The parameters are presented in this report to allow for more direct comparisons of the effectiveness of PEF in reducing different microbial populations, as well as to note the influence of the media on microbial inactivation. In addition, the D-values (k) provide a more direct approach to evaluating the influence of electric field strength on the rate of microbial population reduction. As will be emphasized later in this section of the report, there is a great need to better understand survivor curve shapes for microbial populations exposed to pulsed electric fields.

The results in Table 1C clearly indicate that the D-values are several orders of magnitude smaller than the same parameters for thermal or pressure processes. Assuming first-order kinetics through 6-12Ds, this suggests a significant advantage for PEF, when compared to the other technologies. This assumption may not be valid because inactivation of 99.9% of a cell population is frequently difficult to achieve.

Several investigations have reported data on reduction of *E. coli* populations exposed to PEF. The highest D-values are 4500 μs (k = 0.051×10^{-2} /μs) at 16 kV/cm and 17.8 μs (k = 12.94×10^{-2} /μs) at 70 kV/cm. Using a limited number of D-values, a z(*E*) of 41 kV/cm has been estimated. Note that this magnitude is based on less than ideal data, collected at temperatures ranging from 15 to 37 °C. The D-value of 4500 μs, at 16 kV/cm and 37 °C for *E. coli* would suggest that this microorganism is one of the more PEF resistant vegetative cell populations.

The investigations on the influence of PEF on *Salmonella* Dublin, *S. aureus* and *Zygosaccharomyces bailii* provide only limited amounts of data. The D-values for *S. aureus* are very similar to the magnitudes for *E. coli*, with values of 4000 to 6000 μs at relatively low electric field strength (16 kV/cm) and temperatures of 30 to 37 °C.

The data for the *Listeria* spp. indicate that D-values are as low as 18.8 μs at 50 kV/cm for *Listeria innocua* and as high as 540 μs at 20 kV/cm for *L. monocyctogenes*. Since these data were measured at relatively low temperatures (10 to 50 °C), the parameters would indicate that *Listeria* is one the more resistant vegetative cell populations to a PEF treatment.

The D-values of 50–60 μs (k = 3.84 to 4.61×10^{-2} /μs) at 50 kV/cm for *B. cereus* spores are higher than for other microbial populations at the same field strength and temperature. Two D-values (17.5 to 26.3 ms) for *B. subtilis* spores at the same pressure

Table 1A—Kinetic parameters for inactivation of microbial population for thermal processes

Process Technology	Microorganism	Substrate	Time Parameter (D) (min)	Time Parameter (k) (1/min)	Temperature Coefficient Z(T) (C)	Temperature Coefficient (E) (kJ/mole)	Temperature (C)	Other	References
Thermal									
	Vegetative Cells								
	Salmonella serovars	Milk	0.018-0.56	4.113-127.9	4.4-5.6	392-499	65.6		ICMSF(1996)
	S.Senftenberg	various foods	0.56-1.11	2.075-4.113	4.4-5.6	392-499	65.5		ICMSF(1996)
	S.Typhimurium	TBS + 10-42%MS	4.7 - 18.3	0.126-0.49	4.5-4.6	448-458	55		ICMSF(1996)
	S. Senftenberg	Milk chocolate	276 - 480	0.005-0.008	18.9	120	70-71		ICMSF(1996)
	S.Typhimurium	Milk chocolate	396 - 1050	0.002-0.006	17.7	128	70-71		ICMSF(1996)
	S.Typhimurium	Ground beef	2.13 - 2.67	0.86-1.08			57		ICMSF(1996)
	S.Eastbourne	Milk chocolate	270	0.0085			71		ICMSF(1996)
	Escherichia coli ATCC	Dairy products	1.3-5.1	0.45-1.77			57.2		ICMSF(1996)
	E. coli O111:B4	Skim/Whole milk	5.5-6.6	0.35-0.42			55		ICMSF(1996)
	E. coli O157:H7	Ground beef	4.1-6.4	0.36-0.56			57.2		Line and others (1991)
	E. coli O157:H8	Ground beef	0.26-0.47	4.9-8.86	5.3	401	62.8		Line and others (1991)
	Yersinia enterocolitica	Milk	0.067-0.51	4.52-34.4	4-5.78	367-530	60		ICMSF(1996)
	Vibrio parahaemolyticus	Fish homogenate	10 - 16	0.144-1.05	5.6-12.4	159-352	48		ICMSF(1996)
	V. parahaemolyticus	clam/crab	0.02-2.5	0.92-115	5.6-12.4	166-368	55		ICMSF(1996)
	V. cholerae	crab/oyst	0.35-2.65	0.87-6.58	17-21	101-125	60		ICMSF(1996)
	Aeromonas hydrophila	Milk	2.2-6.6	0.35-1.05	5.2-7.7	256-379	48		ICMSF(1996)
	Campylobacter jejuni	Skim milk	0.74 - 1.0	2.3 - 3.11			55		ICMSF(1996)
	C. jejuni	Beef/Lamb/Chicken	0.62 - 2.25	1.0 - 3.72			55-56		ICMSF(1996)
	Listeria monocytogenes	Milk	0.22 - 0.58	3.97 - 10.47	5.5	386	63.3		ICMSF(1996)
	L. monocytogenes	Meat products	1.6 - 16.7	0.14 - 1.44			60		ICMSF(1996)
	Staphylococcus aureus	Milk	0.9	2.56	9.5	224	60		ICMSF(1996)
	S. aureus	Meat macerate	6	0.384			60	+500 ppm nitrite	ICMSF(1996)
	S. aureus	Pasta	3	0.768			60	aw = 0.92	ICMSF(1996)
	S. aureus	Pasta	40	0.0576				T = 60C, aw = 0.8	ICMSF(1996)
	S. aureus	Phosphate buffer	2.5	0.921			60	pH = 6.5	ICMSF(1996)
	Spores								
	Bacillus cereus	various	1.5 - 36.2	0.064 - 1.535	6.7 -10.1		95		ICMSF(1996)
	Clostridium perfringens	Phosphate buffer	0.015 - 8.7	0.265 - 15.35			90	pH = 7.0	ICMSF(1996)
	C. perfringens	Phosphate buffer	3.15	0.731			104.4	pH = 7.0	ICMSF(1996)
	C. perfringens	Beef gravy	6.6	0.349			104.4	pH = 7.0	ICMSF(1996)
	Clostridium botulinum 62A	Vegetable products	0.61 - 2.48	0.929 - 3.775	7.5 11.6		110		ICMSF(1996)
	C. botulinum 62A	Phosphate buffer	0.88 - 1.9	1.212 - 2.617	7.6 - 10		110	pH = 7.0	ICMSF(1996)
	C. botulinum 62A	Distilled water	1.79	1.287	8.5		110		ICMSF(1996)
	C. botulinum B	Phosphate buffer	1.19 - 2.0	1.152 - 1.935	7.7 - 11.3		110	pH = 7.0	ICMSF(1996)
	C. botulinum B	Vegetable products	0.49 - 12.42	0.185 - 4.7	7.4 - 10.8		110		ICMSF(1996)
	C. botulinum E	Seafood	6.8 - 13	0.177 - 0.339	9.78		74		ICMSF(1996)
	C. botulinum E	Oyster homogenate	72 - 100	0.023 - 0.32	6.8 - 7.5		70		ICMSF(1996)
	Bacillus subtilis	0.1% NaCl	32.8	0.0702	8.74	293	88	Conventional	Cho and others (1999)
	B. subtilis	0.1% NaCl	30.2	0.0763	9.16	282	88	Ohmic	Cho and others (1999)

Table 1B—Kinetic parameters for inactivation of microbial population for pressure processes

Process Technology	Microorganism	Substrate	Time Parameter Coefficient (D) (min)	Time Parameter Coefficient (k) (1/min)	Pressure [z(P)] (MPa)	Pressure (V) (m^3/mole)	Pressure (MPa)	Pressure Threshold	Other	References
Pressure	**Vegetative Cells**									
	Campylobacter		< 2.5	>0.92			300			Smelt and Hellemons (1998)
	Salmonella serovars									
	Salmonella Senftenberg	Buffer	6	0.384			345		T=230C	Metrick and others (1989)
	S. Senftenberg		5	0.461			300			Smelt and Hellemons (1998)
	Salmonella Enteritidis	Meat	3	0.768			450			Patterson and others (1995)
	Salmonella Typhimurium	Milk	3	0.768			350			Patterson and others (1995)
	S. Typhimurium	Meat	1.48	1.556			414		T=25C	Ananth and others (1998)
	S. Typhimurium		0.6	3.838			345		T = 50C	Kalchayanand and others (1998)
	Yersinia enterocolitica	Milk	3	0.768			275			Patterson and others (1995)
	Escherichia coli		7.5 - 15	0.154 - 0.307			300			Smelt and Hellemons (1998)
	E. coli	Milk	1	2.303			400		T=50C	Gervilla and others (1997b)
	E. coli	Meat	2.5	0.92			400			Patterson and Kilpatrick (1998)
	E. coli	Milk	1	2.303			450		T=25C	Gervilla and others (1997a)
	E. coli	Buffer	3	0.768			700			Patterson and others (1995)
	E. coli O157:H7	Milk	3	0.768			400		T=50C	Patterson and Kilpatrick (1998)
	E. coli O157:H8		6	0.384			600			Smelt and Hellemons (1998)
	E. coli O157:H7		0.7	3.29			345		T = 50C	Kalchayanand and others (1998)
	Staphylococcus aureus		150	0.015			400			Smelt and Hellemons (1998)
	S. aureus	Milk	2.5	0.92			500		T = 50C	Patterson and Kilpatrick (1998)
	S. aureus	Meat	3	0.768			500		T = 50C	Patterson and Kilpatrick (1998)
	S. aureus		7.9	0.292			500			Smelt and Hellemons (1998)
	S. aureus		7.14	0.323			600			Smelt and Hellemons (1998)
	S. aureus	Buffer	3	0.768			700			Patterson and others (1995)
	S. aureus 582		0.6	3.838			345		T-50C	Kalchayanand and others (1998)
	Listeria monocytogenes		1.48 - 13.3	0.173 - 1.556			350		101 strains	Smelt and Hellemons (1998)
	L. monocytogenes	Milk	3	0.768			375			Patterson and others (1995)
	L. monocytogenes	Meat	2.17	1.061			414		T=25C	Ananth and others (1998)
	L. monocytogenes ScottA	Meat	3.5	0.658			400		T=Amb.	Mussa and others (1999)
	L. monocytogenes		5.0 - 15	0.154 - 0.461			400			Smelt and Hellemons(1998)
	L. monocytogenes		< 2.5	> 0.92			500			Smelt and Hellemons(1998)
	Listeria innocua	Eggs	3	0.768			450		T=20C	Ponce and others(1998)
	L. monocytogenes	Ground Pork	1.89 - 4.17	0.552 - 1.219			414		T = 25C	Murano and others (1999)
	L. monocytogenes	Ground Pork	0.37 - 0.63	3.656 - 6.224			414		T = 50C	Murano and others (1999)
	L. monocytogenes ScottA		4	0.576			345		T = 50C	Kalchayanand and others (1998)
	Pseudomonas fluorescens	Milk	1	2.303			300		T=50C	Gervilla and others (1997b)
	P. fluorescens	Milk	1	2.303			400		T=10C	Gervilla and others (1997b)
	P. fluorescens		0.6	3.838			345		T = 50	Kalchayanand and others (1998)
	Spores									
	Clostridium sporogenes		12	0.192			680			Crawford and others (1996)
	C. sporogenes		16.772	0.138			600		T=90C	Rovere and others (1996b)
	C. sporogenes		6.756	0.341	725 (90C)		700		T = 93C	
	C. sporogenes		5.306	0.434			800		T = 93C	
	C. sporogenes		3.502	0.658			600		T=100C	
	C. sporogenes		3.186	0.723	962 (100C)		700		T = 100C	
	C. sporogenes		2.857	0.806			800		T = 98C	
	C. sporogenes		1.282	1.796			600		T=108C	Rovere and others (1996b)
	C. sporogenes		0.901	2.556	752 (108C)		700		T = 108C	
	C. sporogenes		0.695	3.314			800		T = 108C	
	Clostridium botulinum Type E Alaska	Buffer	8.77	0.263			758		T = 35C	Reddy and others (1999)
	C. botulinum Type E Alaska	Buffer	2.64	0.872			827		T = 35C	
	C.botulinum Type E Beluga	Crab meat	3.38	0.681			758		T = 35C	Reddy and others (1999)
	C.botulinum Type E Beluga	Crab meat	1.64	1.404			827		T = 35C	
	C. botulinum Type E Alaska	Crab meat	2	1.152			758		T = 35C	Reddy and others (1999)
	C. botulinum Type E Alaska	Crab meat	1.76	1.309			827		T = 35C	
	C.botulinum Type A 62-A	Buffer	13.21	0.174			414		T = 75C	
	C.botulinum Type A 62-A	Buffer	12.6	0.183			551		T = 75C	
	C.botulinum Type A 62-A	Buffer	10.59	0.218	1524	4.39x10-6	689		T = 75C	Reddy and others (1999)
	C.botulinum Type A 62-A	Buffer	9.19	0.251			758		T = 75C	
	C.botulinum Type A 62-A	Buffer	6.7	0.344			827		T = 75C	
	Yeast									
	Saccharomyces cerevisiae	orange juice	10.81	0.21			300		T=34C	Zook and others (1999)
			2.8	0.82			350		T=36.8C	
			0.97	2.37	117	1.241X10-4	400		T=37.2C	
			0.5	4.61			450		T=39.7C	
			0.18	12.79			500		T=43.4C	
	S. cerevisiae	apple juice	9.97	0.231			300		T=34C	Zook and others (1999)
			0.88	2.617	115	1.371X10-4	400		T=37.2C	
			0.28	4.798			450		T=39.7C	
			0.15	15.35			500		T=43.4C	
	S. cerevisiae		1.27	1.813			350		pH=3.7	Parsih and others (1998)
	S. cerevisiae		0.067	34.373			500		pH=3.7	

Table 1C—Kinetic parameters for inactivation of microbial population for PEF processes

Process Technology	Microorganism	Substrate	Time Parameter (D) (microsec)	Time Parameter (k) (x 10-2/microsec)	PEF Coefficient [Z(E)] (kV/cm)	PEF Coefficient (K) (kV/cm)	Field Strength (kV/cm)	Other	Reference
Pulsed Electric Fields	**Vegetative Cells**								
	Escherichia coli	Skim milk	38.4 to 44.8	5.14 to 6.0			20 -45	T=15 C	Martin-Belloso and others (1997b)
	E. coli	SMUF	17.8	12.94			70	T=20 C	Zhang and others (1995a)
	E. coli	Milk	333	0.692			22		Grahl and others (1992)
	E. coli	SMUF	4000 to 4500	0.051 to 0.058			16	T = 37 C	Pothakamury and others (1995)
	E. coli	Buffer	75 to 100	2.3 to 3.07			20	T < 30 C	Hulsherger and Nieman (1980)
	E. coli	Phosphate buffer	270	0.853	~41	6.3 to 8.1	20	T=20 C	Hulsheger and others (1983)
	E. coli	0.1% NaCl	100	2.3			19.5	T = 20 C	Sale and Hamilton (1967)
	E. coli	Phosphate buffer	2	115.15			40		Matsumoto and others (1991)
	E. coli	Potato dextrose	16 to 32	14.39			40	T = 15 C	Zhang and others (1994b)
	E. coli	Skim milk	64 to 96	2.4 to 3.6			40	T = 15 C	Zhang and others (1994b)
	E. coli	Skim milk	27.4 to 49.6	4.64 to 8.41			50	T < 30 C	Qin and others(1995c)
	E. coli	SMUF	26.7	8.63			50	T < 30 C	Qin and others(1995c)
	Salmonella Dublin	Skim milk	4 to 42.4	0.054 to 0.52			15 to 40	T=15-40C	Sensoy and others (1997)
	S. Dublin	Milk	360	0.64			36.7	T = 63 C	Dunn and Pearlman (1987)
	Salmonella Typhimurium	NaCl	4	57.58			83		Gupta and Murray (1989)
	Listeria monocytogenes (Scott A)	Milk	150 to 200	0.012 to 0.015			30	T = 10 to 50C	Reina and others (1998)
	L. monocytogenes	Phosphate buffer	540	0.426		6.4 to 6.5 (2 to 2.8)	20		Hulsheger and others (1983)
	Listeria innocua	Skim milk	76.9	2.995			50	T = 15 to 28 C	Fernandez and others (1999)
	L. innocua	Skim milk	26.7	8.625			50	T = 22 to 34 C	Calderon-Miranda (1998)
	L. innocua	Liquid Whole Egg	18.8	12.25			50	T = 26 to 36 C	Calderon-Miranda (1998)
	Staphylococcus aureus	SMUF	4000 to 6000	0.038 to 0.058			16	T = 37 C	Hulsherger and others (1983)
	S. aureus	SMUF	4000-4500	0.052 to 0.058			16	T < 30 C	Pothakamury and others (1995)
	S. aureus	Phosphate buffer	360	0.64		2.6 (2.0)	20		Hulsherger and others (1983)
	Lactobacillus delbrueckii	SMUF	2000-2400	0.096 to 0.115			16	T < 30 C	Polhakamury (1995)
	Lactobacillus delbrueckii	Buffer	1022	0.225		(1.6)	25	T = 60 C	Jayaram and others (1992)
	Pseudomonas fluorescens	Skim milk	22.2	10.374			50	T = 15 to 28 C	Fernandez and others (1999)
	Pseudomonas auriginosa	Phosphate buffer	308.6	0.746		6.3 (1.8 to 2.6)	20		Hulsheger and others (1983)
	P. fluorescens	Water	3.3	69.79			10	T = 20C	Ho and others (1995)
	Klebsiella pneumoniae	Phosphate buffer	360	0.64		6.6	20		Hulsheger and others (1983)
	Spores								
	Bacillus cereus	0.15% NaCl	50 to 60	3.84 to 4.61			50	T = 25 C	Marquez and others (1997)
	Bacillus subtilis	0.15% NaCl	17.5 to 26.3	8.76 to 13.16			50	T = 25 C	Marquez and others (1997)
	B. subtilis	SMUF	2500 to 3000	0.077 to 0.092			16	T < 30 C	Pothakamury (1995)
	B. subtilis	Pea soup	11.3	20.38	~15.5		33	T < 5.5 C	Vega-Mercado and others (1996a)
	B. subtilis	SMUF	425 to 520	0.44 to 0.54			16		Qin and others (1994)
	Yeast								
	Saccharomyces cerevisiae	NaCl	61.5	3.745			35		Jacob and others (1981)
	S. cerevisiae	Phosphate buffer	270	0.853		(2.3)	20		Hulsheger and others (1983)
	S. cerevisiae	Water	4666	0.049			20		Mizuno and Hori (1988)
	S. cerevisiae	Potato dextrose	8.7	26.47			40	T = 15 C	Zhang and others (1994b)
	S. cerevisiae	Apple juice	102.9 to 135	1.706 to 2.238	~17		12	T = 4-10 C	Zhang and others (1994a)
	S. cerevisiae	Apple juice	42.9 to 428.6	0.537 to 5.368			25	T < 30 C	Qin and others (1995a)
	S. cerevisiae	Apple juice	0.83	277.47			50	T = 22 to 29 C	Qin and others (1995a)
	Candida albicans	Phosphate buffer	240	0.96		2.2 (1.2 to 3.1)	20		Hulsheger and others (1983)
	Zygosaccharomyces bailli	Juices	0.4 to 0.7	3.29 to 5.76			32 to 36.5	T = 20 C	Raso and others (1998)

and from 2 different investigations were considerably lower than the D-values for *B. cereus* spores. Using the D-values for *B. subtilis* spores at 3 different electrical field strengths and within an ambient temperature range, a z(*E*) of 15.5 kV/cm has been estimated. Unexpectedly, this magnitude is much lower than the one estimated for vegetative cell populations (that is, *E. coli* with a z[E] of 41 kV/cm). These observations need more comprehensive investigation before any conclusions are reached.

Several investigations have reported data on inactivation of *S. cerevisiae* when exposed to PEF. Overall, the D-values vary significantly depending on the electric field strength and temperature. In general, the magnitudes are larger than *E. coli*, lower than *L. monocytogenes* and much less than *B. subtilis* spores. An z(*E*) of 17 kV/cm has been estimated from data reported for PEF treatments of *S. cerevisiae* in apple juice, much lower than the value estimated for *E. coli* (41 kV/cm) and similar to the one of *B. subtilis* spores (15.5 kV/cm).

The influence of electrical field strength (*E*) on the rate of microbial population inactivation may also be estimated from the coefficient (K). These parameters have been reported for a limited number of microbial populations. Among them, the populations with greater resistance to PEF would include *Escherichia* spp., *Listeria* spp., *Pseudomonas* spp. and *Klebsiella* spp. The coefficient z(*E*) was highest for *Escherichia* spp., which was higher than the one for *B. subtilis* spores. Data are insufficient to make valid comparisons of the relative resistance for vegetative and spore populations to PEF.

In summary, the survivor data for microbial populations exposed to PEF are too limited to be used in reaching definite conclusions about the magnitude of the kinetic parameters. In addition, data are not adequate to calculate parameters to compare the relative resistance of various microbial populations to PEF. For instance, data based on the same field strength and temperature are lacking. In addition, only a few of the published reports provide information on the threshold field strengths needed to initiate inactivation.

1.3. Future Research Needs

This section focuses on the research needs associated with kinetic parameters to be used for development of food preservation processes to ensure safety. For several technologies discussed in this report, the data necessary to estimate kinetic parameters are lacking. If these technologies are to evolve to industrial applications, kinetic data must be collected in the future.

The following is a list of research areas that need further investigation:

- Evaluation of the adequacy of a linear first-order survivor curve. Although there is evidence of various types of deviation from the historical model, a universally accepted alternative has not evolved. Future research on an appropriate model would be beneficial to all preservation technologies.
- Investigation on the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) and/or activation volumes (V) are quantified. These investigations should also evaluate synergistic effects between pressure and temperature.
- Research on developing an experimental protocol for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to PEF. These studies should incorporate multiple levels of electric field intensity, as well as the potential for synergy with temperature.
- Further research on the PEF microbial inactivation models presented as Eq. (11) or (12). The investigations need to provide reliable kinetic parameters for these models and for the microbial population of interest in food safety.

2. Microbiological Critical Factors

The efficacy of a preservation technology 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 sublethal injury. Each of these factors influences the resistance of a microorganism to a preservation process, independently of the apparent inactivation capacity of that particular process.

Among the food microbial hazards, bacteria are generally the most resistant microorganisms of concern and therefore should be the primary targets in most preservation processes. In most cases, microorganisms other than bacteria will be destroyed before or concurrently with pathogenic and spoilage bacteria; however, in designing processes to inactivate all pathogens, it is also advisable to consider the resistance properties in foods of other microorganisms such as yeasts, molds, parasites and protozoa, that may persist in or grow in foods.

A few genera of foodborne bacteria (for example, *Clostridium* spp. and *Bacillus* spp.) are capable of existing in 2 forms: active vegetative cells and dormant spores. These 2 forms often differ in their resistance properties to heat, chemicals, irradiation and other environmental stresses. In that same manner, studies have shown that spores are typically more resistant than vegetative cells to the alternative processing technologies evaluated in this report. For pasteurization purposes, one is mostly concerned with the inactivation of vegetative cells of disease-producing microorganisms. In order to have a commercially sterile product, however, one must devise a process that inactivates all microbial spores (usually targeting spores of *C. botulinum*) capable of germinating and growing in the food under normal storage conditions.

Differences in resistance of microorganisms may be found not only between genera and species but also between strains of the same species. For instance, some bacterial strains with unique resistance to thermal inactivation, irradiation and high pressure processing have been identified. It is possible that, in the future, a pathogenic "super bug" would emerge. If this occurs, this pathogen should be considered a possible food safety hazard and the process would have to be redesigned to specifically inactivate it. Alternatively, if the "super bug" is not a pathogen or spoilage microorganism, it may be very useful as a possible surrogate during development and validation of a process. Another factor that can affect resistance of bacteria to preservation processes is stage of growth. It appears that cells in exponential or log phase of growth are generally less resistant than cells in stationary phase. The development of stress resistance proteins in stationary phase may be the cause of this phenomenon.

One of the basic principles of microbial genetics is that extreme environments that would kill most bacterial cells result in the selection of mutants resistant to severe conditions. These environmental conditions encountered by a population of cells may induce a stress "defense mechanism" in some of them. This selection process has been scientifically supported by studies suggesting that bacterial stress may induce hypermutability. Hypermutability would in turn lead to a microbial population of greater resistance (Buchanan 1997). Therefore, the exposure of cells to some form of stress may induce and allow the survival of microorganisms with unusually higher durability to a given inactivation process. Mazotta (1999) found that the heat resistance of acid- or salt-adapted, heat-shocked, or starved *E. coli* O157:H7 cells was higher than that of cells grown to exponential or stationary phase under optimum conditions. He suggested that it would be appropriate to use stress-inducing culture conditions

when studying the thermal resistance of vegetative pathogens in specific products in order to add an extra factor of safety to the process. Lou and Yousef (1997) determined that sublethal stresses to ethanol, acid, hydrogen peroxide, heat, or salt had variable effects on subsequent exposure of *L. monocytogenes* to lethal levels of the same stressors. For example, heat shocking increased the resistance of the microorganism to ethanol, hydrogen peroxide and salt, but not to acid. Davidson (1999) reviewed the impact of stress induction on resistance to food antimicrobials. He stated that resistance could be acquired through previous exposure or adaptation due to cross-protection from environmental or processing factors including stresses such as heat or acid. A number of studies like the ones described have demonstrated the occurrence of stress-induced enhanced resistance to inactivation. The questions relative to process design and verification are: (1) Are the microorganisms and food environments likely to be of the type involved in stress induction? (2) Would stress induced resistance possibly occur at any point in the food processing operation? and (3) If it did, would it significantly impact the inactivation process leading to possible underprocessing? Considering that in most food processing systems the design is to have microorganisms exposed only once to a stress-inducing factor (for example, heat, acid, antimicrobials and so on), the development of a resistant population is not likely to occur. One possible exception might be the case of previously processed material that is reprocessed into the streamline. In those cases, in-depth studies of the impact of stress induction during the processing are needed.

Another microbial-related factor that influences the effectiveness of a process is the susceptibility of the microorganism to cellular injury. The effectiveness of an inactivation process is often measured by enumerating any surviving organisms (using biological indicators or surrogates) in a selective medium. Under the best circumstances, a processed microorganism would be either viable or dead; however, inactivation often results in a continuum of effects with some degree of injury. Injured cells can be easily underestimated, resulting in misleading conclusions about the efficiency of the method. The detection and enumeration of injured microorganisms require special procedures. Often, injury is identified when surrogate organisms are enumerated using a selective culturing medium (generally a medium containing a chemical inhibitor that allows growth only of the particular microorganism being enumerated) in contrast to a nonselective medium. It is often desirable to use selective media in the field to ensure growth of only the surrogate microorganism, and not of background microflora. The choice of "best" method to enumerate the test organism will largely depend on the experimental variables and the researcher's experience with field studies.

3. Pathogens of Public Health Concern

In the United States, foodborne diseases caused by microorganisms can be attributed primarily to pathogenic bacteria, enteric viruses and protozoa (Anonymous 1999; Carsberg 1999; Jackson and others 1997; Katsuyama 1993; Varnam and Evans 1991). The following bacteria are known to be responsible for causing foodborne disease: *Aeromonas hydrophila, Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens,* pathogenic *Escherichia coli, Listeria monocytogenes, Salmonella, Shigella, Staphylococcus aureus,* pathogenic *Vibrio* spp. and *Yersinia enterocolitica.* The viruses of concern in foods are Hepatitis A, Norwalk, Norwalk-like and Rotavirus (CDC 2000; Mead and others 1999). *Cryptosporidium parvum, Cyclospora cayetanensis, Giardia lamblia* and *Toxoplasma gondii* are all parasites of concern, in part because they produce resistant cysts. When exploring the new preservation technologies, their preservation level should be compared to that of classical pasteurization or commercial sterilization technologies. Therefore, in an attempt to determine the pathogens of greatest public health concern for new technologies, the resistance of pathogens to heat will be examined.

3.1. Vegetative Bacteria Inactivated by Cooking and Pasteurization

Salmonella. Bacteria of the genus *Salmonella* is one of the most well-known and frequently encountered pathogens in foods. Approximately 2,200 serovars of *Salmonella enterica* subsp. *enterica* exist and can be isolated from meats, poultry, eggs, raw milk, water, fish, shellfish, feeds, fruits and vegetables. Because *Salmonella* serovars are natural contaminants of intestinal tracts of animals, birds and reptiles, they may contaminate food and equipment through secondary contamination. Therefore, inactivation of this pathogen through processing and avoidance of post-processing contamination is very important. The infectious dose of this microorganism can be very low: It has been demonstrated that it may take only one cell to cause a person to become ill. This makes effective pasteurization critical to produce safe food.

Inadequate heating of foods of animal origin or cross contamination are the primary vehicles for salmonellosis outbreaks. Meat and poultry (that is, beef, turkey, chicken and pork) and homemade ice cream (generally due to the use of raw eggs), fruits and vegetables and salads have been the most frequently reported items (CDC 2000). Raw or improperly pasteurized milk and eggs, as well as other foods have also been associated with salmonellosis. Inadequate cooking or processing, improper cooling, ingestion of raw products and cross contamination of foods after cooking seem to be the major sources of *Salmonella* serovars.

The maximum growth temperature for *Salmonella* serovars is 49.5 °C. The microorganism is considered to be sensitive to heat and is killed easily by pasteurization of milk equivalent to 71.7 °C for 15 s. The heat resistance of *Salmonella* serovars depends on factors such as serovar type, water activity, pH and heating medium. The heat resistance of serovars in various foods is shown in Table 2. *Salmonella* serovar Senftenberg is generally considered to be the most resistant strain (Tables 2 and 3). Therefore, any heat-resistant studies using *Salmonella* serovars should include serovar Senftenberg strain 775W, unless it is not relevant for the application. Then it would serve only as a point of reference for heat resistance.

Shigella. Bacteria of the genus *Shigella,* the causative microorganism for shigellosis, is a member of the *Enterobacteriaceae.* It is a gram-negative, nonsporeforming nonmotile rod. The organism has a growth range of 10 to 47.2 °C with an optimum of 37 °C. The 2 primary foodborne pathogens are *S. flexneri* and *S. sonnei.* The microorganism is carried by humans and primates and is spread to food by carriers and contaminated water. *Shigella* strains are not particularly heat resistant. Approximately 5 min at 63 °C inactivates most strains of *S. flexneri* and *S. sonnei.* The main foods implicated in outbreaks of *Shigella* spp. are salads and seafoods that become contaminated during handling by infected workers or by unclean and unsanitized food contact surfaces. Control of *Shigella* is best accomplished by hygiene, health education, water disinfection and sanitation along with mild heat treatment where necessary.

Pathogenic *Escherichia coli.* *Escherichia coli* is a gram-negative, motile, facultative anaerobe nonsporeforming rod. The source of the microorganism is generally the gastrointestinal tract of warmblooded animals but it can also be found in water. Five to 6 types of diarrheagenic *E. coli* are known today, including enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroadherent and enteroaggregative. These strains may cause neonatal, infantile, traveler's, or bloody diarrhea. Some produce toxins while others are invasive. Enterohemorrhagic *E. coli* (EHEC) causes a sequela called hemolytic uremic

syndrome. Foods implicated in outbreaks of EHEC include ground beef, roast beef, alfalfa sprouts, raw milk, apple cider, meat sandwiches, mayonnaise, lettuce and dry salami. Food process inactivation of this bacterium is best accomplished by: 1) adequate cooking of all meat products to a center point temperature of 165–180 °F and 2) a professional sanitation program in place to inactivate the bacteria and prevent post-processing contamination or cross-contamination of processed food with raw product. The heat resistance of *E. coli* is equivalent to or slightly lower than *Salmonella* serovars (Table 4). Therefore, a heat process sufficient to inactivate *Salmonella* serovars will also likely inactivate *E. coli*.

Yersinia enterocolitica. *Yersinia enterocolitica* is a pathogen that causes a foodborne infection with an onset time of 3–7 d. The symptoms of yersiniosis include severe abdominal cramps which mimic appendicitis, watery diarrhea, vomiting and fever. Pork and pork products, milk and foods washed in contaminated water (for example, tofu) have all been implicated in outbreaks. Cross contamination can also cause a problem in ready-to-eat foods. *Yersinia enterocolitica,* as a psychrotroph can grow at refrigeration temperatures. In fact, cold storage can be selective for the microorganism. *Yersinia enterocolitica* has very low heat resistance in milk (Table 5).

Vibrio. Three species of *Vibrio* are potential pathogens in food: *V. cholerae, V. parahaemolyticus* and *V. vulnificus.* All are found in the marine environments and contaminate foods via contaminated water. Foods associated with *Vibrio* spp. foodborne infections include seafoods, raw vegetables, milk and inadequately sanitized water. Keeping raw product at low temperatures prior to processing helps to slow growth, and heating above 60 °C should easily inactivate this heat-sensitive organism (Table 6).

Aeromonas hydrophila. *Aeromonas hydrophila* has many pathogenic properties resembling *V. vulnificus,* that is, gastroenteritis in healthy individuals or septicemia in individuals with underlying chronic disease (for example, leukemia, carcinoma and cirrhosis) and those treated with immunosuppressive drugs or who are undergoing cancer chemotherapy or with impaired immune systems. Infections among healthy people are generally self-limiting whereas children are at the greatest risk. Species of *A. hydrophila* are ubiquitous in freshwater environments. Although *A. hydrophila* can be isolated from a wide range of foods at the retail level, outbreaks are generally small. The organism is a well-established component of raw meat spoilage and is found on beef, pork, lamb, poultry, fish and shellfish. It is also a common component of raw milk and raw vegetables. *Aeromonas hydrophila* is eliminated by mild heat treatments (Table 7).

Campylobacter jejuni. *Campylobacter jejuni* is the leading cause of foodborne illness in humans in the United States (CDC 1999). The organism causes a diarrheal infection but can also have a more severe sequela known as Guillain-Barre Syndrome (0.2 to 2 cases/1000 cases of *C. jejuni*; paralysis, demyelination of nerves). The microorganism is generally unable to grow in foods; however, it often finds entry to food via human carriers or contamination. In heat-treated or dehydrated foods, contamination may not be a problem, but raw refrigerated foods of animal origin can be a source. The organism can be isolated from all common food animals and birds. Foods commonly associated with infection include raw milk, poultry, red meat and contaminated water sources. The microorganism is extremely sensitive to heat and would be inactivated at temperatures as low as 55 °C (Table 8).

Listeria monocytogenes. *Listeria monocytogenes* is the cause of a foodborne illness known as listeriosis. There are 13 serovars of pathogenic *L. monocytogenes,* but according to Raccourt and Cossart (1997), 95% of human isolates are serotypes 1/2a, 1/2b and 4b. The microorganism often attacks persons with suppressed immune systems, including pregnant women, neonates, the elderly and persons immunosuppressed by medications. Manifestations of listeriosis include abortion, perinatal septicemia and meningitis. The mortality rate associated with listeriosis is ca. 30% (Rocourt and Cossart 1997; V.J. Scott, personal communication). Although rarely described, *L. monocytogenes* can grow to high populations in temperature-abused food resulting in severe gastroenteritis after consumption. *Listeria monocytogenes* is truly ubiquitous and can survive for long periods of time under extreme and adverse conditions. *Listeria monocytogenes* can multiply in foods stored at refrigeration temperatures so risk may increase during storage. It has been found in raw milk, raw milk cheese, soft-ripened cheeses, raw meats and seafood. There have been cases of illness from coleslaw and other raw vegetables that have been fertilized with animal manure or wastewater, and then not rinsed and cleaned prior to preparing and eating. Recently, an outbreak of listeriosis caused by *L. monocytogenes* serotype 4b resulted in 50 cases and 8 deaths linked to consumption of hot dogs and/or deli meats (CDC 1998). *Listeria monocytogenes* may be controlled in food processing plants with a good sanitation program and prevention of cross-contamination between raw and finished product. In addition, heating equivalent to milk pasteurization (71.7 °C for 15 s) or heating to 62.8 °C for 30 min or above should inactivate the microorganism in milk or other foods (Tables 9 and 10).

3.2. Vegetative Bacteria Inactivated by Pasteurization but Able to Produce a Heat-stable Toxin

Staphylococcus aureus. *Staphylococcus aureus* can produce a toxin in improperly stored food that, if ingested, will produce mild to severe symptoms of nausea, cramps, vomiting, diarrhea and prostration in 2–7 h, lasting 1 to 2 d. The enterotoxins produced by *S. aureus* are resistant to heating (up to a D_{110C} = 10 min as measured by bioassays), including low-acid canned food processing. Many healthy people harbor *S. aureus.* It can be found in the nose, throat, hands, fingertips, hair and skin. Any food that is contaminated with the organism and supports growth can potentially develop this bacterial toxin. Proteinaceous foods (for example, chicken, turkey, meat, fish, dairy products, salad vegetables, ham), potatoes, cream-filled products and cream sauces are commonly involved in outbreaks. As the microorganism does not compete well in mixed populations, it is generally not a problem in unheated foods; however, when other naturally occurring bacteria are killed by cooking or inhibited from growth and *S. aureus* is later introduced by humans, it survives and grows. Consequently, it may be found in prepared foods such as salads, custards and cream-filled products. *Staphylococcus aureus* is also resistant to low water activities and survives curing solutions that contain salt or sugar. The cells of the microorganism are not heat resistant (Table 11) and should easily be killed by any mild heat treatment. *Staphylococcus aureus* is best controlled by preventing contamination.

3.3 Sporeforming Bacterial Organisms That Survive Cooking and Pasteurization and Produce Toxin

Bacillus cereus. *Bacillus cereus* is a sporeforming organism that produces 2 types of illness: the diarrheal syndrome, which develops within 20 h following ingestion, or the emetic (vomiting) response, which occurs 1 to 5 h after ingestion. The illnesses are the result of toxins associated with growth of the microorganism in foods (emetic) or the gastrointestinal tract (diarrheal). The diarrheal (enterotoxin) is produced during exponential growth in the gastrointestinal tract, while the emetic toxin is produced by cells growing in the food product (Granum 1997). Dairy products, cereals, meats and fried rice are commonly the foods involved in foodborne illness. Since the microorganism does produce spores,

it is considered resistant to at least pasteurization conditions. In fact, the spores of *B. cereus* have D_{95C} values ranging from 1.5 to 36.2 min (Table 12). One thing that is apparent concerning the heat resistance of *B. cereus* spores is how variable their heat resistance is in the same food product. It is generally thought that *B. cereus* do not survive the low-acid canned food process. For instance, using the highest z(T) (10.1) and D-value (36.2) in Table 12, the D_{121C} is 5.8 s, which confirms that this heat susceptibility is the case (ICMSF 1996).

Clostridium perfringens. *Clostridium perfringens* food poisoning is the result of an enterotoxin produced in the gastrointestinal tract by *C. perfringens*. After ingestion, the microorganisms multiply and sporulate in the small intestine, releasing the enterotoxin and causing symptoms including abdominal cramps and diarrhea (McLane 1997). Meat and poultry are the most common foods associated with *C. perfringens* food poisoning. Foodborne illness caused by this microorganism is generally the result of poor refrigeration and inadequate reheating of cooked foods. Spores are often found on raw meats and may survive cooking of beef or poultry. If foods are inadequately cooled, the spores may then germinate and outgrow. If the food is then inadequately reheated, cells may continue to grow and reach large numbers. Ingestion of large numbers of organisms is necessary both for the microorganism to survive passage through the stomach and to initiate growth and toxin production in the intestines. As can be seen in Table 13, the D-value of *C. perfringens* spores at 98.9 °C may be as high as 31.4 min in beef gravy. Therefore, a low number of spores could potentially survive cooking of a meat in sauce; however, the preferred method of control of this microorganism is not necessarily initial heating but rather adequate cooling and adequate reheating following cooling to inactivate any cells produced during cooling.

Clostridium botulinum. *Clostridium botulinum* is a common soil bacterium that produces heat-resistant spores. This organism produces a potent neurotoxin that may be toxic to both humans and animals. The toxin is considered heat-labile and can be inactivated by heating to 80 °C for 10 min. When ingested, the toxin is absorbed and irreversibly binds to peripheral motor nerves causing paralysis and possible death without antitoxin treatment. Vegetables can carry heat-resistant Type A, B, and F *Clostridium botulinum* spores that are a major concern in low-acid canned foods. Type E spores also can be found in fish and seafood products. The heat resistance as measured by D-values of *C. botulinum* Types A and proteolytic B spores generally ranges from 0.6 to 3 min at 110 °C (Tables 14 and 15). A comparison of Type A and B spores in similar products is shown in Table 16. *Clostridium botulinum* Type E spores are much less resistant than Type A or B and can be inactivated at or below 100 °C (Table 17).

3.4. Cyst-producing Protozoa That Can Remain Infectious in Unpasteurized Foods

Cryptosporidium* and *Cyclospora. The protozoa, *Cryptosporidium parvum* and *Cyclospora cayetanensis*, are not able to replicate in foods, but they do produce cysts that can remain infectious in foods for extended periods of time. Since these organisms appear to have a low infective dose, their presence can contribute to infection, causing diarrhea in the general population. These protozoa may contaminate water systems since the cysts have a high tolerance for disinfectants, such as chlorine. Washing food with contaminated water can infect foods with cysts. Thermal resistance values for *Cryptosporidium* oocysts of $D_{60\,°C}$ of 20 s in distilled water and $D_{71.7\,°C}$ of <1 s have been reported in milk. According to Rose and Slifko (1999), the heat resistance of *Cyclospora* may be similar to that of *Cryptosporidium* (Table 18).

3.5 Enteric Viruses That Can Cause Foodborne Infection from Unpasteurized Foods

Hepatitis A virus, Rotavirus and Norwalk virus. Several outbreaks of foodborne illness have been attributed to the viral contamination of shellfish and of unprocessed fruits. Hepatitis A virus and other enteric viruses may be found in shellfish taken from waters polluted by sewage. Fruits grown in fields where human waste or sludge is used as fertilizer have the potential for contamination by enteric viruses. Foods most vulnerable to viral contamination would be those not receiving a heat pasteurization step.

4. Surrogate Organisms to Validate Processing Parameters

4.1. Thermal, electrothermal and nonthermal food processing

The establishment of traditional thermal processes for foods has been based on 2 main factors (Anonymous 1989): 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, generally defined by a heat transfer rate. These 2 factors are used to calculate the scheduled process, thereby ensuring inactivation of pathogen(s) in that product. The validity of the established process is often confirmed using an inoculated test pack study. An inoculated pack study would be tested under actual plant conditions (this includes processing and control equipment, product and packaging) to reproduce the process in every detail. Since it is unwise to introduce viable pathogens into the production area, surrogate organisms are often utilized in the inoculated pack study, and their inactivation is measured to validate the process. Surrogates play an important role as biological indicators that can mimic the thermal inactivation properties of a pathogen and can help to detect peculiarities or deviations in the processing procedure.

One of the challenges in using new processing technologies for food preservation and pathogen inactivation is to determine if traditional methodologies can be used to establish and validate the new process. For practical purposes, the mechanism of microbial inactivation under electrothermal processes is basically the same as under conventional thermal processes: that is, heat inactivation. Thus, the 2 factors described above, which are well established for thermal processes (Anonymous 1996), should be used as a basis for establishing and validating scheduled electrothermal processes. It is also appropriate to use surrogate organisms to assist in determining and validating the process effectiveness. Regarding other preservation processes not based on heat inactivation (that is, high pressure, pulsed electric field, pulsed light), nonpathogenic surrogates still need to be identified and their significance evaluated. To accomplish this, more research needs to be done in the area of inactivation kinetics of pathogens by new technologies as well as in the identification of nonpathogenic candidates useful as surrogate organisms.

4.2. Importance of Surrogates

Surrogate organisms are invaluable in confirming the efficacy of thermal-based processes. Their use, as opposed to using actual pathogens, derives from the need to prevent the introduction of harmful organisms into the production facility area. The consequences of mishandling a pathogen in the presence of workers, product and equipment (from safety to legal liability issues) could be devastating. Therefore, the use of surrogates by processing companies is of great importance to ensure microbiological safety of the process. For instance, surrogates have been used for many years in the low-acid canning industry to establish and

validate the destruction of *C. botulinum* spores. The use of nonpathogenic spores of the putrefactive anaerobe *C. sporogenes*, or spores of the flat-sour thermophilic organism *Bacillus stearothermophilus* as surrogates for *C. botulinium*, have helped the industry develop processes that ensure products are safe and commercially sterile.

4.3. Criteria for Surrogates

The ideal surrogate would be the pathogen (or target organism) itself that had been transformed into a nonpathogenic form using genetic engineering techniques. Such an approach to surrogate selection is generally not followed due to possible reversion to pathogenicity or possible detection of false positives during routine testing. Generally, surrogates are selected from the population of well-known organisms that have well-defined characteristics and a long history of being nonpathogenic. In selecting surrogates, the following microbial characteristics are desirable:

- Nonpathogenic.
- Thermal or other inactivation characteristics that can be used to predict those of the target organism.
- Durability to food and processing parameters similar to target organism (for example, pH stability, refrigeration stability, oxygen tolerance and so on.).
- Stable thermal and growth characteristics that are similar from batch to batch.
- Easily prepared into high-density population forms.
- Once prepared, population is constant until utilized.
- Easily enumerated using rapid and inexpensive detection systems.
- Easily differentiated from other natural flora.
- Follows inactivation kinetics in a manner similar to pathogens receiving the same inactivation treatment.
- Genetically stable so results can be reproduced independently of laboratory or time of experiment.
- Will not establish itself as a "spoilage" organism on equipment or in the production area.
- Not susceptible to injury or reversible inactivation properties.

Ideal surrogates, with all of the features described above, are scarce. Generally, surrogates will have many of the criteria, as is the case with the traditional surrogates used in low-acid canned foods processing validation.

4.4. Surrogates for Pasteurized Products

Rather than using biological indicators as a basis for process validation, pasteurization processes have traditionally been evaluated and monitored using enzymatic destruction. Milk pasteurization has relied on the inactivation of the naturally occurring phosphatase enzyme as confirmation that product has received the proper heat treatment. This approach is not quantitative and is specific to pasteurization of milk. To obtain quantitative information to support the development and validation of thermal processes, the use of bacterial surrogates is preferred to the use of naturally occurring enzymes. Research, however, is progressing on the identification and use of proteins and enzymes with inactivation kinetics comparable to microorganisms of concern, and there is a great potential in using them as chemical indicators.

Selection of surrogate organisms for pasteurization of products is a relatively new task for food scientists. The literature basically lacks information on recommendations for useful surrogate organisms. Therefore, a good deal of research and development may be required for progress to be made. Efforts are needed to identify and establish surrogates that meet many of the criteria listed above and can be utilized in process development for pasteurized foods.

Pathogens of public health significance in foods are vegetative cells of both gram-positive and gram-negative organisms as well as protozoan cysts. These organisms are inactivated fairly rapidly at pasteurization temperatures and are not good candidates as surrogates. Thermoduric lactic acid bacteria are also present in many foods. These organisms may survive the process and should be evaluated for their possible value as surrogates. They include the lactic streptococci (*Streptococcus thermophilus*), the lactobacilli (*Lactobacillus delbrueckii* spp., *Lactobacillus bulgaricus*, *Lactobacillus lactis*) and *Pedioccocus* spp. Other related lactic acid bacteria to consider as possible surrogates would include strains of *Lactococcus* and *Leuconostoc*. In cases where *Listeria monocytogenes* is the pathogen of interest, strains of *Listeria innocua* have served as nonpathogenic surrogates. In addition, nonpathogenic strains of *E. coli* have served as surrogates for *E. coli* O157:H7. In cases such as these, where surrogates are utilized, it should be proven that the surrogates are suitable for use based upon the above criteria.

4.5. Surrogates for Low-acid Canned Food Products

Methodologies for validating low-acid-canned food (LACF) processes have been in existence for quite some time. The regulatory agencies require that scheduled processes for LACFs be established by a person or organization having expert knowledge of thermal processing requirements for foods packaged in hermetically sealed containers. The biological validation of an electrothermal process such as ohmic heating or inductive heating may be designed and performed following conventional heating biological validation procedures; however, the mode of heat generation, heat distribution and location of coldest point(s) need special considerations when validating an electrothermal process and cannot be extrapolated from conventional thermal processes, as will be described in the subsequent sections of this report. These issues need to be investigated by experts in electrothermal processes.

Where novel nonthermal processes are being investigated, the application of traditional and classical approaches, such as the use of the formula described by C.O. Ball in the 1920's, may not be applicable. The approaches to process development for novel processes may initially be mostly empirical and strongly supported by biological validation until the nature of the kinetics of inactivation is more fully understood and hence predictable. It remains to be seen if the use of organisms like *C. sporogenes* or *B. stearothermophilus* will be acceptable surrogates for *C. botulinium* in many of these novel nonthermal processing applications. As will be described in the following sections of this report, future research needs to address the resistance of pathogens of concern and the identification of appropriate surrogates for the specific nonthermal processes.

4.6. Other Considerations

The use of surrogate organisms to determine and validate processes for electrothermal processes will be challenging, especially for pasteurization processes. The following are some further points to consider while undertaking this endeavor:

- Keep the approach as easy, accurate, and simple as possible.
- Design the process so that the surrogate exhibits a predictable time-temperature profile that correlates to that of the target pathogen.
- Be attentive to the introduction of system modifications or variables, which could alter the heat-transfer properties of the food leading to inaccurate results (for example, thermocouple probes, changing heating rates, nutrients added to the product for surrogate growth, altering viscosity and so on).
- Validate the susceptibility or tolerance of a surrogate, if it is

known.

• When first developing a process, working with a mixture of potential surrogate strains may be useful in narrowing the parameters toward establishing an effective process.

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Thermal Resistance Tables

Table 2—Heat resistance of serovars of *Salmonella* in various foods (ICMSF 1996)

Serovar	Food	Temp (°C)	D-value (min)	Approx. z-value (°C)
Senftenberg	beef boullion	65.5	0.66	
	pea soup	65.5	1.11	5.6
	skim milk	65.5	1.11	
	Milk	65.6	0.56	4.4
	Milk chocolate	70	480	18.9
	Milk chocolate	71	276	
Typhimurium	Milk chocolate	70	1050	17.7
	Milk chocolate	71	396	
	TSB[1] + 10% MS[2]	55.2	4.7	4.5
	TSB + 30% MS	55	11	4.6
	TSB + 42% MS	55.1	18.3	
	ground beef	57	2.13-2.67	
Eastbourne	Milk chocolate	71	270	
none specified	Ground beef	57.2	4.2	

[1]Trypticase soy broth
[2]Milk solids

Table 3—Heat resistance as measured by the D-value at 65.6 °C (150 °F) and approximate z-values of *Salmonella* serovars in milk (ICMSF 1996)

Serovar	$D_{65.6C}$ (s)	Approx. z-value (°C)
Anatum	1.4	5.0
Binza	1.5	5.3
Cubana	1.8	5.6
Meleagridis	1.1	5.4
Newbrunswick	1.3	4.5
Senftenberg	34.0	4.4
Tennessee	1.4	4.9

Table 4—Effect of heating on pathogenic and non-pathogenic strains of *Escherichia coli* (ICMSF 1996)

Product	Strain	Temp (°C)	D-value (min)
Raw milk	ATCC 9637 (NP)[1]	57.2	1.3
Chocolate milk	ATCC 9637 (NP)	57.2	2.6
40% Cream	ATCC 9637 (NP)	57.2	3.5
Ice cream mix	ATCC 9637 (NP)	57.2	5.1
Skim milk	O111:B4	55	5.5
Whole milk	O111:B4	55	6.6
Ground beef [2]	O157:H7	57.2	4.5
Ground beef [3]	O157:H7	57.2	4.1-6.4
Ground beef [2]	O157:H7	62.8	0.4
Ground beef [3]	O157:H7	62.8	0.26-0.47

[1]NP, non-pathogenic
[2]Doyle and others 1984
[3]Line and others 1991

Table 5—Effect of heating on *Yersinia enterocolitica* in milk; where measured, z-value ranged from 4.0-6.78 °C (ICMSF 1996)

Temperature (°C)	D-value
51.7	23.4-29.9
55	1.8-2.2
57.2	4.6-14.7
58	1.4-1.8
60	0.067-0.51
62	0.15-0.19
62.8	0.012-0.96
65	0.028
68.3	0.09

Table 6—Heat resistance of *Vibrio* species (ICMSF 1996)

Species	Product	Temp (°C)	D-value (min)	z-value (°C)
parahaemolyticus				5.6-12.4
	fish homogenate	48	10-16	
	clam homogenate	55	0.02-0.29	
	crab homogenate	55	2.5	
cholerae				
	1% peptone	60	0.63	17
	crab homogenate	60	2.65	21
	oyster homogenate	60	0.35	
vulnificus	SIMILAR TO *V. parahaemolyticus*			

Table 7—*Aeromonas hydrophila* heat resistance (ICMSF 1996)

Product	Temp (°C)	D-value (min)	z-value (°C)
Raw milk	48	3.3-6.2	5.2-7.7
Saline	48	2.2-6.6	5.2-7.7

Table 8—*Campylobacter jejuni* heat resistance (ICMSF 1996)

Product	Temp (°C)	D-value (min)
Skim milk	50	1.3-5.4
	55	0.74-1.0
Ground beef	50	5.9-6.3
	56	0.62-0.96
Lamb	50	5.9-13.3
	55	0.96-1.26
Cooked chicken	55	2.12-2.25

Table 9—Resistance of *Listeria monocytogenes* to heat in milk products (ICMSF 1996)

Products	Temp (°C)	D-value (min)
Raw milk, raw skim milk, raw whole milk, cream	52.2	24.08-52.8
	57.8	3.97-8.17
	63.3	0.22-0.58
	66.1	0.10-0.29

Table 10—Heat resistance of *Listeria monocytogenes* in various products at 60 °C (ICMSF 1996)

Product	D60 °C - value (min)
Ground meat	3.12
Ground meat, cured	16.7
Fermented sausage	9.2-11
Roast beef	3.5-4.5
Beef	3.8
Beef homogenate	6.27-8.32
Naturally contaminated beef	1.6
Weiner batter	2.3
Chicken leg	5.6
Chicken breast	8.7
Chicken homogenate	5.02-5.29
Carrot homogenate	5.02-7.76
RANGE	*1.6-16.7*

Table 11—*Staphylococcus aureus* vegetative cell heat resistance (ICMSF 1996).

Product	Temp (°C)	D-value (min)	Notes
Milk	50	10	z = 9.5 °C
	55	3	
	60	0.9	
	65	0.2	
	70	0.1	
	75	0.02	
Meat macerate	60	6.0	+ 500 ppm nitrite
Pasta, semolina-egg dough	60	3	a_w = 0. 92
		5	a_w = 0. 87
		8	a_w = 0. 85
		12	a_w = 0. 83
		>40	a_w = 0. 80
		32	a_w = 0. 76
		22	a_w = 0. 61
Phosphate buffer	60	2.5	pH = 6.5

Table 12—*Bacillus cereus* spores heat resistance in various media at 95 °C (ICMSF 1996)

Heating medium	D95 °C (min)	z-value (°C)
0.06 M phosphate, pH 7.0	2.6-21.7	10.0-10.1
0.05 M phosphate, pH 7.0[1]	2.1-3.4	
Phosphate, pH 7.0	13	
Distilled water	1.5-36.2	
Water	36	6.7-8.3
Infant formula, pH 6.3	2.7-15.3	8.1-8.7
Milk	1.8-19.1	9.4
Bread	2.9-36.2	
RANGE	*1.5-36.2*	*6.7-10.1*

[1]Contains sorbitol, glycerol or NaCl

Table 13—Heat resistance of Clostridium perfringens spores (ICMSF 1996)

Product/Heating Medium	Temperature (°C)	D-value (min)
Phosphate buffer, pH 7.0	90	0.015-8.7
Phosphate buffer, pH 7.0	98.9	18.6
	104.4	3.15
	110	1.29
Beef gravy, pH 7.0	98.9	31.4
	104.4	6.6
	110	0.5

Table 14—Heat resistance of Clostridium botulinum strain 62A (Type A) spores at 110 °C. (ICMSF 1996)

Product	D-value (min)	z-value (°C)
Asparagus, canned, pH 5.04	1.22	8.8
Asparagus, canned, pH 5.42	0.61	7.9
Corn, canned	1.89	11.6
Macaroni creole, pH 7.0	2.48	8.8
Peas, puree	1.98	8.3
Peas canned, pH 5.24	0.61	7.6
Peas, canned, pH 6.0	1.22	7.5
Spanish rice, pH 7.0	2.37	8.6
Spinach, canned, pH 5.37	0.61	8.4
Spinach, canned, pH 5.39	1.74	10.0
Squash	2.01	8.2
Tomato juice, pH 4.2	1.50-1.59[1]	9.43
Tomato juice, pH 4.2	0.92-0.98	—
Phosphate buffer, M/15, pH 7.0	0.88	7.6
	1.74	10.0
	1.34	9.8
	1.6-1.9	8.1-9.2
	1.01	9.1
Distilled water	1.79	8.5

[1]Strain A16037

Table 15—Heat resistance of *Clostridium botulinum* proteolytic Type B spores at 110 °C (ICMSF 1996)

Product	D-value (min)	z-value (°C)	Strain
Asparagus, canned, pH 5.04	1.09	9.7	213B
Asparagus, canned, pH 5.42	1.06	7.9	213B
Beans, snap	0.86	9.7	213B
Beets	1.17	10.8	213B
Carrots, fresh	0.94	9.4	213B
Corn	1.03	10.0	213B
Corn, puree	2.88	10.6	213B
Corn, canned	2.15	9.6	213B
Milk solids, whole, 20%, pH 6.34	0.93	7.9	213B
Mushrooms, puree	0.49-0.99	—	7 strains
Peas, puree	2.14-12.42	8.3	213B
Peas, canned, pH 5.6	3.07	10.1	213B
Peas, canned, pH 5.94	1.52	7.4	213B
Rock lobster, liquor	2.97-3.33	10.6	A35
Spinach, fresh	1.75	10.3	213B
Spinach, canned, pH 5.39	1.54	8.6	213B
Spinach, canned, pH 5.37	1.19	—	213B
Phosphate buffer, M/15, pH 7.0	1.85	7.7-11.3	213B
	1.4	8.5	213B
	1.6	8.3	213B
	1.19	9.1	213B
	2.0	9.1	213B
	1.5	10.1	Amanna
	2.0	9.0	169B

Table 16—Comparison of heat resistances as measured by D-value in minutes of Clostridium botulinum Type A and B spores in similar products at 110 °C (ICMSF 1996)

Product	Type A	Type B (Proteolytic)
Asparagus, canned	0.61-1.22	1.06-1.09
Corn, canned	1.89	2.15
Peas, puree	1.98	2.14-12.42
Peas canned	0.61-1.22	1.52-3.07
Spinach, canned	0.61-1.74	1.19-1.54
Phosphate buffer, M/15, pH 7.0	0.88-1.9	1.19-2.0

Table 17—Heat resistance of *Clostridium botulinum* non-proteolytic Type E spores in seafood products (ICMSF 1996)

Product	Temp (°C)	D-value (min)	z-value (°C)
Blue crabmeat	74	6.8-13.0	-
Oyster homogenate	70	72	7.5
Oyster homogenate + 1% NaCl	70	100	6.8
Oyster homogenate + 0.13% K sorbate	70	72	7.4
Oyster homogenate + NaCl + K sorbate	70	79	7.3
Menhaden surimi	73.9	8.66	9.78

Table 18—Heating resistance of protozoa in water systems and in foods

Organism	Heating Med	Survival	Time/Temp.	Reference
Anisakis pseudoterranova	fish/medium	maximum survival time	1 min, 60 °C	1
Anisakis anisakis	fish/medium	maximum survival time	10 s, 55 °C	1
Cryptosporidium oocysts	distilled water	3-log reduction	1 min, 60 °C	2
Cryptosporidium *oocysts*	Milk	6-log reduction	5 s, 71.7 °C	2
Taenia cysticerci		inactivated	60 °C	1

[1]ICMSF 1996
[2]Rose and Slifko 1999

Microwave and Radio Frequency Processing

ASHIM K. DATTA AND P. MICHAEL DAVIDSON

SCOPE OF DELIVERABLES: **The use of microwave and radio frequencies to heat food for commercial pasteurization and sterilization in order to enhance microbial safety is discussed here. Although not under FDA regulations, use of microwave technology to enhance microbial food safety in the home is also discussed briefly. Mechanisms of heating food and destroying pathogens, and the validation of industrial processes are also discussed, followed by conjecture on handling deviations during industrial processing. This document summarizes information obtained through published literature and personal contacts with industry, academia, and government. Although radio frequency is covered whenever possible, very little information on radio frequency heating for commercial pasteurization or sterilization of food is available in the published literature and no commercial facility for this purpose could be located. The microbial inactivation mechanisms of radio frequency heating are also quite similar to those of microwave heating. Thus, this document refers mostly to microwave processing with the implicit assumption that the principles are generally applicable to radio frequency. Specific information on radio frequency is included whenever available.**

1. Introduction

1.1. Definition, Description and Applications

1.1.1. Definition

Microwave and radio frequency heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material (Metaxas 1996; Metaxas and Meredith 1988; Roussy and Pearce 1995). The frequencies allocated by the Federal Communications Commission (FCC) for the purposes of heating are listed in Table 1. Typically, microwave food processing uses the 2 frequencies of 2450 and 915 MHz. Of these two, the 2450 MHz frequency is used for home ovens, and both are used in industrial heating. It is worthwhile to note that outside of the United States, frequencies of 433.92, 896, and 2375 MHz are also used.

Radio frequency heating in the United States can be performed at any of the 3 frequencies listed in Table 1. As mentioned earlier, there is not much commercial use of these frequencies for food pasteurization or sterilization, although they are used in baking and other processes in the food industry. An overview of food and chemical processing uses of radio frequency can be seen in Kasevich (1998) and Minett and Witt (1976).

1.1.2. How the microwaves and radio frequencies generate heat

Heating with microwave and radio frequency involves primarily 2 mechanisms—dielectric and ionic. Water in the food is often the primary component responsible for dielectric heating. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the very high frequencies listed in Table 1. Such oscillations of the water molecules produce heat. The second major mechanism of heating with microwaves and radio frequency is through the oscillatory migration of ions in the food that generates heat under the influence of the oscillating electric field.

The rate of heat generation per unit volume, Q, at a particular location in the food during microwave and radio frequency heating can be characterized by (Buffler 1993; Datta and Anantheswaran 2000)

$$Q = 2\pi f \epsilon_0 \epsilon'' E^2$$

where E is the strength of electric field of the wave at that location, f is the frequency (Table 1) of the microwaves or the radio frequency waves, ϵ_0 the permittivity of free space (a physical constant), and ϵ'' is the dielectric loss factor (a material property called dielectric property) representing the material's ability to absorb the wave. Not apparent from the above equation, there is another dielectric property called the dielectric constant that affects the strength of the electric field inside the food. The dielectric properties depend on the composition (or formulation) of the food, moisture and salt being the two primary determinants of interest (Mudgett 1994; Datta and others 1994). The subsequent temperature rise in the food depends on the duration of heating, the location in the food, convective heat transfer at the surface, and the extent of evaporation of water inside the food and at its surface.

1.1.3. Advantages of microwave and radio frequency processing

Microwave and radio frequency heating for pasteurization and sterilization are preferred to the conventional heating for the primary reason that they are rapid and therefore require less time to come up to the desired process temperature. This is particularly true for solid and semisolid foods that depend on the slow thermal diffusion process in conventional heating. They can approach the benefits of high temperature-short time processing whereby bacterial destruction is achieved, but thermal degradation of the desired components is reduced. This is illustrated in Fig. 1 for typical time-temperature histories of microwave and conventional heated processes.

Microwave and radio frequency heating may be relatively more uniform than conventional heating, depending on the particular heating situation (Datta and Hu 1992); however, heating uniformity is hard to predict. Figure 2 illustrates a scenario in which microwave heating is spatially more uniform than conventional heating and helps demonstrate the reasoning behind it. The information shown in Fig. 2 is computed from mathematical models of a conventional and a comparable microwave heating

Table 1 Frequencies assigned by the FCC for industrial, scientific, and medical use

	Frequency
Radio	13.56 MHz ± 6.68 kHz
	27.12 MHz ± 160.00 kHz
	40.68 MHz ± 20.00 kHz
Microwaves	915 MHz ± 13 MHz
	2450 MHz ± 50 MHz
	5800 MHz ± 75 MHz
	24125 MHz ± 125 MHz

process for a solid for input parameters given in Datta and Hu (1992). Figure 2a shows that the range of temperatures reached by the 2 processes are approximately similar (as read from the horizontal axes) at the heating times shown. The vertical axis shows the cumulative volume fractions of the food associated with a temperature, that is, for any temperature, the value on the curve signifies the volume fraction of food that has temperatures at or below this value. Figure 2b shows that the range of F_0 values (signifying time-temperature histories) are quite different for the same conventional and microwave heated food as in Fig. 2a, for which temperatures are approximately similar. The conventional heat process shows a much larger spread of F_0, which primarily signifies its tremendous nonuniformity of temperatures and long processing times leading to significant over-processing of the surface regions of the food.

Other advantages of microwave and radio frequency heating systems are that they can be turned on or off instantly, and the product can be pasteurized after being packaged. Microwave and radio frequency processing systems also can be more energy efficient.

1.1.4. Industrial pasteurization and sterilization systems

Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 y (Jeppson and Harper 1967; Kenyon and others 1970; Mudgett and Schwartzbenrg 1982; Decareau 1985; Schlegel 1992; Harlfinger 1992; Anonymous 1996; Tops 2000). Studies with implications for commercial pasteurization and sterilization have also appeared for many y (Proctor and Goldblith 1951; Hamid and others 1969; Knutson and others 1988; Burfoot and others 1988; 1996; Kudra and others 1991; Cassanovas and others 1994; Villamiel and others 1997; Zhang and others 1999). Early operational systems include batch processing of yogurt in cups (Anonymous 1980) and continuous processing of milk (Sale 1976). A very significant body of knowledge has been developed related to these processes. As of this writing, 2 commercial systems worldwide could be located that currently perform microwave pasteurization and/or sterilization of foods (Akiyama 2000; Tops 2000). As a specific example, 1 company (Tops 2000) produced over 13 million ready meals in 1998 and have installed a newly designed system in 1999. Although continuous microwave heating in a tube flow arrangement has been studied at the research level, no commercial system is known to exist for food processing.

Commercial radio frequency heating systems for the purpose of food pasteurization or sterilization are not known to be in use, although it has been researched over the y (Bengtsson and Green 1970; Houben and others 1991; Wig and others 1999). The primary advantage of improved uniformity of heating was shown for in-package sterilization of foods in large packages using radio frequency at 27.12 MHz, although enhanced edge heating continued to be an issue (Wig and others 1999).

Implementation of a microwave sterilization process can vary significantly among manufacturers. Unlike conventional heating, the design of the equipment can more dramatically influence the critical process parameter—the location and temperature of the coldest point. This uncertainty makes it more difficult to make general conclusions about processes, process deviations, and how to handle deviations. For example, in one implementation (Harlfinger 1992) the process design consisted of heating, equilibration, holding, and cooling stages. The equilibration stage between heating and holding was to equilibrate the temperatures and avoid nonuniformities within the product. Hot air temperature and time are the factors controlling the equilibration process. All 4 stages are done under pressure to reach sterilization temperatures. The parameters recorded for the process were delivered power, temperature, pressure, speed, and cycle time.

Another system (Tops 2000) consists of microwave tunnels with several launchers in relation to the number of products (ready meals). Microwave-transparent and heat-resistant trays are used with shapes adapted for microwave heating. Exact positioning of the package is made within the tunnel and the package receives a precalculated, spatially varying microwave power profile optimized for that package. The process consists of heating,

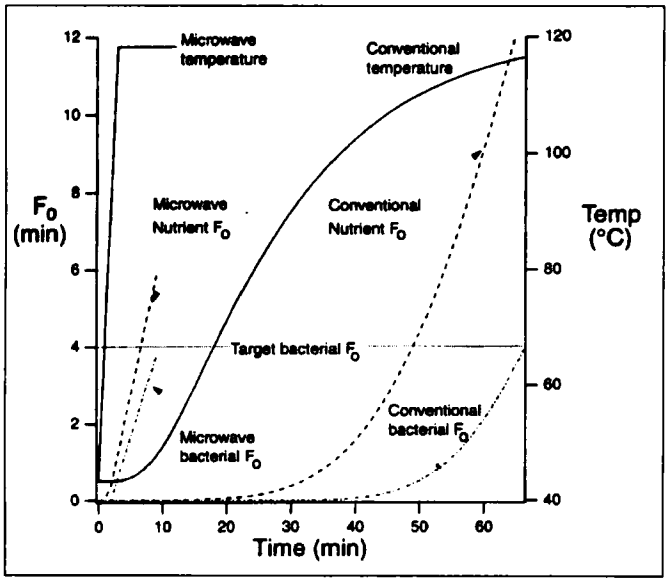

Fig. 1—Quality parameters for microwave and conventional heating compared using computed values for typical heating situations (Datta and Hu 1992). F_0 represents the accumulated lethality (see Section 4)

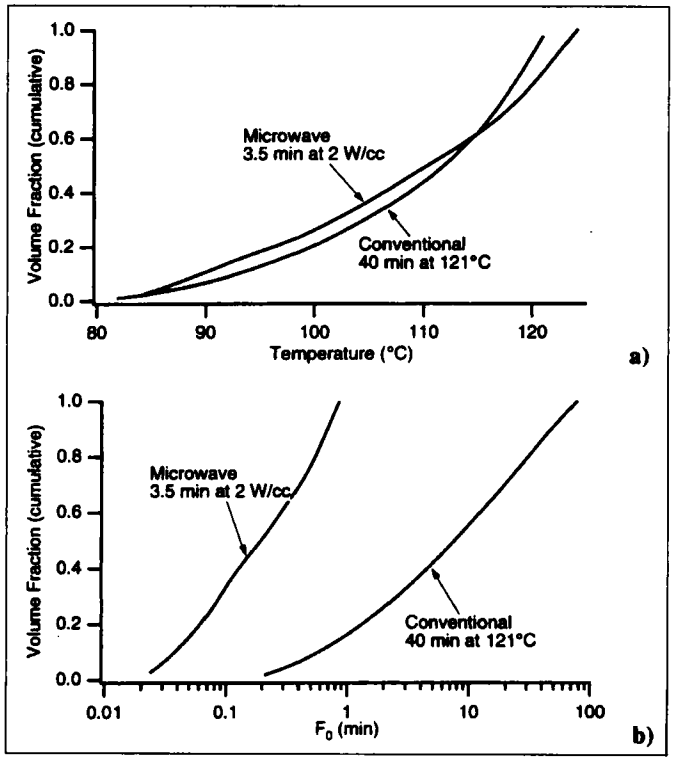

Fig. 2—Illustration of how F_0 values (b) are typically quite different for microwave processing against conventional processing even when the range of temperatures are similar (a) (Datta and Liu 1992)

holding and cooling in pressurized tunnels. The entire operation is highly automated (see monitoring of process deviations later).

For in-package pasteurization or sterilization, packaging materials need to be microwave transparent and have a high melting point. Also, because metal reflects microwaves, packages with some metal component can considerably change the food temperatures (critical process factor). In some situations, metals have been deliberately added to the package to redistribute microwave energy to achieve increased uniformity of heating. The most common packages that have been tried are individual pouches made of microwave transparent rigid films such as polypropylene with an ethylene vinyl alcohol (EVOH) barrier or a polyethylene terephthalate (CPET) film.

1.1.5. Shelf-life extension at home

Almost every U.S. household owns a microwave oven that uses microwaves at a frequency of 2450 MHz. Reheating in a microwave oven, which the FDA does not regulate, is perhaps the most widespread use of the microwaves and has been known to involve serious microbiological safety issues. Researchers have also reported the use of home microwave ovens for pasteurization or for increasing shelf life (Chiu and others 1984; Knutson and others 1988; Thompson and Thompson 1990).

1.1.6. Future processes

Many techniques have been tried to improve the uniformity of heating. These include rotating and oscillating the food package (O'Meara and others 1977), providing an absorbing medium (such as hot water) surrounding the product (Stenström 1974; Ohlsson 1991; Lau and others 1998), equilibrating after heating (Fakhouri and Ramaswamy 1993), and cycling the power. In the past, success of these processes has been limited due to the tremendous dependence of temperature and its distribution on food and oven factors. Use of the 915 MHz and radio frequencies to improve uniformity of heating may have potential for the future (Lau and others 1999b; Wig and others 1999). Future possibilities to improve the uniformity of heating include variable frequency microwave processing and phase control microwave processing. Although these 2 techniques have been applied to microwave heating of nonfood materials, they are yet to be applied to food in any significant way. Combinations of microwave and conventional heating in many different configurations have also been used to improve heating uniformity. The critical process factor in combination heating or any other novel processes would most likely remain the temperature of the food at the cold point, primarily due to the complexity of the energy absorption and heat transfer processes.

In the future, microwaves may be combined with conventional heating or chemical treatments for surface treatment, for example, meat processing (KSU 1999) or food contact surface (Anonymous 1996).

1.2. Summary of Mechanism of Inactivation

The energy absorption from microwaves and radio frequency can raise the temperature of the food high enough to inactivate microorganisms for effective pasteurization or sterilization. A number of studies have proven that the thermal effect is the essential contributor to the destruction of microorganisms (Goldblith and Wang 1967; Rosén 1972; Fujikawa and others 1992) as well as the degradation of vitamin B_1, thiamin (Welt and Tong 1993).

Since the beginning of microwave processing, there has been controversy about the possible nonthermal (also called "athermal") effects of microwave processing (these are effects unrelated to the lethality caused by the heat) (McKinley 1936; Burton 1949; Cross and Fung 1982; Fung and Cunningham 1980). Researchers have repeatedly reported nonthermal effects, although detailed discussions on those mechanisms are difficult to locate in the literature. As many as 4 separate effects have been proposed—selective heating of microorganisms, electroporation, cell membrane rupture, and cell lysis due to electromagnetic energy coupling. These mechanisms are discussed later in more detail; however, the general consensus (Heddleson and Doores 1994) is that the reported nonthermal effects are likely to be due to the lack of precise measurements of the time-temperature history and its spatial variations.

1.3. Summary of Microbial Inactivation Kinetics

Since the studies reporting nonthermal effects have been inconclusive, only thermal effects are presumed to exist. Thus, microbial inactivation kinetics for microwaves are essentially the same as the inactivation kinetics of conventional thermal processing. The microwave nonthermal effects have been reported to add to the destruction of microorganisms. Thus, ignoring the possible nonthermal effect can only provide an extra safety factor. To date, there do not appear to be any microwave-resistant foodborne pathogens.

1.4. Summary of Critical Process Factors

Since the thermal effect is the sole lethal mechanism assumed in this processing technology, time-temperature history at the coldest location will determine the safety of the process. Both the magnitude of time-temperature history and the location of the cold points are functions of the composition (ionic content, moisture, density, and specific heat), shape, and size of the food, the microwave frequency, and the applicator (oven) design. Time is also a factor in the sense that, as the food heats up, its microwave absorption properties can change significantly and the location of cold points can shift (Fig. 3).

1.5. Synergistic Effects

Microwave processes are sometimes combined with conventional heating. Synergistic effects, where the total effect of the combined process on the microorganism is more than the sum of individual effects of microwave and the other process, have not been reported.

1.6. Current Limitations/Status

As mentioned earlier, only 2 companies (Tops 2000; Akiyama 2000) could be located worldwide that are currently using microwave technology for pasteurization/sterilization of foods. Other systems may be operational, but details were not available (Bassani 1999; Anonymous 1999). Some reasons given for the lack of success in commercial operation are complexity, expense, nonuniformity of heating, inability to ensure sterilization of the entire package, lack of suitable packaging materials, and unfavorable economics when compared to prepared frozen foods in the United States. Current research at several universities (Washington State University, Cornell University) and a government agency (U.S. Army Laboratories at Natick, MA) is aimed at further commercial use of microwave sterilization, particularly in the context of providing improved quality rations for soldiers.

2. Pathogens of Public Health Concern Most Resistant to the Technology

2.1. Identification of Pathogens Resistant to Microwaves

Numerous studies address the effect of microwave heating on pathogenic microorganisms in foods. Bacteria reported to be inactivated by microwave heating include *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens,* pathogenic *Escherichia coli, Enterococcus, Listeria monocytogenes, Staphylococcus aureus,*

and *Salmonella* (Heddleson and others 1994; Rosenberg and Bogl 1987; Knutson, and others 1987; Chipley 1980). The nematode *Trichinella spiralis*, the organism that causes trichinosis, may also be inactivated (Zimmerman 1983). Foodborne pathogens have been shown to be inactivated by microwave heating in various poultry, beef, fish, and pork products, milk, and eggs; however, Heddleson and Doores (1994), reported that "in-home pasteurization" of milk was "problematic" and "potentially dangerous" due to nonuniform heating and lack of standardization of home microwave ovens.

It is very difficult to precisely compare the effectiveness of microwave heating to conventional heating based on the literature, because of the different techniques employed or the lack of detail in the methods or materials used, especially in relation to temperature monitoring (Heddleson and Doores 1994). A recurring conclusion in the literature is that nonuniform heating by microwaves may lead to survival of foodborne pathogens, including *Salmonella* and *L. monocytogenes*, in certain locations of foods heated at selected internal locations to endpoint temperatures that would normally be lethal (Schnepf and Barbeau 1989; Harrison and Carpenter 1989). For example, several studies have demonstrated that the measured internal temperature of poultry does not indicate the extent of inactivation of surface-inoculated *Salmonella* on poultry due to lower temperatures at the product surface (Schnepf and Barbeau 1989).

There do not appear to be any obvious "microwave-resistant" foodborne pathogens. Various studies have shown increased resistance of *S. aureus*, *C. perfringens*, or *Enterococcus faecalis* but not necessarily to the point that these could be labeled as resistant. As with conventional heating, bacteria are more resistant to thermal inactivation by microwave heating than yeasts and molds and bacterial spores are more resistant than vegetative cells.

2.2. Effects of Critical Process Factors on Inactivation

As with other thermal processes, the primary factors that determine safety are temperature and time (that is, integrated time-temperature history). A number of critical process factors affect time-temperature history. These are discussed in detail in Section 4.1. Some of these critical process factors are moisture, ionic content, microwave frequency, product parameters (including mass, density, geometry), specific heat, and the temperature achieved. It is important to note that in the context of microwave processing, these critical process factors do not change the rate of inactivation per se. Rather, these factors change the spatial distribution of microwave absorption and, therefore, the spatially distinct heating rate and time-temperature history. The spatial distribution of time-temperature history, in turn, changes the distribution of the extent of inactivation within the food, thus generally changing the total inactivated population within a given food sample. For example, the population of cells heated for 47 s at 700 W in a microwave oven in phosphate buffer were reduced by 99.8%, while those in 1% sodium chloride were reduced only by 62.4% (Heddleson and others 1994). Such a difference is attributed to the effect of salt in decreasing the penetration of microwaves. Less microwave penetration leads to a lower internal temperature and a lesser destruction in the interior regions, resulting in an overall lower destruction.

2.3. Shape of Inactivation Curves

The shapes of the inactivation curves are expected to be similar to those for conventional heating.

3. Mechanisms of Inactivation

3.1. Pathogen Culture Maintenance

As stated above, microwave or radio frequency processing causes microbial inactivation predominantly through thermal effects. In reviewing the literature, no pathogen is identified as uniquely resistant to these processing methods. Therefore, maintenance of cultures (pathogen, surrogate vegetative cells, or spore crops) for evaluating the process or processing unit effectiveness should follow generally accepted culturing procedures for thermal process evaluation. Conditions used for preparing, culturing, or storing vegetative cells or spores should be such that they produce the most resistant cell or spore. Appropriate conditions may be determined by consulting thermal resistance literature (see Section 4.3.). Generally, specific conditions for the growth of the particular test microorganism should be defined. Cells incubated to stationary phase usually demonstrate maximum resistance. As suggested in the Overarching Principles Section 2, sublethal stress conditions also need to be evaluated, as they may increase resistance. As a rule, one should ensure that the test microorganism has a heat resistance equivalent to that generally recognized for the particular genus, species, and strain used.

3.2. Microbial Enumeration Conditions and Methods

Once the vegetative cell or spore is treated with microwaves, it must be enumerated to determine if it is still viable. The objective of the recovery process is to provide optimum conditions for treated cells or spores to grow to obtain a measure of the maximum number of noninjured and injured survivors (Overarching Principles Section 2). For thermal processes, the length of incubation may be important in recovering viable cells or spores, because thermally treated cells or spores generally grow slower than nontreated ones. As with other process studies, experimentation will be necessary to determine the optimum conditions and methods for microbial enumeration.

3.3. Detailed Analysis of Inactivation Mechanisms

Two mechanisms are proposed for inactivation of microorganisms by microwaves. The first states that microwaves inactivate microorganisms entirely by heat through mechanisms comparable to other biophysical processes induced by heat, such as denaturation of enzymes, proteins, nucleic acids, or other vital components, as well as disruption of membranes (Heddleson and Doores 1994). There is no question as to the validity of this mechanism. A second proposed mechanism for inactivation by microwaves involves nonthermal effects. Four predominant theories have been used to explain nonthermal inactivation by microwaves or "cold pasteurization": selective heating, electroporation, cell membrane rupture, and magnetic field coupling (Kozempel and others 1998). The selective heating theory states that solid microorganisms are heated more effectively by microwaves than the surrounding medium and are thus killed more readily. Electroporation is caused when pores form in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. Cell membrane rupture is related in that the voltage drop across the membrane causes it to rupture. In the fourth theory, cell lysis occurs due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell.

These mechanisms have been studied extensively since the 1970s by a number of researchers. Culkin and Fung (1975) reported earlier studies that suggested microwave heating at 2450 MHz caused greater destruction of *Aspergillus*, *Penicillium*, *Rhizopus*, aerobic microorganisms, *Salmonella* and *Proteus* in foods than heating alone. Culkin and Fung (1975) exposed *E. coli* and *Salmonella* Typhimurium in soups to 915 MHz microwaves and then determined survivors in the top, middle, and bottom regions of the product. The temperatures were measured using temperature-sensitive strips. They found that the

greatest survival in the soups was in the top layer, which was also shown to have the lowest temperature. A series of studies by Khalil and Villota (1988; 1989a;b) suggested nonthermal effects of microwave heating. They first determined that *Bacillus stearothermophilus* spores in various media (water, NaCl, sucrose, phthalate, or phosphate buffers) had lower $D_{100\,°C}$ values when 2450 MHz microwaves were used compared to using a heated water bath. The experiment appears to have involved 6 tubes at a single temperature with no replication. In addition, the come-up times, although a small part of the overall heating times (microwaves: 58–83 s out of 90–190 min, conventional heat: 100–135 s out of 113– 240 min), were not considered. Heddleson and Doores (1994) disputed the above conclusions due to inaccuracies in temperature measurement. Khalil and Villota (1988) further studied the effect of microwaves (2450 MHz assumed) on injury of *S. aureus* FRI-100. They heated cells at a sublethal temperature of 50 °C and maintained microwave temperature using recirculated cooled kerosene. Microwave heating caused a greater amount of cellular injury as determined by plating on trypticase soy agar plus 7% sodium chloride, increased loss of ultraviolet-absorbing cellular material, and extended time for enterotoxin production. Their findings also showed that microwave-injured cells recovered better when microwave heating was carried out anaerobically. This effect was not seen with conventional heating. They speculated that the microwaves catalyzed oxidative reactions, possibly in membrane lipids, decreasing recovery of exposed cells. In another study, Khalil and Villota (1989b) demonstrated that while both conventional and microwave heating destroyed the 16S subunit of RNA of sublethally-heated *S. aureus* FRI-100, only microwave heating affected the integral structure of the 23S subunit. Moreover, when cells were allowed to recover following injury, it took longer for the microwave treated cells to restore their 23S RNA. Heddleson and Doores (1994) again concluded that these studies suffered from the lack of proper method of temperature measurement due to the unavailability of fiber optic thermometry.

Kozempel and others (1998) designed a system in which various fluids were exposed to microwave energy (5.0 to 5.4 kW) and then cooled so as to maintain temperatures of the fluids at 40 °C. The fluids were inoculated with a bacterium reported to be *Pediococcus* strain NRRL B-2354 prior to exposure. The greatest kill took place in apple juice (up to 4.6-logs in 1 pass), with moderate lethality (up to 0.7-logs in 1 pass), occurring in water and 10% glucose. The amount of kill with multiple passes through the system was not constant. In some products the kill rate was reduced following the first pass. Little or no lethality was demonstrated with skim milk, pineapple juice, tomato juice, apple cider, or beer. In addition to the influence of the product itself, the medium used to grow the *Pediococcus* strain also appeared to affect cells counts; however, none of the product characteristics such as insoluble solids, pH, and conductivity could fully explain the variation. The authors concluded that they had demonstrated "significant microorganism kills in some fluids using microwave energy at sublethal temperatures." Kozempel and others (2000) subsequently designed a new system that was capable of isolating thermal and nonthermal effects of microwave energy. The system was a double tube that allowed input of microwave energy but removed thermal energy with cooling water. With this system, the researchers found no inactivation of *Enterobacter aerogenes, E. coli, Listeria innocua, Pediococcus*, or a yeast in various fluids including water, egg white, whole egg, tomato juice or beer at sublethal temperatures. They concluded that, in the absence of other stresses such as pH or heat, microwave energy did not inactivate microorganisms; however, they did suggest that microwave energy may complement or magnify thermal effects. In tests with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in apple juice, Ramaswamy and others (2000) also found that the nonthermal effect of microwave energy at sublethal temperatures is insignificant. However, they determined that, at equivalent heat treatments, microwaves enhanced inactivation. They demonstrated in a continuous flow system that *E. coli* K - 12 had significantly lower D-values (12.98 s at 55 °C, 6.31 s at 60 °C, 0.78 s at 65 °C) using microwave energy than equivalent heat treatments with hot water (44.7 s at 55 °C, 26.8 s at 60 °C, 2.00 s at 65 °C) or steam (72.71 s at 55 °C, 15.61 s at 60 °C, 2.98 s at 65 °C). They concluded that, while there was no nonthermal effect of microwaves, there was a significant enhancement of thermal treatments.

Apart from the described studies, most research has concluded that there is little or no nonthermal effect of microwaves on microorganisms (Rosenberg and Bögl 1987; Knutson, and others 1987) and that inactivation of microorganisms is due only to heat. Goldblith and Wang (1967) heated suspensions of *E. coli* and *Bacillus subtilis* under conventional heating and with microwaves at 2450 MHz. The degree of inactivation of both microorganisms was identical with conventional and microwave heating. Vela and Wu (1979) heated various bacteria, fungi, and bacteriophages in 2450 MHz microwaves in water and as lyophilized cultures. There was no inactivation of dry cultures even after extended exposure. Similarly, Jeng and others (1987) found no difference in inactivation of *B. subtilis* spores under conventional or microwave (2450 MHz) heating in automated computer-controlled temperature monitoring systems. Kazbekov and Vyacheslavov (1978) found that thymidine and thymine uptake, leakage of potassium and hydrogen ions, and uptake of DNA by cells of *E. coli* or *B. subtilis* under low power microwaves were typical of that shown for heating. Fujikawa and others (1992) found no major differences in inactivation kinetics of *E. coli* in phosphate buffer between microwaves and conventional heating. Welt and others (1993a) demonstrated no difference between conventional and microwave inactivation of *Clostridium sporogenes* PA3679 at 90, 100, and 110 °C. A suspension of spores that was exposed to microwaves, but continuously cooled in silicone tubing demonstrated no detectable inactivation.

While there is some controversy as to the additional inactivation of microorganisms over the thermal effect of microwaves, this additional inactivation is small and inconsistent. In many studies comparing microwave heating to conventional heating, microwave heating appears less effective due to nonuniform heating effects from unpredictability of cold spots and changing product parameters, such as specific heat. Therefore, when developing methods for describing the inactivation kinetics of microwave heating, it is recommended that only thermal effects be included in the model.

Under the assumption of only thermal effects, the kinetic parameters presented in Table 1A of the Overarching Principles are recommended for use in design of processes involving the microwave treatment of foods. The kinetic parameters used to design thermal processes have been presented and defined in an introductory chapter of the main document. The pathogens of concern will be the ones defined for thermal processing and described in the Overarching Principles Section 3 of this report.

4. Validation/Critical Process Factors

4.1. Identification and Description of Critical Process Factors

Time-temperature history at the coldest point determines the microbiological safety of the process, as in other thermal process-

ing. Once temperature is known at the coldest point as a function of time, accumulated lethality can be calculated following the well-known equation

$$F_0 = \int_0^{t_f} 10^{(T-250)/z}\, dt$$

where T is the cold point temperature at any time t, z is the z-value in °F and t_f is the total duration of heating. There are, however, major differences between conventional and microwave heating in terms of the location of the cold point and how time-temperature history of the cold point is affected by a number of critical process factors. *Note that the effect of the factors are discussed in a simplistic way in order to illustrate the concepts—the actual influence of the factors can be quite complex and are only known from detailed experiment or mathematical modeling.* Such effects are discussed in detail in books such as Datta and Anantheswaran (2000), Buffler (1993) and Decareau (1985) or review papers such as Saltiel and Datta (1998).

Time-temperature history at the coldest point for a conventional thermal process is generally quite predictable for a food that is all solid or all fluid. For example, for a conduction-heated (solid) food, it is usually the geometric center. In microwave heating, even for a solid food, the coldest point is less straightforward to predict and can change during the heating process (Fig. 3), depending on a number of food and oven factors (Fleischman 1996; Zhang and others 1999). Accordingly, relatively sophisticated modeling, based on measured properties of the foods, needs to be used and subsequently validated to ascertain the location of the point of lowest integrated time-temperature history. Well-developed but simple procedures, such as the Ball formula (Ball and Olson 1957) would be much harder to achieve for microwave heating. Changes in properties during heating have a more pronounced effect in microwave heating as compared to conventional heating. As the food heats, its microwave absorption capability typically increases, which increases the rate of temperature rise and therefore further increases the rate of microwave absorption. Such coupling could lead to runaway heating (Zhang and others 1999; Zhang and Datta 1999). Figure 3 also illustrates the coupling effect. Initially, at lower temperatures, microwave absorption is lower, so the waves are able to penetrate a lot further into the material. As the material heats up, it absorbs microwaves more readily and the waves are not able to penetrate as far. Especially in foods with high ionic concentrations, the surface at higher temperatures can act as a shield.

Since heat is constantly generated everywhere in the food, but at different rates, the difference between the temperature at the coldest and the warmest points in the food keeps increasing with time. This is unlike conventional heating where the coldest point approaches the warmest temperature of the system (typically the heating medium temperature) with time.

In conventional heating, the surface is at the highest temperature, corresponding to the temperature of the heating medium. In microwave heating, the food heats up while the surrounding air stays cold (Datta 2000). The cold air keeps the surface temperature lower than locations near the surface of food. Surface evaporation, especially when heating an unpackaged food, can further decrease the surface temperature. In some heating applications, such as with frozen foods that are spherical, the surface could be the coldest location.

In conventional heating, the maximum temperature is limited by the heating medium temperature, such as steam in a retort. Since microwave absorption continuously generates heat, temperature keeps increasing in the microwave heating process. To keep the temperature within reasonable limits, microwaves need to be turned on and off (cycled) once the target temperature has been reached.

One of the advantages of microwave heating is that the come-up time is short. It is this shorter come-up time that helps retain the organoleptic qualities and that is the basis for preferring microwave processing to conventional thermoprocessing. In calculating the process time, the come-up time in microwave heating cannot be given nearly as much importance as in conventional heating (see Fig. 1).

4.1.1. Factors related to product and package

Food shape, volume, surface area, and composition are critical factors in microwave heating (Zhang and others 1999). These fac-

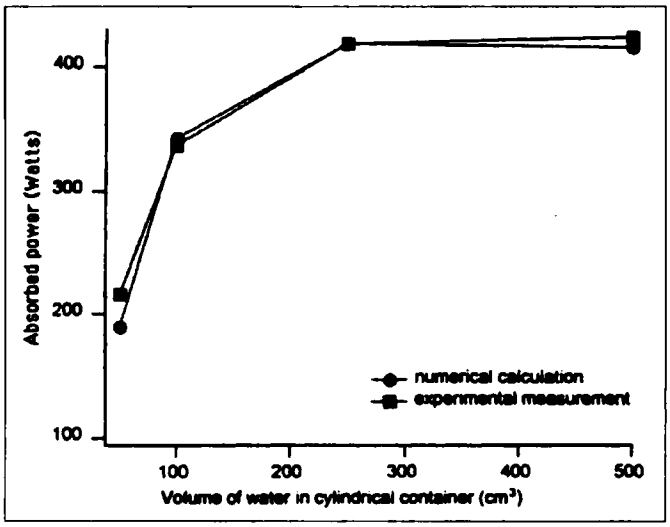

Fig. 4—Magnitude of total absorbed power as a function of volume of food, obtained from experiments and electromagnetic simulations (Zhang and others 1999)

Fig. 3—Microwave power absorption (Q in equation 1) patterns in a sterilization process can change dramatically during heating, as shown by the migration of hottest locations (in red) from interior (a) to surface (b). Shown are computed results for a ham cylinder (0.7% salt) heated in a microwave oven similar to domestic microwave ovens (Zhang and others 1999)

tors can affect the amount and spatial pattern of absorbed energy, leading to effects such as corner and edge overheating, focusing, and resonance. For example, a curved shape can focus microwaves and produce a higher internal rate of heating than near the surface (Ohlsson and Risman 1978). Such heating patterns can also change with time, as illustrated in Fig. 3. The effect of food volume on total amount of energy absorbed by the food for a given setting of power level is typically as shown in Fig. 4. Since the total energy absorbed lags the increase in volume, average temperature rise drops (however, food as a whole heats slower).

Composition, in particular moisture and salt percentages, has a much greater influence on microwave processing than in conventional processing, due to its influence on dielectric properties. High salt and moisture content increases the efficiency of microwave absorption, thereby decreasing the depth of penetration. Thus, interior locations generally get heated less in foods with high salt or moisture content, reducing microbial destruction. Composition can also change the thermal properties such as specific heat, density, and thermal conductivity and, thereby, change the magnitude and uniformity of the temperature rise. For example, the temperature of a low specific heat oil increases at a much faster rate than that of water when compared at the same level of absorbed power.

The different components of mixed food products, such as multi-compartment frozen dinners, will heat differently (Ryynanen and Ohlsson 1996; Zhang and others 1999). Packaging material is also a critical process factor. In contrast to commercial canning, where metal containers offer minimum thermal resistance and are not a critical process factor, metallic components present in a package, such as aluminum foil and susceptors, can dramatically influence the heating rates of the packaged food.

4.1.2. Factors related to process and equipment

Several process and equipment factors are critical in microwave heating (Zhang and others 1999). Design (size, geometry, and so on) of the microwave oven can significantly affect the magnitude and/or spatial variation of the power absorption in the product. In addition, presence or absence of devices added to improve uniformity, such as mode stirrers and turntables, are major factors affecting temperature distribution.

The placement of the food inside the oven can also have a significant influence on the magnitude and uniformity of power absorption (Zhang and others 1999). Other factors related to the equipment are the temperature of the medium surrounding the product and the level of food surface evaporation (especially significant for unpackaged food), both affecting food surface temperature. Cooling effects due to surface evaporation have been shown to lead to survival of *Trichina* larvae in pork (personal communication with J. Gerling cited in Datta 1991).

Heating of containerized liquids with microwaves without agitation causes flow and thermal stratification inside the container. Warmer liquid moves to the top, much like in conventional heating. Due to variations in product characteristics (such as viscosity), in package components (such as metal in aluminum foil), and in equipment factors, the patterns of temperature distribution in heated liquids (static or flowing) can be quite complex, and the slowest heating location needs to be determined for distinctly different situations (Prosetya and Datta 1991; Anantheswaran and Liu 1994a 1994b; Datta and Hu 1992; Tajchakavit and Ramaswamy 1995).

In addition to the aspects discussed above, power level and cycling of the microwave input are critical process factors in microwave heating. Also, power output by the magnetron (the component in an oven generating the microwaves) changes as the magnetron heats up over time. Thus, equipment specific "wait time" may be necessary before the power output becomes stable. Due to differences in penetrating ability, the frequency of the microwaves can dramatically affect the heating rates and their spatial distribution. In a simplified view, a lower frequency of 915 MHz has a higher depth of penetration than the 2450 MHz used for home microwaves. At this lower frequency, uniformity of heating can improve with reduced edge heating (Lau and others 1998).

Table 2 Critical Process Factors in Microwave Heating

Food	Shape, size, composition (moisture, salt, and so on), multiple components (as in a frozen dinner), liquid against solid
Package	Presence of metallic elements such as aluminum foil, susceptor
Process	Power level, cycling, presence of hot water or air around the food, equilibration time
Equipment	Dimensions, shape and other electromagnetic characteristics of the oven, frequency, agitation of the food, presence of mode stirrers and turntables

Equilibration of the product following heating can help to level the temperature distribution and improve uniformity. Its important effects have been demonstrated (Fakhouri and Ramaswamy 1993; Ramaswamy and Pilletwill 1992).

A summary of the various product, package, process, and equipment factors discussed above is provided in Table 2. Due to the number of critical factors implicated, none of them alone can be treated as a critical process factor by itself, unless all others are held constant.

4.1.3. Identification of the effect of process factors on cold point using mathematical simulation

Due to the complexity of the system where the heating pattern depends on such a large number of factors, simulation-based design can save significant time and resources in developing microbiologically safe processes. Such simulation-based design can drastically reduce the number of experiments needed to predict the location of cold points and the time-temperature history at these locations for the actual food and equipment combinations. State of the art commercial software simulating the electromagnetic and heat transfer properties has been used for microwave food process design (Dibben 2000; Zhang and Datta 2000). Such software can provide a comprehensive insight into the heating process by showing interior power absorption (that is, heating rates) in a 3D object (Fig. 3), difficult using experimentation. Simulation-based design can allow the process and equipment designers to judiciously choose proper combinations of food and process parameters in an efficient manner, reducing some of the time and expenses in prototype building. Location of coldest point (the critical process factor) and its time-temperature history can be predicted this way. As the user friendliness, accuracy, and linkages with other software improve, more food processors are expected to use these programs routinely.

4.2. Description of Methods to Measure/Monitor Critical Process Factors

Monitoring the temperature of microwave processed food poses a challenge. Thermocouples and other metallic probes used to record temperatures for conventional processing in static systems are generally unsatisfactory for precision temperature measurements in microwave ovens for several reasons. Firstly, metallic probes reflect and absorb the energy of incident microwaves, and require special grounding and installation to withstand microwave operations. Secondly, electromagnetic field disturbances caused by the presence of metallic probes create localized changes in heating patterns that can produce variability in overall heating patterns.

Valuable alternatives to metallic probes, however, are fiber-

optic temperature probes. These are on the market and have been used to monitor temperatures during microwave heating. They are inert to the electric and magnetic fields of the microwaves or radio frequencies. Additional advantages are their accuracy (from 0.2 to 0.05 °C) and fast response (milliseconds). Some disadvantages of fiber-optic probes are their current price (although it is dropping) and somewhat fragile nature.

4.3. Description of Microbial or Chemical Surrogates/ Indicators

For determining the kinetics and efficiency of microwave inactivation of microorganisms, surrogate/indicator microorganisms could be selected from those traditionally used in thermal processing studies. No microorganisms with unique resistance to microwave processing have been reported in the literature, suggesting that classical surrogates (vegetative cells or spores) would be appropriate for process determination and validation.

Since microwave or radio frequency processing is primarily a heat process, microbiological validation tests should be designed using procedures that parallel those used for thermal processing. Important considerations for an inoculated pack study of a new thermal process include selection of a surrogate microorganism, preparation and handling of the test microorganism, size and volume of inoculum, method of inoculation, processing levels and conditions, number of containers, product data collection, statistical techniques and methods for determining survivors (for example, incubation, microbiological recovery). A more detailed discussion of these procedural considerations is available in the *Laboratory Manual for Food Canners and Processors* (National Canner's Association 1968). The principles and methods are similar, whether the objective is pasteurization, pathogen reduction, or commercial sterilization.

One problem that needs to be carefully considered is the method of inoculation. Since microwave heating can be nonuniform and the cold spot is not easy to identify, the inoculum should be distributed throughout the food product. This could be a particular problem with solid products, unless made homogeneous by grinding or placed in locations where significant spatial variation in heating rates is expected.

The use of history indicators or time-temperature integrators (see reviews by Hendrickx and others 1995; Van Loey and others 1996), either biological (microbiological or enzymatic) or chemical, could be a way to monitor the process impact and could be helpful in identifying critical process parameters. As in thermal processing, proper calibration of the kinetic parameters of the surrogates/indicators is required. The same principles as for thermal processing apply and extra care should be given to critical factors associated with the microwave/radio frequency heating process in that the presence of the indicator should not influence the heating process. An example is the validation of sterilization patterns by correlating thermally induced chemical changes in the food (a history indicator) to bacterial destruction. Intrinsic chemical markers (Kim and Taub 1993; Prakash and others 1997, Zhang and others 1999) whose extent of formation is a function of time-temperature history have been used recently in several processing situations, including microwave sterilization (Lau and others 1999a; Zhang and others 1999; Wig and others 1999). This approach can provide information on spatial distribution of the integrated time-temperature history within a packaged system and on any variation among packages in a continuous process operation.

5. Process Deviations

Process deviations in microwave processing present some special issues and challenges. Temperatures are generally more difficult to monitor and measurement of power output from microwave generators may not accurately reflect product temperature, unless the sensitivity of the heating process to changes in food composition, size, shape, placement in the oven and other factors discussed earlier are taken into consideration. Due to the complex nature of the process, adjustments such as extending the time or increasing the power level will not be simple. It is generally believed that complete reprocessing would be the most reliable way to handle underprocessed material.

5.1. Basic Detection Methods for Process Deviations

Process parameters under direct control of the operator are the power level (including cycling), spatial distribution of power (number and positioning when multiple microwave generators are used) and time of exposure. As mentioned above, once a deviation has taken place, adjustments could be complex.

In one implementation (Tops 2000), the exact placement of a product in the tunnel is known and power levels of multiple microwave generators are programmed precisely to provide the custom-tailored heating profile for that tray and product. For example, the center of the tray is provided with higher microwave power. The detection system consists of a variety of monitors. For example, broken generators and insufficient power level delivered by a generator is automatically recorded. Infrared surface measurement of each tray can be made while they are being transported to the hold system. Swelling of the top surface of individual packages due to internal steam generation during heating is monitored using a distance tracer—adequate heating produces enough steam for the package to swell sufficiently. Visual control is also made by placing maxi-thermometers (that measure maximum temperatures) at precise locations in the package. An endoscope is also used to observe the heating process inside the microwave tunnel and therefore to monitor it manually.

In another implementation (Harlfinger 1992), power settings for individual magnetrons are stored over time. If the power delivered varies from the set values, an alarm warns the operator. An additional warning signal comes from any blockage of the product feeding system that may lead to unintended cooling of the preheated products. This publication also reported the use of a DataTrace metallic temperature data-logger inside the package by 1 company to monitor the time-temperature history. Use of such metallic data-loggers requires careful considerations and interpretations.

5.2 Methods to Assess and Correct Deviations

See general discussions under Section 5.1. above. Details about process deviations are hard to obtain. Only 1 company was willing to share information about current production of microwave pasteurized and sterilized food (Tops 2000). In this company's implementation, a product is rejected based on an automated control system. Rejection is done at the end of the cooling system based on infrared surface temperature measurement, detection of broken microwave generators, and other means described in Section 5.1. Further loading of food into that tunnel is automatically stopped following a rejection. The temperature control system for each microwave tunnel can also be adjusted, if necessary, following a deviation. The control system is also programmed for each individual product.

In general, extensive experimentation would be needed to validate the effectiveness and reliability of the methods to assess and correct deviations.

6. Research Needs

Research needs have been identified in the following areas:

- Effects of food formulation on heating patterns.

- Effects of equipment design factors, including frequency (for example, 915 MHz is sometimes proposed instead of the commonly used 2450 MHz for better uniformity of heating).
- Development of variable frequency ovens (although currently more expensive for food applications) for improved uniformity of heating.
- Understanding factors affecting heating patterns, including qualitative changes occurring with frequency changes.
- Monitoring and real-time adjusting for process deviations in microwave and radio frequency processing.

Glossary

A complete list of definitions regarding all the technologies is located at the end of this document.

Conventional heating. Heating of a substance by transfer of thermal energy from a heating medium at higher temperature to a low temperature product.

Focussing. Concentration of electromagnetic waves inside a food due to its curved surface, much like a lens focussing light waves. It leads to enhanced heating at the interior.

Internal energy generation. Heat generation within a material and throughout its volume due to the presence of an energy source that is dissipated throughout the volume (see volumetric heating).

Liquid crystals. Materials with properties that are useful for thermal sensing. Liquid crystals typically change color with temperature.

Magnetron. The physical component of a microwave system that generates the microwaves.

Microwaves. Electromagnetic waves at frequencies 915, 2450, 5800, and 24225 MHz.

Nonthermal effects. Effects due to the exposure to a process that are not of thermal origin, that is, cannot be explained by measured temperature changes.

Penetration depth. The distance the electromagnetic waves (of a certain frequency) travel in a material before it loses 63% of its energy.

Power cycling. The process of the microwave source turning on and off.

Radio frequency. Electromagnetic waves at frequencies of 13.56, 27.12 and 40.68 MHz.

Runaway heating. A cycle of increasing temperature in food causing an increasing rate of energy (microwave/ohmic) absorption that further increases the rate of temperature rise. It is more prominent in foods undergoing phase change from ice to water and in foods containing significant salt and other ions.

Specific heat. The ability of a material to store heat. Technically as the amount of energy required to raise the temperature of unit mass of an object by a unit increment in temperature.

Variable frequency. Sweeping over a range of frequencies during the microwave heating process to improve uniformity.

Volumetric heating. Heating by internal energy generation throughout the volume of a material (see also internal energy generation).

Waveguide. The physical component of a microwave system that guides the microwaves from magnetron to the cavity where the food is heated. When applied in the form of pulses, it reverses the charge for each pulse and pulse intensity gradually decreases.

We particularly appreciate the following individuals who have either reviewed the document and provided comments or provided relevant information: M. C. Hendrickx, Katholieke Universiteit, Leuven, Belgium; Juming Tang, Washington State University; Irwin Taub, U.S. Army Natick Soldier Center; Tom Yang, U.S. Army Natick Soldier Center; K.P. Sandeep, North Carolina State University; R. C. Swamy Anantheswaran, The Pennsylvania State University; H. S. Ramaswamy, McGill University; Rudy Tops, Tops Foods, Lammerdries 26, Olen, Belgium

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Ohmic and Inductive Heating

SUDHIR K. SASTRY AND JEFFREY T. BARACH

Scope of Deliverables: This section covers information, where available, on kinetics and relevant safety considerations for processing commercially sterile or extended shelf-life products produced by ohmic and inductive heating. It does not include combinations of other alternate process technologies with ohmic and inductive heating, since the number of potential combinations is infinite but no information is available.

1. Introduction

1.1. Definition, Description and Applications

Ohmic heating (sometimes also referred to as Joule heating, electrical resistance heating, direct electrical resistance heating, electroheating, and electroconductive heating) is defined as a process wherein (primarily alternating) electric currents are passed through foods or other materials with the primary purpose of heating them. The heating occurs in the form of internal energy generation within the material. Ohmic heating is distinguished from other electrical heating methods either by the presence of electrodes contacting the food (as opposed to microwave and inductive heating, where electrodes are absent), frequency (unrestricted, except for the specially assigned radio or microwave frequency range), and waveform (also unrestricted, although typically sinusoidal).

In inductive heating, electric coils placed near the food product generate oscillating electromagnetic fields that send electric currents through the food, again primarily to heat it. Such fields may be generated in various ways, including the use of the flowing food material as the secondary coil of a transformer. Inductive heating may be distinguished from microwave heating by the frequency (specifically assigned in the case of microwaves), and the nature of the source (the need for coils and magnets for generation of the field, in the case of inductive heating, and a magnetron for microwave heating).

Information on inductive heating is extremely limited. A project was conducted in the mid-1990s at the Technical University of Munich (Rosenbauer 1997), under sponsorship from the Electric Power Research Institute. No data about microbial death kinetics under inductive heating were published. Thus, the succeeding discussion focuses on ohmic heating.

A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation, and extraction. The present discussion, however, concerns primarily its application as a heat treatment for microbial control. In this sense, the main advantages claimed for ohmic heating are rapid and relatively uniform heating. Ohmic heating is currently being used for processing of whole fruits in Japan and the United Kingdom. One commercial facility in the United States uses ohmic heating for the processing of liquid egg.

The principal advantage claimed for ohmic heating is its ability to heat materials rapidly and uniformly, including products containing particulates. This is expected to reduce the total thermal abuse to the product in comparison to conventional heating, where time must be allowed for heat penetration to occur to the center of a material and particulates heat slower than the fluid phase of a food. In ohmic heating, particles can be made to heat faster than fluids by appropriately formulating the ionic contents of the fluid and particulate phase to ensure the appropriate levels of electrical conductivity.

1.2. Summary of Mechanisms of Microbial Inactivation

The principal mechanisms of microbial inactivation in ohmic heating are thermal in nature. Occasionally, one may wish to reduce the process requirement or to use ohmic heating for a mild process, such as pasteurization. It may then be advantageous to identify additional nonthermal mechanisms. Early literature is inconclusive, since temperature had not been completely eliminated as a variable. Recent literature that has eliminated thermal differences, however, indicates that a mild electroporation mechanism may occur during ohmic heating. The principal reason for the additional effect of ohmic treatment may be its low frequency (50 – 60 Hz), which allows cell walls to build up charges and form pores. This is in contrast to high-frequency methods such as radio or microwave frequency heating, where the electric field is essentially reversed before sufficient charge buildup occurs at the cell walls.

1.3. Summary of Microbial Inactivation Kinetics

A summary of the available kinetic data is presented in Tables 1 through 3 in Section 3.4. While some evidence exists for nonthermal effects of ohmic heating, a larger body of research is needed to understand more fully the mechanisms for microbial inactivation. For most ohmic processes, which rely on heat, it may be unnecessary for processors to claim this effect in their process filings; however, if sufficient data is available, it may be possible for future processes to be reduced.

1.4. Summary of Critical Process Factors

Temperature is the principal critical process factor in ohmic heating. As in conventional thermal processes, the key issue is determining the zone(s) of minimum thermal treatment. Fundamentally, there is only 1 critical factor: temperature-time history of the coldest point. Temperature of an ohmic process, however, is significantly affected by other factors, which will be discussed in greater detail in section 4.1.

1.5. Other Considerations

While it is possible to envision creative combinations between ohmic heating and other alternative process technologies, no data are available at this time.

2. Pathogens of Public Health Concern Most Resistant to the Technology

2.1. Identification of Pathogens Resistant to Ohmic Heating

Since the purpose of this technology is to use the rapid, volumetric heating provided by the electrothermal methods, the most resistant pathogens would likely be the same as those for thermal processes. Results to date show that the electric field can

only enhance microbial kill, so any additional beneficial effect will add an extra factor of safety.

2.2. Effects of the Critical Process Factors on Inactivation

Since the main critical process factor is the thermal history and location of the cold spot, the effects on microbial inactivation are the same as for thermal processes. Locating cold zones during ohmic heating, however, cannot be extrapolated from current knowledge of conventional heating and requires special consideration.

2.3. Shape of Inactivation Curve

Microbial inactivation curves of ohmic heating processes are similar to thermal curves, except for differences in slope (the rate may be enhanced by the electric field) on some occasions. Thus, the conventional D- and z-value kinetics, (or, alternatively, rate constants and activation energies) are relevant. These are summarized in Section 3.4.

3. Mechanisms of Inactivation

3.1. Pathogen Culture Maintenance

A review of the literature describing the application of ohmic processing to foods revealed that there are no particular pathogen strains with a unique resistance to the technology. Again, since the predominant method of microbial inactivation is thermal, thermal death kinetics of pathogens and target spoilage organisms would be followed. Therefore, standard published and generally accepted preparing and culturing procedures (National Canners Association 1968) should be used for pathogenic, surrogate cultures or spores in order to evaluate an ohmic processing unit's ability to inactivate them. Depending on the case, culture maintenance will be done either by in-house trained staff or by outside consulting firms that will provide materials for testing and system validation. As a rule, a surrogate should have the highest heat resistance recognized for that type of microorganism. A biological test culture with substandard heat resistance is of little use in evaluating a thermal process. For a detail discussion of the characteristics of surrogate microorganisms, see Overarching Principles Section 4.

Once the key pathogen(s) are selected and the surrogates for them identified, literature and experts in the field can help identify suitable culture maintenance and preparation conditions for a process evaluation.

3.2. Microbial Enumeration Conditions and Methods

See Overarching Principles Section 2.

3.3. Detailed Analysis of Inactivation Mechanism(s) for Pathogens Identified, as a Function of Process Variables, Microbial Repair and Regeneration, and Effects of Post-processing Storage

Because the principal mechanisms of microbial inactivation are thermal in nature, processors intending to file ohmic treatment processes with FDA should be able to file processes based on thermal inactivation. At times, one may wish to reduce the process requirement or to use ohmic heating as a mild processing method, such as pasteurization. It may then be advantageous to determine if additional nonthermal mechanisms exist.

Early literature on nonthermal effects has been inconclusive (Palaniappan and others 1990). Most studies either did not specify sample temperatures or failed to eliminate temperature as a variable. It is critically important that any studies comparing conventional and ohmic heating are conducted under similar temperature histories. Palaniappan and Sastry (1992) found no difference between the effects of ohmic and conventional heat treatments on the death kinetics of yeast cells (*Zygosaccharomyces bailii*), under identical histories. In some cases, however, a mild electrical pretreatment of *Escherichia coli* decreased the subsequent inactivation requirement.

More recent studies suggest that a mild electroporation mechanism may contribute to cell inactivation during ohmic heating. For example, studies on fermentation of *Lactobacillus acidophilus* under the presence of a mild electric field (Cho and others 1996) have indicated that although the fermentation lag phase can be significantly reduced, the productivity of the fermentation is also lowered by the presence of the electrical field. This may be due to the presence of mild electroporation, which improves the transport of substrates at the early stages of fermentation, thereby accelerating it. At the later stages, the electroporation effect would improve the transport of metabolites into the cell and thereby would inhibit fermentation. The presence of pore-forming mechanisms on cellular tissue has been confirmed by recent work (Imai and others 1995; Wang 1995; Kulshrestha and Sastry 1999). Another recent study (Cho and others 1999), conducted under near-identical temperature conditions, indicates that the kinetics of inactivation of *Bacillus subtilis* spores can be accelerated by an ohmic treatment. A 2-stage ohmic treatment (ohmic treatment, followed by a holding time prior to a second heat treatment) further accelerated death rates. A recent study (Lee and Yoon 1999) has indicated that leakage of intracellular constituents of *Saccharomyces cerevisiae* was enhanced under ohmic heating, as compared to conventional heating in boiling water.

The principal reason for the additional effect of these ohmic treatments may be the low frequency (50 – 60 Hz) used, which allows cell walls to build up charges and form pores (electroporation mechanism). This is in contrast to high-frequency methods such as radio or microwave frequency heating, where the electric field is essentially reversed before sufficient wall charge buildup occurs. Some contrary evidence has also been noted. In particular, the work of Lee and Yoon (1999) has indicated that greater leakage of *S. cerevisiae* intracellular constituents is detected under high frequencies. The details of temperature control within this study, however, are not available at this time, so it is not known if these researchers had adequately eliminated temperature effects.

Little or no information is available regarding the effects on survivor counts from ohmic heating processes on post-processing storage. In 1 industry study, survivor counts in a pasteurized liquid egg product decreased over storage time, apparently due to injury effects (Reznik 1999). In this study, initial post-pasteurization plate counts from conventional (plate) and electroheating were similar; however, after 12 wk of storage, many conventionally heated samples were reported to reach counts of 10,000 cfu/ml, while the electroheated samples exhibited less than 10 cfu/ml in most samples. No detailed statistical information on this study is available.

3.4. Description of Methods to Measure or Mathematically Model Pathogen Inactivation

A summary of the available kinetic data of microbial inactivation is presented in Tables 1 through 3.

While some evidence exists for nonthermal effects of ohmic heating, a larger body of research is needed to understand more fully the inactivation mechanism of the various microorganisms. For most ohmic processes, which rely on heat, it may be unnecessary for processors to claim this effect in their process filings; however, future processes may be reduced, if sufficient data are available.

Table 1—D-values and kinetic reaction rate constants (k) for Bacillus subtilis spores under conventional and ohmic heating (Cho and others 1999)

Temperature (°C)	D-values, conventional heating (min^{-1})	D-values, ohmic heating (min^{-1})	k, conventional heating (s^{-1})	k, ohmic heating (s^{-1})
88	32.8	30.2	0.00117	0.001271
92.3	9.87	8.55	0.003889	0.004489
95	5.06		0.007586	
95.5		4.38		0.008763
97	3.05		0.012585	
99.1		1.76		0.021809
z-value (°C) or Activation energy (E_a)(kcal/mol)	8.74*	9.16*	70.0**	67.5**

* - z-value;
** - Activation Energy

Table 2—D-values and reaction rate constants for inactivation of Bacillus subtilis spores during single and double stage conventional and ohmic heating at 90 °C (Cho and others 1999)

Stage nr	D-values, conventional heating (min^{-1})	D-values, ohmic heating (min^{-1})	k, conventional heating (s^{-1})	k, ohmic heating (s^{-1})
1	17.1	14.2	0.002245	0.002703
2	9.2	8.5	0.004172	0.004516

Table 3—Kinetic reaction rate constants (k) for Zygosaccharomyces bailii under conventional and ohmic heating (Palaniappan and others 1992)

Temperature (°C)	D-values, conventional heating (min^{-1})	D-values, ohmic heating (min^{-1})	k, conventional heating (s^{-1})	k, ohmic heating (s^{-1})
49.8	294.6	274.0	0.008	0.009
52.3	149.7	113.0	0.016	0.021
55.8	47.21	43.11	0.049	0.054
58.8	16.88	17.84	0.137	0.130
z-value (°C) or Activation energy (E_a)(kcal/mol)	7.19*	7.68*	29.63**	27.77**

* - z-value;
** - Activation Energy

4. Validation/Critical Process Factors

4.1. Identification and Description of Critical Process Factors

Since ohmic heating is fundamentally a thermal-based process, temperature and time are the principal critical process factors. Ohmic heating is an internal energy generation process, so there is theoretically no upper temperature limit to the process. Thus, if product hold-up occurs, it is possible for boiling to occur within the system even with a high degree of pressurization.

As in conventional thermal processes, the key problem is locating the zone(s) of minimum thermal treatment. Fundamentally, then, there exist only 2 critical factors, temperature and time of treatment at the coldest zone(s).

Several other factors, however, significantly affect the temperature within an ohmic process. Within a continuous flow ohmic heating system, the critical parameters affecting ohmic heating include the electrical conductivity(ies) of the respective phases of the food, the temperature dependence of electrical conductivity, the design of the heating device, the extent of interstitial fluid motion, the residence time distribution (if any), thermophysical properties of the food, and electric field strength. Although 2 commercial manufacturers currently produce ohmic systems, no standard or preferred designs are available. Therefore, while a detailed treatment of these factors cannot be attempted within the scope of this document, some general comments may be made.

The rate of heating is directly proportional to the square of the electric field strength, E, and the electrical conductivity, σ (Sastry 1994). Since the electrical conductivity of most foods increases with temperature (Palaniappan and Sastry 1991a, 1991b; Halden and others 1990), ohmic heating becomes more effective as temperature increases; however, this also raises the possibility of runaway heating, (similar to microwaves), and the possibility of arcing due to deposition of proteinaceous material on electrode surfaces. Another major factor affecting electrical conductivity is ionic content. It is therefore possible to formulate products with salt levels designed for effective ohmic heating. The electric field strength may be varied either by changing the electrode gap or the applied voltage, although the latter is much more easily controlled for any given design.

Other product properties that may effect temperature distribution include the density and specific heat of the food product. High densities and specific heats are conducive to slower heating. The thermal conductivity of the food may be of relevance if the material is heterogeneous, but is of relatively low importance for homogeneous materials.

Equipment design is another critical factor that should be considered. Some of the designs that are commercially available include electrodes that are located at various positions along the length of the product flow path (in-line field), or those located perpendicular to the flow (cross-field). The considerations in each of these cases are somewhat different. In the in-line field design, the device generally operates at high voltage and relatively low current. The product heats as it flows through the heater, so its electrical conductivity changes over the length. Since the total voltage drop in the heater must equal the applied voltage, the material at the upstream end experiences high field strengths, and downstream locations experience lower field-strengths. In the cross-field design, the field strength is constant throughout the system. While these are basic designs, a number of other possibilities exist, or are in development at this time; thus, it is not possible to present a comprehensive discussion here.

As in other thermal processes, it is necessary to identify the location(s) of cold zones and their motion. The thermal process must be sufficient to inactivate target pathogens to the desired extent at the location(s) of least lethal treatment. For a homogeneous fluid medium, such a position would likely be at the fastest moving region.

For the processing of solid-liquid mixtures, the question of the location of least lethal treatment has received considerable attention. Modeling work (de Alwis and Fryer 1990a,b; de Alwis and others 1989; Fryer and others 1992; Fu and Hsieh 1999), conducted for a static system, showed the effects of differences in electrical conductivity between a single low- or high-conductivity particle (inclusion particle) located within a medium of significantly different electrical conductivity. Sastry (1992) and Sastry and Palaniappan (1992) showed that the less conductive phase could heat faster than the fluid, if it was present in sufficiently high concentration. They also noted that an inclusion particle may provide a worst-case scenario. Since that time, considerable effort has gone into determining the worst-case scenario within an ohmic processing system (Zhang and Fryer 1993; Sastry and Salengke 1998). Unlike conventional heating systems, it is not clear that the worst case is necessarily associated with a static situation. Indeed, Khalaf and Sastry (1996) and Sastry and Salengke (1998) indicated that in situations where the solid is less conductive than the fluid, the worst case is associated with a well-mixed fluid surrounding the solid; however, when the solid phase is more conductive than the fluid, the worst case appeared to be the static condition. Further studies by Salengke and Sastry (1999) show that, when the situation favors a cold spot within the solid phase, the worst-case occurs with a well-mixed fluid (Fig. 1), but when the cold-spot occurs within the

fluid phase, the worst case is static (Fig. 2). Davies and others (1999) have shown the effects of shadow formation around individual solid particles, under a static condition in 2 dimensions.

As the above discussion suggests, 1 critical factor is likely to be fat content. If a fat globule is present within a highly electrical conductive region, where currents can bypass the globule, it may heat slower than its surroundings due to its lack of electrical conductivity. Under such conditions, any pathogens potentially present within the fat phase may receive less treatment than the rest of the product. Heating of the fat phase may then depend on the rate at which energy is transferred from the surroundings. Based on the foregoing discussion, a high heat transfer coefficient may not necessarily relate to the worst case, since fluid motion tends to moderate heating in such situations. If a fat-rich (low conductivity) phase is aligned to significantly intercept the current, it is possible for such a zone to heat faster than the surrounding fluid. In any case, care must be taken in establishing the process.

Whatever the scenario under consideration, the worst-case must be accounted for. Particle electrical conductivity and its temperature dependence are critical control factors that define the worst-case scenario. Additionally, the cold zone may shift considerably during the course of processing, depending on the relative electrical conductivities of phases. The exact details are likely to be equipment-specific; thus, a separate analysis may need to be conducted for each new system and product.

4.2. Describe Methods to Measure/Monitor Critical Process Factors

For single-phase homogeneous fluid foods, temperature may be monitored at the cold zone, which may typically be the fastest-moving region of fluid. Temperature measurements are complicated by the presence of an electrical field; thus, the temperature sensor must be electrically isolated from the surrounding environment. An example of such isolation is a teflon-coated thermocouple.

Currently, there is no physical method available for measurement of temperature within real particulate food products undergoing continuous ohmic heating. Measurements have been conducted with liquid crystal (Sastry and Li 1996), which works only for transparent foods, and MRI (magnetic resonance imaging) (Ruan and others 1999), which is limited by the need to turn off the electrical field for 14 s during data acquisition.

The only other approach appears to be the use of history indicators (time temperature integrators), either biological (microbiological or enzymatic) or chemical. The same principles as thermal processing apply and extra care should be given to critical factors associated with the ohmic heating process (the presence of the indicator should not influence the heating process). For recent reviews on time temperature integrators, see Hendrickx and others (1995) and Van Loey and others (1996). An application to ohmic heating has recently been described by Kim and others (1996). If the worst-case scenario for a product could be pre-established by mathematical models, it would be possible to evaluate total process treatment by such indicators.

4.3. Description of Microbial or Chemical Surrogates/ Indicators

To determine processing kinetics and efficiency of ohmic in-

Fig. 1—Color mapping of modeled temperature distributions within an ohmic heater after 150 s of ohmic heating, for a single solid cylindrical "inclusion" particle 1/3 as conductive as the fluid; (a) well-mixed fluid (b) static fluid

Fig. 2—Color mapping of modeled temperature distributions within an ohmic heater after 150 s of ohmic heating, for a single solid cylindrical "inclusion" particle twice as conductive as the fluid; (a) well-mixed fluid (b) static fluid

activation of microorganisms, a surrogate/indicator organism would be selected from those microbes traditionally used in thermal processing studies. No organism(s) with unique resistance to ohmic processing has been reported in the literature, suggesting that classical surrogates (vegetative cells or spores) would be appropriate for process determination and validation (see Overarching Principles Section 2).

5. Process Deviations and How to Handle Them

Process deviations in this technology are equipment-specific. Since no standard equipment design currently exists, the character of the deviation and the corrective action cannot be characterized in a simple manner within the scope of this document.

5.1. Basic Detection Methods for Process Deviations

Depending on the system, the detection methods may involve monitoring of particle conductivities, system power input, and inlet and outlet temperatures. Significant deviations from operating conditions may indicate an abnormality that is equipment-specific. Further, it is important to protect against undetectable hazards.

One approach to preventing process deviations would be to monitor electrical conductivity of the process stream on-line. Solids of unusually low or high electrical conductivity could be detected, in principle, and diverted before entering the heater; however, much depends on the sensitivity of the detection technique and the background noise created by natural variations in product electrical conductivity.

5.2. Methods to Assess and Correct Deviations

Assessing and correcting deviations is not simple, since the knowledge base is incomplete at this time. As stated above, this is a subject that needs further research and separate, design-specific evaluation. If deviations occur beyond the safety factors set by design, then complete product reprocessing may be necessary. As stated above, the information will depend on design, and insufficient information is available at this time.

6. Research Needs

THE FOLLOWING AREAS OF RESEARCH NEED TO BE ADDRESSED before implementing ohmic heating as a preservation method:

- Develop a more complete body of knowledge about the combined influence of temperature and electric fields on the destruction kinetics of key pathogenic microorganisms.
- Develop the knowledge base to assess the impact of deviations for specific designs of ohmic heaters. This would include improved models for ohmic processes.
- Develop methods for monitoring temperatures within individual solids.

Glossary

A complete list of definitions regarding all the technologies is located at the end of this document.

Conductivity (Electrical), σ. Physical property of a food material that determines its ability to conduct electricity and is expressed in Siemens per cm (S/cm). In ohmic heating, it enables heating to occur.

Conductivity (Thermal). Physical property of a food material which determines its ability to conduct heat. Expressed in Watts/meter °C.

Conventional heating. Heating of a substance by transfer of thermal energy from a heating medium to a low temperature product.

Cross-field. An ohmic heating system where the electric field is aligned across the product flow path.

Electroheating. See ohmic heating

Inclusion particle. A food particle of significantly different electrical conductivity than its surroundings.

Interstitial fluid motion. The motion of fluid in the spaces between solid particles.

Nonthermal effects. Effects due to the exposure to a process that are not of thermal origin, i.e., cannot be explained by measured temperature changes.

Specific heat. The ability of a material to store heat. Described technically as the amount of energy required to raise the temperature of unit mass of an object by a unit increment in temperature.

Thermophysical properties. Properties that influence the heating rate of a material. Examples of thermophysical properties are thermal conductivity (the ability of the material to conduct heat), specific heat (the ability of the material to store heat), and density (the mass per unit volume of the material).

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High Pressure Processing

DANIEL F. FARKAS AND DALLAS G. HOOVER

Scope of Deliverables: This section covers high pressure processing as an alternative technology for preservation of foods. It includes critical proc`ess factors, their effect on inactivation levels and mechanisms of inactivation, as well as pathogens of concern and recommendations for surrogates. Methods to handle deviations are also described.

1. Introduction

1.1. Description of Technology

1.1.1. Process physical description

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 0 °C (to minimize any effects of adiabatic heat) to above 100 °C. Vessels are uniquely designed to safely withstand these pressures over many cycles. Commercial exposure times at pressure can range from a millisecond pulse (obtained by oscillating pumps) to a treatment time of over 1200 s (20 min). In contrast to thermal processing, economic requirements for throughput may limit practical exposure times to less than 20 min. Pressures used in the HPP of foods appear to have little effect on covalent bonds (Tauscher 1998; 1999); thus, foods subjected to HPP treatment at or near room temperature will not undergo significant chemical transformations due to the pressure treatment itself. HPP may be combined with heat to achieve an increased rate of inactivation of microbes and enzymes. Chemical changes in the food generally will be a function of the process temperature and time selected in conjunction with the pressure treatment.

HPP differs from the homogenization of liquids in that decompression is achieved by expanding the compressed food against a constraining liquid causing it to do work and thus lowering its temperature towards its original value. Homogenization dissipates compression work as heat by expanding the product through an orifice or capillary.

HPP acts instantaneously and uniformly throughout a mass of food independent of size, shape, and food composition. Thus, package size, shape, and composition are not factors in process determination. The work of compression during HPP treatment will increase the temperature of foods through adiabatic heating approximately 3 °C per 100 MPa, depending on the composition of the food. For example, if the food contains a significant amount of fat, such as butter or cream, the temperature rise can be larger. Foods cool down to their original temperature on decompression if no heat is lost to or gained from the walls of the pressure vessel during the hold time at pressure. Figure 1 shows typical temperature rises for water and fat as a function of compression pressures. A uniform initial temperature is required to achieve a uniform temperature increase in a homogenous system during compression.

While the temperature of a homogenous food (one with less than 25% fat) will increase uniformly due to compression, the temperature distribution in the mass of food during the holding period at pressure can change due to heat transfer to or from the walls of the pressure vessel. The pressure vessel must be held at a temperature equal to the final food temperature increase from compression for truly isothermal conditions. Temperature distribution must be determined in the food and reproduced each treatment cycle if temperature is an integral part of the HPP microbial inactivation process specification.

Foods decrease in volume as a function of the imposed pressure as shown in Fig. 2. An equal expansion occurs on decompression. For this reason the packaging used for HPP-treated foods must be able to accommodate up to a 15% reduction in volume, and return to its original volume, without loss of seal integrity and barrier properties.

Regarding HPP as a food-processing technology, the greater the pressure level and time of application, the greater the potential for changes in the appearance of selected foods. This is especially true for raw, high-protein foods where pressure-induced protein denaturation will be visually evident. High hydrostatic pressures also can cause structural changes in structurally fragile foods such as strawberries or lettuce. Cell deformation and cell membrane damage can result in softening and cell serum loss. Usually these changes are undesirable because the food will appear to be processed and no longer fresh or raw. Food products

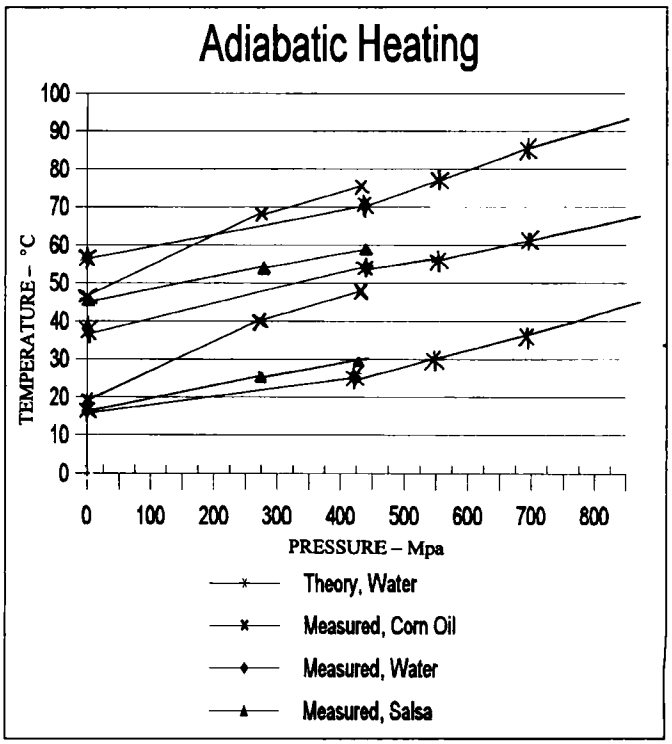

Fig. 1—Increase in temperature of water, corn oil, and salsa as a result of Adiabatic Compression (Ting 1999). Note that the increase in temperature upon compression is also a function of initial Temperature

that have been brought to market or that currently employ HPP in their manufacture include fruit jellies and jams, fruit juices, pourable salad dressings, raw squid, rice cakes, foie gras, ham, and guacamole. Raw oysters shucked and pasteurized by HPP may become available in 2000.

1.1.2. The influence of pH, water activity, and temperature on HPP

Compression of foods may shift the pH of the food as a function of imposed pressure. Heremans (1995) indicates a lowering of pH in apple juice by 0.2 units per 100 MPa increase in pressure. The direction of pH shift and its magnitude must be determined for each food treatment process. Instrumentation for the routine measurement of pH at pressures between 100 and 800 MPa must be developed as it is not available at the time of this review.

The magnitude and direction of the shift of water activity, if any, as a function of pressure has not been reported. Oxen and Knorr (1993) showed that a reduction of water activity (measured at one atmosphere) from 0.98 – 1.0 to 0.94 – 0.96 resulted in a marked reduction in inactivation rates for microbes suspended in a food. Reducing the water activity appears to protect microbes against inactivation by HPP; however, it is to be expected that microbes may be sublethally injured by pressure, and recovery of sublethally injured cells can be inhibited by low water activity. Consequently, the net effect of water activity may be difficult to predict.

Linton (1999) has shown that pH has a marked effect on inactivation rates of *Escherichia coli* O157H:7. As pH is lowered, most microbes become more susceptible to HPP inactivation, and sublethally injured cells fail to repair. These observations indicate pH and water activity are critical process factors in the inactivation of microbes of public health significance in foods treated by HPP. HPP treatments, in the absence of significant temperature increases, do not break covalent chemical bonds. Ionic bonds such as those responsible for the folding of proteins can be disrupted. The influence of pH on the survival of pressure-damaged microbes is illustrated by the work of Garcia-Graells and others (1998) and Pagan and others (1999). The latter workers treated *E. coli* C9490, a pressure-resistant strain taken from stationary phase cultures, at 100, 200, 300, 400, 500, and 600 MPa for 10 min in pH 7.0 phosphate-buffered saline (PBS). The treated cells were transferred to pH 3.5 tryptone soy broth and held at 37 °C for 3 h. Cells treated at pressures of 200 MPa and below showed no loss of viability. Cells treated at 300 to 600 MPa were found to die at a rate which increased as a function of pressure treatment. Studies using a pressure treatment of 400 MPa for 10 min and subsequent holding in media with pH values between 7.0 and 3.5 showed that cells were inactivated at pH 4.5 or lower. The internal pH of the pressure-damaged cells was not a factor in their loss of viability. This work shows that acid pH values can cause inactivation of pressure-damaged cells.

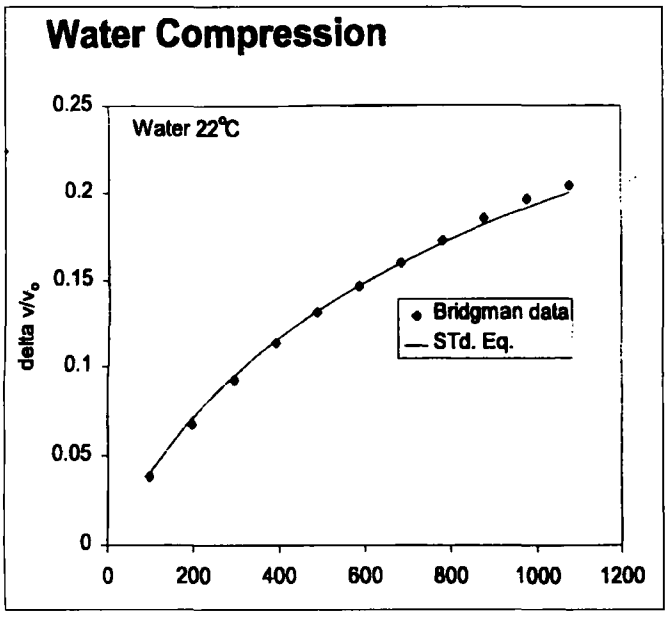

Fig. 2—Fractional decrease in volume of water as a function of imposed Pressure (Bridgeman 1912, Ting 1999)

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 45 to 50 °C appear to increase the rate of inactivation of food pathogens and spoilage microbes and thus merit the development of processes which incorporate a uniform initial food temperature in this range. Process temperatures in the range of 90 – 110 °C in conjunction with pressures of 500 – 700 MPa have been used to inactivate spore-forming bacteria such as *Clostridium botulinum*. The use of elevated temperatures as part of a specified HPP process will require monitoring the food temperature during the process to insure every element of the food is at or above the specifieD-value. The effect of temperature on the rate of inactivation of microbes and enzymes subjected to pressure treatment is discussed more fully in the section on mechanisms of inactivation (Heinz and Knorr 1999). Meyer (2000) has proposed that the heat of compression be used in a high-temperature, short-time combined thermal and pressure treatment, since the temperature of a product can be raised from 100 to 120 °C by a quick compression to 700 MPa and brought back to 100 °C by a quick decompression.

1.2. Equipment for HPP Treatment

Equipment for batch HPP treatment of foods is shown schematically in Fig. 3 and consists of (1) a pressure vessel of cylindrical design, (2) two end closures, (3) a means for restraining the end closures (for example, yoke, threads, pin), (4) a low pressure pump, (5) an intensifier which uses liquid from the low pressure pump to generate high pressure process fluid for system compression, and (6) necessary system controls and instrumentation. The six components of a high pressure processing system can be arranged to treat unpackaged liquid foods in a semi-continuous manner and packaged foods in a batch configuration. Semi-continuous equipment is described in Section 1.2.2.

1.2.1. Batch HPP equipment technology

Batch HPP systems are similar in operation to batch thermal processing retort systems in that both process cycles consist of filling the process vessel with product, closing the vessel, bringing the vessel to pressure process conditions, decompressing the vessel and removing the product. High pressure vessels may operate in a vertical, horizontal, or tilting mode. Pressure vessels capable of routine operation at pressures over 400 MPa can be built of two or more concentric cylinders of high tensile strength steel. The outer cylinders compress the inner cylinders such that the wall of the pressure chamber is always under some residual compression at the design operating pressure. Safety codes (ASME Section 8, Division 3 of the Boiler and Pressure Vessel Code) require the inner cylinders to crack to allow leakage to relieve pressure and thus avoid catastrophic failure of the pressure vessel ("leak before break"). The outer cylinder of a pressure vessel may be wire wound or encapsulated in a liquid-filled, permanently pressurized, outer cylinder to ensure a cycle life of over 100,000 cycles at pressures of 680 MPa or higher. The inner cylinder and all parts exposed to water or food should be made of

stainless steel to avoid corrosion. Systems using high tensile strength steel (non-stainless) may use a food-approved oil or water containing FDA- and USDA-approved lubricants, anti-corrosion agents, and antimicrobial compounds as pressurizing fluids. Packaged foods treated in systems using a lubricant can be protected during HPP treatment by over-wrapping in a sealed bag. Preferred practice is to design high pressure food processors with stainless steel food contacting parts so that filtered, potable, water can be used as the isostatic compression fluid.

Pressure vessels are available as laboratory units with volumes of 0.1 to 2 liters. Pilot plant vessels have capacities of 10 to 25 liters while batch production pressure vessels can be supplied with volumes of several hundred liters. Two or more pressure vessels can be driven by a single intensifier.

For batch operation, packaged food is loaded into the pressure vessel, the vessel is sealed, and process water is pumped into the vessel to displace any air. When the vessel is full, the pressure relief valve is closed, and water is pumped into the vessel until the process pressure is reached. The rate of compression is directly proportional to the horsepower of the low pressure pump driving the intensifier. When the process time is completed, the pressure relief valve is opened and the water used for compression is allowed to expand and return to atmospheric pressure. The vessel is opened and the packaged food is removed and is ready for shipment. The displacement of air prior to HPP treatment is done to reduce pumping costs by eliminating air compression. Residual air in the treatment chamber has no effect on microbial inactivation kinetics of HPP-treated, packaged foods. The amount of air in the system is not a critical process factor.

A 100-horsepower pump can bring a 50-liter vessel to an operating pressure of 680 MPa in 3 – 4 min. Compression time is a function of pump horsepower. Work must be supplied to compress water at pressures above 200 MPa. Figure 1 can be used to estimate the additional water needed to bring a vessel of known volume to process pressure. The data shown in Fig. 1 neglect the expansion of the pressure vessel and associated piping during compression. Vessel expansion may add several percent to the vessel volume. A filled 100-liter vessel will require an additional 15 liters of water to bring it to a pressure of 680 MPa.

The high cost of pressure vessels, pumps, intensifiers, and sealing systems requires that the system cycle as many times per hour as is possible given the hold time at pressure needed to treat the food. Systems that can perform product loading, vessel sealing, compression, decompression, unsealing, and unloading in under 2 min are under design. Target pressure hold times of 5 min or less are desirable. HPP treatments will probably be limited to hold times no longer than 10 min. This is in contrast to batch thermal processes which many require 60 min to complete a process cycle.

1.2.2. Semi-Continuous HPP equipment technology

Current semi-continuous systems for treating liquids use a pressure vessel containing a free piston to compress liquid foods. A low-pressure food pump is used to fill the pressure vessel. As the vessel is filled the free piston is displaced. When filled, the inlet port is closed and high pressure process water is introduced behind the free piston to compress the liquid food. A process pressure of 680 MPa will result in a 15% compression of the liquid treated. After an appropriate process hold time, the system is decompressed by releasing the pressure on the high pressure process water. The treated liquid is discharged from the pressure vessel to a sterile hold tank through a sterile discharge port. A low pressure water pump is used to move the free piston towards the discharge port. The treated liquid food can be filled aseptically into pre-sterilized containers.

1.2.3. Continuous HPP process equipment

At the time of this writing no commercial continuous HPP systems are operating. A continuous system must compress the liquid food, provide a plug flow hold tube or hold vessel to achieve a specified process time. Next there must be a means to decompress the liquid such that the liquid is caused to do work to avoid excessive shear and heating. The decompressed, treated liquid could be sent to a sterile hold tank for eventual aseptic filling.

Homogenizers operating above 100 MPa have been proposed as a means for the inactivation of microbes in liquid foods (Moorman and others 1996). Experimental data must demonstrate the efficacy of this equipment as a function of operating pressure. Heating effects during decompression must be separated from the contribution made by pressure.

1.2.4. Pulsed HPP processing systems

Semi-continuous and batch equipment can be adapted to pulsed operation by programming a series of treatment cycles of short duration prior to discharging the treated food. Preliminary studies (Aleman and others 1996) observed an increase in the inactivation rate of yeast with multiple-pulsed pressure treatments. The total pulsed exposure time was equal in duration to a single constant pressure treatment. Pulse frequency, and the ratio of time intervals at pressure and off pressure, must be considered. Pulse shape (ramp, square, sinusoidal, or other wave form) must be considered. Section 2.6 (HPP Pulsed Applications) provides a more detailed review of the literature and includes the effect of pulsed pressure treatments on spore inactivation.

1.3. Summary of Critical Process Factors

The critical process factors in HPP will be discussed later in the report. They include pressure, time at pressure, time to achieve treatment pressure, decompression time, treatment temperature (including adiabatic heating), product initial temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity, and concurrent processing aids. Other processing factors present in the process line before or after the pressure treatment were not included. Pressure pulsing would require additional monitoring of pulse shape frequency, and high and low pressure values of the pulse.

2. Pathogens of Public Health Concern Most Resistant to HPP

2.1. Historical perspective

The first report of high hydrostatic pressure killing bacteria was by H. Roger in 1895; however, in food science and technology, the most important work involving microbial inactivation was that by Bert Hite, published in June of 1899 (Hite 1899). Hite originally experimented with the application of high hydrostatic pressure on foods and food microorganisms. He showed that the shelf-life of raw milk could be extended by about 4 d after pressure treatment at 600 MPa for 1 h at room temperature. Souring was delayed for about 24 h after treatment at 200 MPa. In later work, Hite and others (1914) found most pressure-treated fruits remained commercially sterile for at least 5 y after processing at pressures ranging from 400 to 820 MPa. Hite's last contribution to the field was in 1929 (Giddings and others 1929) in which tobacco mosaic virus was treated at pressures above 930 MPa with inconsistent inactivation.

Larsen and others (1918) confirmed that HPP can inhibit microbial growth and cause cells to die. Vegetative types were killed after 14 h at 607 MPa. It was recognized that spores of bacteria were extremely resistant to inactivation by pressure, but could be killed at 1,214 MPa.

In later years, Timson and Short (1965) pressurized milk at 1,034 MPa/35 °C for 90 min and learned that approximately 0.05% of the bacterial population was capable of surviving this pressure. Microbial analysis identified the survivors as spores of *Bacillus subtilis* and *Bacillus alvei*. It was suggested that the lethal effect of pressure was more evident in the solid phase than the liquid phase of water. That is, *B. subtilis* survived solid-phase transitions from Ice II, III, and V to Ice I. They found a neutral pH more protective to the spores than acid pH. Additionally, the presence of NaCl or glucose provided protection against the damaging effect of pressure encountered at acid and alkaline pH. In their 1965 article, W.J. Timson and A.J. Short noted a 1932 paper by J. Basset and M. A. Macheboeuf who reported the survival of spores of *B. subtilis* exposed to more than 1,724 MPa (250,000 psi) for 45 min.

At the Institute of Food Technologists Annual Meeting in 1974, D.C. Wilson presented a paper reestablishing use of pressure and elevated temperatures as a food preservation method. Low pressures of around 140 MPa combined with temperatures of 82 to 103 °C were effective for the sterilization of low-acid foods in sealed containers. The combination of mild heat with hydrostatic pressure produces a synergistic effect. At 0.35 MPa and 100 °C the D-value is 280 min for gram-positive sporeforming bacteria, while at 138 MPa and 100 °C the D-value is 2.2 min. Consequently, substantial reductions in microorganisms can be achieved when co-treatments of heat and pressure are utilized. In the 1980s, there was a dearth of information regarding inactivation kinetics of HPP for important food microorganisms; however, the current literature is relatively voluminous concerning the inactivation of microorganisms (and enzymes) in foods processed by HPP.

2.2. Microorganisms with greatest pressure resistance

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. The most heat-resistant pathogen, 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. Spores of *C. botulinum* are among the most pressure-resistant known. Spore suspensions of strains 17B and Cap 9B tolerated exposures of 30 min to 827 MPa and 75 °C (Larkin and Reddy 1999). Among the sporeformers of concern, *Bacillus cereus* has been the most studied because of its facultatively anaerobic nature and very low rate of lethality.

Normally, gram-positive vegetative bacteria are more resistant to environmental stresses than vegetative cells of gram-negative bacteria. This observation commonly applies to pressure resistance as well. Among the pathogenic non-sporeforming gram-positive bacteria, *Listeria monocytogenes* and *Staphyloccocus aureus* are the two most well-studied regarding the use of HPP processing. *Staphyloccocus 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 *E. coli* O157:H7 that possesses pressure resistance comparable to spores. Some strains of *Salmonella* spp. have demonstrated relatively high levels of pressure resistances. 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.

2.2.1. Nonsporeforming bacteria

Heat-resistant bacteria are usually more pressure-resistant than heat-sensitive types, but there are notable exceptions. For

example, *Salmonella* Senftenberg 775W is the most heat-resistant *Salmonella* known (Ng and others 1969). Comparison with a heat-sensitive strain of *Salmonella* Typhimurium (D-value at 57.5 °C = 3 min) showed *Salmonella* Senftenberg 775W to be consistently more pressure-sensitive (Metrick and others 1989). It was also found that significant metabolic injury occurred in *Salmonella* that survived pressurization. Recovery of these cells was possible with incubation at 37 °C in a non-selective enrichment medium. These data suggest that cells sublethally stressed by pressure may be more susceptible to other means of inactivation.

Vibrio parahaemolyticus, a marine bacterium that is also an important foodborne pathogen, is substantially more sensitive to the effects of high hydrostatic pressure than *L. monocytogenes*, an important gram-positive pathogen common in raw foods (Styles and others 1991). A 10^6-cfu/ml population of *L. monocytogenes* is inactivated within 20 min by a 345-MPa pressurization in buffer at 23 °C, while a similar concentration of *V. parahaemolyticus* is eliminated in half the time (10 min) at half the pressure (173 MPa) in clam juice. Milk, as compared to buffer, offers a protective effect for *L. monocytogenes*. This was similar to the protection afforded to pressurized *Salmonella* by strained-chicken baby food (Metrick and others 1989). Pressure in combination with low pH between 3.0 and 4.0 in citrate buffer destroyed *Listeria monocytogenes* populations of approximately 10^7 cfu/mL within 30 min (Stewart and others 1997). Treatment at pressures above 304 MPa at less than pH 6.0 also resulted in no detectable survivors when Trypticase soy agar plus 0.6% yeast extract was used as the plating medium; however, when *Listeria* recovery agar was used, approximately 10^2 cfu/mL were recoverable. These surviving cells represent an injured subpopulation that cannot recover at pH less than 5.6.

Fujii and others (1995) evaluated several plating media to judge the effect of pressure-induced injury of *E. coli*, *V. parahaemolyticus*, and *L. monocytogenes*. These bacteria were pressure-treated to generate survival rates of 10 to 50% of the starting viable concentration. Their results showed that plating media such as Trypticase soy agar and nutrient agar were superior to brain heart infusion agar (BHI) and plate count agar (PCA) in the detection of sublethally treated cells exposed to pressure. Detection levels could be improved for BHI and PCA by the addition of horse blood. As anticipated, detection was relatively low for selective media and variable depending on the selective ingredients in these media.

Satomi and others (1995a) observed a sharp drop in survivors and injury rate in *E. coli* above 182 MPa that corresponded to release of UV-absorbing substances. Pressure resistance in *E. coli* was not affected by the type of growth media used to propagate the cells nor the presence of oxygen; however, resistance did increase with age of the culture and increase of osmotic pressure in the pressurizing menstruum. Pressure resistance was reduced with a decline of pressure menstruum pH and pressure treatment at 44 °C. Additional studies by Satomi and others (1995b) assessed conditions of optimal recovery for *E. coli* and *V. parahaemolyticus* following exposure to debilitating levels of HPP. Parameters for most rapid recovery of *E. coli* were nutrient medium with <1.0% NaCl, pH 7.0 at 30 to 37 °C. Most rapid recovery of *V. parahaemolyticus* occurred when incubation was in aerobic conditions and nutrient medium of 0.5 to 3.0% NaCl, pH 7.0 at 37 °C.

Patterson and others (1995) examined the response to HPP of several vegetative types of food-poisoning bacteria. *Yersinia enterocolitica* was the most sensitive bacterium in the study. It was reduced 5-log cycles with 275 MPa for 15 min in phosphate-buffer-saline (PBS). For comparable 5-log reductions using 15-min treatments, *Salmonella* Typhimurium required 350 MPa, *L. monocytogenes* required 375 MPa, *Salmonella* Enteritidis 450 MPa, *E. coli* O157:H7 required 700 MPa, and *S. aureus* 700 MPa. The bacteria tended to be more pressure resistant in UHT milk than meat or buffer. The authors remarked that the variability of pressure response in bacteria depended upon bacterial strain differences and different suspending media.

Patterson and Kilpatrick (1998) used HPP against *E. coli* O157:H7 NCTC 12079 and *S. aureus* NCTC 10652 in milk and poultry. Their findings showed a practical necessity for combined use of pressure and elevated temperatures. Alone, neither treatment displayed effective inactivation of the pathogens. In UHT milk, 400 MPa/50 °C/15 min reduced populations of *E. coli* approximately 5 log cfu/g, and 500 MPa/50 °C/15 min delivered reductions of approximately 6 log cfu/g for *S. aureus*. In minced irradiation-sterilized poultry meat, *E. coli* was reduced by approximately 6 log cfu/g by 400 MPa/50 °C/15 min, and *S. aureus* exposed to 500 MPa/50 °C/15 min was reduced by approximately 5 log cfu/g. Also, polynomial expressions derived from the Gompertz equation were used to devise models to predict inactivation of each pathogen at different pressure-temperature combinations.

HPP of *L. monocytogenes* and *Salmonella* Typhimurium in fresh pork loin was investigated by Ananth and others (1998), who found that at 25 °C the D-values at 414 MPa were 2.17 min for *L. monocytogenes* and 1.48 min for *Salmonella* Typhimurium. A treatment of 414-MPa/13-min/25 °C inactivated either pathogen inoculated at levels of approximately 10^6 per chop. There were also no detectable psychrotrophic plate counts from the pork loin after 7 d of storage at 4 °C. After 7 d, plate counts climbed, and at 33 d reached nearly 10^6 cfu/g. Interestingly, sensory analysis (triangle test of difference) showed that samples cooked after pressurization were different ($P>0.05$) from controls, but only for samples pressure-treated at 2 °C, not at 25 °C. It was determined that, generally, pressure-treated meat was not significantly different from controls in sensory quality, and HPP did extend the shelf-life of the product. The effects of HPP on *L. monocytogenes* and pork chops were also studied by Mussa and others (1999) with pressure treatments apparently conducted at ambient temperature. Strain Scott A was found to have a D-value at 400 MPa of 3.5 min while the indigenous microbiota of the pork was found to have a D-value at 400 MPa of 1.3 min.

The effects of HPP on microbial inactivation of *E. coli* and *Pseudomonas fluorescens* in 6%-fat ovine milk was investigated by Gervilla and others (1997a). The strain of *E. coli* was most resistant when pressure-treated at 10 °C and strain of *P. fluorescens* was most pressure resistant when pressure-treated at 25 °C. *E. coli* was more pressure resistant than *P. fluorescens*. Inactivation of >6 log cfu/mL was attained for *E. coli* when treated at ≥450 MPa/ 25 °C for 5 min and for *P. fluorescens* when treated at ≥400 MPa/10 °C for 5 min. When treatment temperatures of 50 °C were used, equivalent reductions of bacterial populations were obtained with pressures of 400 MPa for *E. coli* and 300 MPa for *P. fluorescens*.

In another study by Gervilla and others (1997b), 6%-fat ovine milk inoculated with *Listeria innocua* 910 CECT was investigated with special regard to pressure-treatment temperatures. Pressure treatments at 2 °C were more effective inactivating *L. innocua* than at ambient temperature (25 °C), but less effective than at 50 °C. Complete elimination of starting inocula of 10^7 to 10^8 cfu/mL in ewes' milk was accomplished by the following conditions: 2 °C/450 MPa/15 min, 10 °C/450 MPa/15 min, 25 °C/450 MPa/15 min, and 50 °C/350 MPa/15 min. Five- and 10-min treatment periods were also examined, and found to require an additional 50-MPa increase in pressure for complete inactivation, with the exception of 5-min treatments at 50 °C. The authors noted that the fat in ewes' milk has been shown to confer thermal protection for *L. monocytogenes* and *L. innocua*, and voiced concern that this same character may increase the resistance of *Liste-*

ria spp. and other detrimental bacteria treated with combinations of pressure and temperature in ovine milk.

Listeria innocua 910 CECT was examined in liquid whole egg by Ponce and others (1998); however, in this product, starting inocula of approximately 10^6 cfu/mL could not be totally inactivated by 300 to 450 MPa at −15 to 20 °C for up to 15 min. The most effective treatment examined in this study (450 MPa/20 °C/15 min) showed a reduction of about 5 log cfu/mL. The effects of these treatments on the functional properties of the liquid whole egg was not noted.

Three strains of *L. monocytogenes* showed a wide range of pressure sensitivities (Simpson and Gilmour 1997a). Scott A was not eliminated by exposure to 450 MPa for 30 min at ambient temperature, whereas another strain (a poultry isolate) was eliminated at 400 MPa after 15 min (starting concentration for both 5 $\times 10^8$ cfu/mL). A third strain, NTC11994, was completely eliminated when pressurized at 450 MPa for 30 min. These cultures were pressurized in phosphate-buffer-saline (PBS) modified with bovine serum albumin (protein), glucose (carbohydrate), and olive oil (lipid). These components were found to protect *Listeria* against pressure inactivation when compared to PBS alone. Simpson and Gilmour (1997b) examined the pressure resistance of 13 enzymes from 3 strains of *L. monocytogenes* that demonstrated a range of sensitivities to HPP. They found no evident trends between the pressure resistance of any specific enzyme and the strain from which it was derived, suggesting that none of the selected enzymes was the primary site of pressure inactivation in *L. monocytogenes*.

The variability of pressure resistances within strains of *S. aureus, L. monocytogenes, Salmonella* and *E. coli* O157:H7 was demonstrated by Alpas and others (1999); however, the range of pressure resistances with species diminished significantly when the pressure treatment temperature was raised from 25 to 50 °C. This finding represents another reason to conduct HPP treatment with mild heat.

As exemplified above in inoculated trials with HPP treatment of milk products, *L. innocua* is a preferred surrogate organism for *L. monocytogenes*. *Listeria innocua* fills this role because in addition to its very similar physiology and metabolism with *L. monocytogenes*, the nonpathogen is equally resistant to low pH, drying, heating and salt. Such hardiness makes L. innocua an excellent indicator in inoculated pack studies at dairy facilities and other food processing plants.

In the selection of a gram-negative bacterium as an HPP indicator organism, a nonpathogenic member of Enterobacteriaceae would appear to be a valid choice. A nonpathogenic variety of *E. coli*, selected for notable pressure resistance, would probably be the most applicable selection given the concern for *E. coli* O157:H7.

2.2.2. Bacterial spores

Unless high hydrostatic pressures in excess of 800 MPa are used, heat in conjunction with HPP is a requirement for effective elimination of bacterial endospores in low-acid foods. The articles reviewed in this section indicate the pressure levels, treatment temperatures, and exposure times necessary to inactivate spores. The references describe the complex role spore germination plays in this process. Spores present the greatest challenge for inactivation by HPP.

Clouston and Wills (1969) examined the effect of hydrostatic pressure up to 1700 MPa at 25 °C on the heat and radiation resistance of spores of *Bacillus pumilus*. Initiation of germination occurred at pressures exceeding 500 MPa and was the prerequisite for inactivation by compression. It was assumed that there was a net decrease in the volume of the system during initiation of germination as a result of increased solvation of the spore components.

Butz and others (1990) investigated the effects of pressures between 150 and 400 MPa at temperatures of 25 to 40 °C on bacterial spores and showed that pretreatment at relatively low pressures (60 – 100 MPa) led to accelerated inactivation of spores at high pressure. Several papers on the use of HPP to inactivate spores have made similar suggestions for a two-exposure treatment with HPP to enhance the inactivation of spores. The first exposure germinates or activates the spores, and the second exposure at a higher pressure inactivates the germinated spores and vegetative cells (Heinz and Knorr 1998).

Effects of combined pressure (200 and 400 MPa) and temperature (20 and 90 °C) on the reduction of *Bacillus stearothermophilus* spores have been examined (Seyderhelm and Knorr 1992). Limited effects were found when spores were pressurized at 0.1 MPa (1 atmosphere) in conjunction with temperatures up to 90 °C, or 400 MPa and 20 °C. Marked effects on spore counts, however, were observed when pressurized between 200 and 400 MPa at temperatures between 60 and 90 °C. Initial counts of 3×10^6 were reduced to <10 at 90 °C and 200 MPa, at 80 °C and 350 MPa, or at 70 °C and 400 MPa. Kakugawa and others (1996) also examined heat and pressure effects on spore suspensions of *B. stearothermophilus*. Viable counts could be reduced from 10^6 to 10^2 spores/mL in 30 min by treatment at 110 °C and 200 MPa, and in 10 min by exposure to 100 °C and 400 MPa. Attempts to reduce the viable spore counts below 10^2/mL could not be accomplished even after 50 min at 120 °C and 400 MPa.

The effect of hydrostatic pressure on activation of *Bacillus* spp. spores as a preparatory state for synchronous germination was investigated by Nishi and others (1994). These workers found that activation of *Bacillus subtilis* spores in milk by 200 MPa from 25 to 60 °C resulted in a greater rate of spore germination than exposure to 80 °C. They reported that most of the pressure-activated spores germinated within 1 h of exposure to 37 °C as indicated by loss of heat resistance.

Okazaki and others (1996) examined spores of *B. subtilis, Bacillus coagulans*, and *Clostridium sporogenes* PA3679 at pressures up to 400 MPa in combination with temperatures ranging from 25 to 110 °C. In phosphate buffer, it was found that for the strains selected, spores of *B. subtilis* were more pressure-resistant than spores of *B. coagulans*, and spores of *B. coagulans* were more pressure-resistant than spores of *C. sporogenes*. As a result, high treatment temperatures were required to eliminate spores suspensions of about 10^7/mL. At ambient temperature, Crawford and others (1996) were able to reduce *C. sporogenes* by 5-log cycles after 60 min at 680 MPa.

Rovere and others (1996a) examined pressure-treatment parameters for inactivation of spores of *C. sporogenes* PA3679 starting with concentrations of approximately 10^5 spores/mL and pressure-hold times of 5 min. Elimination of these spore levels was possible with processes of 1,400 MPa/54 °C and 800 MPa/75 °C in different model food systems. In a study involving spore suspensions of PA3679 in meat broth, Rovere and others (1996b) noted that pressure acts as a complementary synergistic process to allow reduction of the thermal processing parameters necessary to eliminate problematic sporeformers in foods. Processing at 108 °C/800 MPa was found to be the most effective treatment with a calculated D-value of 0.695 min. Heat treatment (110 °C) alone generated a D-value of 13.3 min for spores of PA3679.

The pressure sensitivity of strains of several species of bacilli and *C. sporogenes* PA3679 were evaluated by Gola and others (1996). Pressure treatments of 900 MPa for 10 min at 30 °C were unable to completely destroy 8.4×10^2 *C. sporogenes* spores/mL in truffle cream. Total inactivation of *B. cereus* (starting concentration 4×10^5 spores/mL), *Bacillus licheniformis* (6×10^6 spores/mL) and *B. stearothermophilus* (4×10^5 spores/mL), in phosphate buffer, were successful using a 20 °C double-pulse treatment

(200 MPa/1 min followed by 900 MPa/1min), 800 MPa for 3 min at 60 °C, and 800 MPa for 3 min at 70 °C, respectively.

Ludwig and others (1992) found that the best conditions to germinate spores of *Bacillus* sp. were medium pressure, high temperature, and some additives such as salts, amino acids, and glucose. For spore suspensions of *B. stearothermophilus* exposed to 250 MPa and 60 °C, a biphasic survivor curve was evident. It featured a rapid decrease in viability that represented the inactivation of vegetative cells, followed by a "slow step" that represented the spores. Similar results were obtained using a strain of *B. subtilis*. Ludwig and others (1996) noted that pressure only kills the germinated forms of the spores. Data were presented showing kinetics of germination as measured by the release of dipicolinic acid (DPA). Release was greatest at an ionic strength of 0.14 M NaCl and pressures between 100 and 250 MPa. Full germination (100% DPA release) was strongly dependent on treatment temperature. Optimum germination at 40 °C was obtained within 30 min at 100 MPa. Additional work by Ludwig and others (1996) showed that *Clostridium sticklandii* ATCC 12662, a gram-positive sporeformer, was quite susceptible to HPP. Cultures of 10^9 cfu/mL were eliminated with a 10-min exposure to 300 MPa at 37 °C, while treatment at 25 °C and 300 MPa required 30 min for complete destruction. This strain of *C. sticklandii* also showed biphasic kinetics. There was a large, very sensitive population and a smaller, more resistant fraction in the ratio of 10^6:1 representing vegetative cells to spores. In addition, Ludwig and others (1996) examined the release of dipicolinic acid (DPA) and amino acids (as a measurement of germination) by *B. subtilis* and found that optimal release of these components occurred at 110 MPa and 50 to 60 °C. The authors recommended that for maximum inactivation of spores of *B. subtilis*, pressure cycles between low (0.1 to 60 MPa) and high pressures (500 MPa) and temperatures as high as possible would represent the best approach. They added that spore inactivation is best achieved by a complex interplay between temperature and pressure effects on germination and inactivation processes.

Raso and others (1998c; 1998d) presented data indicating that the temperature of sporulation affected the pressure resistance of spores of the food pathogen, *B. cereus*. They found that *B. cereus* sporulated at a lower temperature (for example, 20 °C) was more pressure-resistant than *B. cereus* sporulated at a higher temperature (for example, 37 °C) at any water activity (0.92 to 0.99) or pH (3.5 to 7.8) of pressure treatment. When germination was measured they noted that initiation of spore germination was also affected by sporulation at lower temperatures. The basis for heightened resistance of the spores was stated to be due to the mechanism of pressure inactivation. That is, it occurs in two stages; exposure to pressure first germinates the spores, then pressure inactivates the germinated forms. High concentrations of sucrose were found to protect the spores from pressure inactivation.

Work by Wuytack and others (1998) added further to the clarification of the mechanism of germination and induced pressure resistance in spores of *B. subtilis*. They found that germination can be initiated at low (100 MPa) and high (500 MPa) treatments of 30 min; however, germination is arrested by exposure to 500 MPa resulting in a significant portion of the spores becoming pressure-resistant, as well as more resistant to hydrogen peroxide and UV light. They suggested that the UV and hydrogen peroxide resistance after high pressure treatment was due to the presence of more small, acid-soluble proteins after high pressure treatments. Such findings indicate that exposure of bacterial spores to pressure can result in spores not only more resistant to the pressure process itself, but also more resistant to other accompanying food preservative methods, which can worsen conditions for effective elimination or reduction of spores.

This phenomenon of acquiring resistance by previous exposure to a certain condition is well known in the case of heat exposure. It appears that the high variability of heat resistance of spores of clostridia is caused by the immediate environmental history of the spores (Jay 1996). For example, spores of the food pathogen, *C. perfringens*, vary dramatically in their resistance to heat (Weiss and Strong 1967). It is assumed that the wide range of heat resistances is due to the diversity of environments from which *C. perfringens* has evolved, as well as the inducible nature of spore heat resistance triggered by compounds in the environment (Alderton and Snell 1969). As such, the heat resistance of the spore of *C. perfringens* is chemically reversible between the resistant and sensitive states. Heredia and others (1997) demonstrated that not only will spores of *C. perfringens* show increased heat resistance by a sublethal heat shock of 55 °C/30 min, but the vegetative cells will become more heat-resistant as well (at least two-to three-fold). Spores of *C. botulinum* held in calcium acetate solutions (0.1 to 0.5 M) for 140 h at 50 °C raise heat resistance five to ten times, while heat resistance can be lowered by holding the spores in 0.1 N HCl at 25 °C for 16 h (Alderton and others 1976). Such phenomena have been indicated by exposure of spores to the natural acid conditions of some foods. The extent of variability of clostridial spores to pressure is not well known as a function of food composition. This is important, not only because of the pathogenic nature of these two species of *Clostridium*, but because strains of *C. botulinum* can produce very pressure-resistant spores. As stated earlier, spore suspensions of *C. botulinum* 17B and Cap 9B have shown little, if any reduction in viability after exposure to 827 MPa at 75 °C (Larkin and Reddy,1999).

Hayakawa and others (1994a; 1994b) found that six cycles of oscillatory pressurization (5 min each) at 600 MPa and 70 °C were required to eliminate 10^6 spores/mL of *B. stearothermophilus* IFO 12550. Continuous treatments at pressures up to 800 MPa and 70 °C for 60 min showed that some spores survived. Attempts were made to reduce the treatment temperature for inactivation of the spore suspensions (10^6/mL), but the need for an elevated treatment temperature could not be eliminated. The only treatment that resulted in complete destruction of the spores was the oscillatory approach with a treatment temperature of 70 °C. Additionally, it was found that a synergistic effect of spore existed with a sucrose palmitic acid ester (<10 ppm) used in combination with 60 °C for 60 min against spores of *B. stearothermophilus* IFO 12550 (Hayakawa and others 1994b).

In the comparison of spore suspensions from six strains representing five different species of *Bacillus*, Nakayama and others (1996) found no correlation between pressure and heat resistances. Spores remained viable after a treatment of 981 MPa at 5 to 10 °C and neutral pH for 40 min. These findings indicated that pressure and elevated temperature will inactivate spores (Gould and Sale 1970; Clouston and Wills 1969). Work of Sale and others (1970) showed that exposure to elevated temperature germinated spores and made them susceptible to pressure inactivation. Not all germinated spores appeared to be inactivated by pressure. Combined heat and pressure sterilization of low-acid foods must be developed to ensure a reliable and safe process.

For green infusion tea, Kinugasa and others (1992) found that 700 MPa at 70 °C for 10 min resulted in a product that could be held at room temperature without spoilage. This was true even in tea inoculated with spores of *B. licheniformis*, *B. coagulans*, and *B. cereus* added at 10^6/mL. HPP was deemed superior to retort processing in that HPP had little or no effect on tea components, including catechins, vitamin C, and amino acids, as well as taste attributes.

As described above, it is evident that in addition to being extremely pressure-resistant, bacterial spores are also highly vari-

able regarding the level of pressure resistance. This variability depends on the conditions of their sporulation and pressure treatment. Given these characteristics, consistency of spore crop preparation and standardization of methods are quite important for inoculated pack studies using nonpathogenic sporeforming indicator organisms. PA 3679 is a logical choice, given this strain's long history in serving as an indicator organism for *C. botulinum* in the canning industry; however, *B. subtilis* may be a better choice because spore suspensions of *B. subtilis* are highly pressure resistant, and as a facultative anaerobe, easier to grow and handle. These considerations make spores of *B. subtilis* a good candidate as an HPP indicator or surrogate.

2.2.3. Yeasts and Molds

Yeasts are an important group of spoilage microorganisms, but none is an important food pathogen. Toxic mold growth is a safety concern in foods. Butz and others (1996) examined responses of the heat-resistant molds, *Byssochlamys nivea*, *Byssochlamys fulva*, *Eurotium* (*Aspergillus fischeri*), *Eupenicillium* sp. and *Paecilomyces* sp. to HPP (300 to 800 MPa) used in combination with different treatment temperatures (10 to 70 °C). All the vegetative forms were inactivated by exposure to 300 MPa/25 °C within a few minutes; however, ascospores required treatment at higher pressures. A treatment of 600 MPa at 60 °C eliminated all ascospores within 60 min except for the ascospores of *B. nivea* and *Eupenicillium*. *B. nivea* required 800 MPa and a processing temperature of 70 °C to destroy a starting inoculum of $<10^6$/mL within 10 min. A pressure of 600 MPa at 10 °C was adequate to eliminate 10^7 cfu/mL of *Eupenicillium* within 10 min. In the range of 4.0 to 7.0, pH was found to have little effect on pressure inactivation of *Byssochlamys* sp. On the other hand, low water activities (a_w = 0.89) increased pressure sensitivity of ascospores as did treatment in grape juice (as compared to saline solution).

Although HPP inactivation of molds has not been studied as thoroughly as HPP inactivation of bacteria, a non-producer of mycotoxins of the *Aspergillus* spp. would be a logical choice for a surrogate mold. Selection of an indicator with a characteristic pigmentation, such as *Aspergillus niger*, would assist in differentiating an indicator mold from background fungi. Processors may not desire release of a "visually vivid" mold in their processing facilities. A less obtrusive aspergilli may be preferred.

2.2.4. Viruses

As stated earlier, the first attempt to estimate the pressure sensitivity of viruses was by Giddings and others (1929) with tobacco mosaic virus (TMV). In that study, pressures of 135,000 psi (920 MPa) were necessary to demonstrate any kind of effectiveness in the inactivation of TMV; however, among viruses there is a high degree of structural diversity and this is reflected in a wide range of pressure resistances (Smelt 1998). Human viruses appear more pressure sensitive than TMV. Human immunodeficiency viruses are reduced by 10^4 to 10^5 viable particles from exposure to 400 to 600 MPa for 10 min (Otake and others 1997). Brauch and others (1990) showed that bacteriophages (DNA virus) were significantly inactivated by exposures to 300 to 400 MPa, while Butz and others (1992) found Sindbis virus (a lipid-coated virus) relatively unaffected by pressures of 300 to 700 MPa at −20 °C. Shigehisa and others (1996) found that an 8-log plaque-forming unit (PFU) population of herpes simplex virus type 1 was eliminated by a 10-min exposure to 400 MPa, and a 5-log PFU population of human cytomegalovirus was destroyed by a 10-min exposure to 300 MPa. Shigehisa and others (1996) also evaluated pressure effects on human immunodeficiency virus (HIV) type 1 and found that a 5.5 log tissue culture infectious dose of HIV type 1 was eliminated after a 10-min exposure to 400 MPa at 25 °C. Exposure to lower levels of pressure were essentially ineffective. Overall, these results suggest that most human viruses will be eliminated in pressure treatments designed for elimination of problematic bacteria (for example, 400 Mpa); however, this area requires further investigation before such conclusions can be drawn.

Selection of a bacteriophage as a nonpathogenic indicator virus seems to follow current logic. Among viruses, bacteriophage are relatively easy to handle and enumerate, and would carry no risk of infection to humans. The biology of l phage of *E. coli* is very well studied and readily available, making it a strong candidate.

2.2.5. Parasites

Information is lacking on the pressure resistances of oocysts and spores of *Cryptosporidium* and *Cyclospora*, and of protozoans *Entamoeba histolytica* and *Giardia lamblia*. It is reasonable to assume that the survival forms of these parasites will be significantly more sensitive to pressure than bacterial spores and cells; however, these determinations remain to be completed.

The parasitic worms of *Trichinella spiralis* are killed by a 10-min exposure to 200 MPa (Ohnishi and others 1993). These results were obtained by observing the motility of larvae recovered from muscle tissue following pressure treatment. Even though further studies are necessary to evaluate the pressure resistance of *Cryptosporidium* and *Cyclospora*, it is relatively safe to assume that parasites are not as pressure-resistant as bacteria. It would seem reasonable to use a nonpathogenic bacterium as an HPP indicator organism to judge survival of foodborne parasites. Parasites are generally quite difficult to obtain and maintain in high quantities for process development work involving inoculated pack studies.

2.3.Inactivation of microorganisms in foods

Horie and others (1991) presented work on the development of pressure-processed jams from the Meidi-ya Food Factory Co. in Japan, whose jams and preservatives, marketed in 1991, were the first commercial foods that incorporated HPP for preservative purposes. Elimination of yeasts was reported (*Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*) as well as bacteria (*Staphylococcus* spp., *Salmonella* spp. and a coliform). Jams were processed at 294 MPa for 20 min with a starting inoculum between 10^5 and 10^6 cfu/mL. Refrigeration of the jam, after processing, was necessary due to browning and flavor changes caused by enzymatic activities and chemical reactions involving oxygen. Taste panels were reported to prefer the pressure-processed varieties to the jams prepared in the conventional manner. Nutritionally, the pressure-processed strawberry jam retained 95% of its vitamin C compared to the fresh product.

Parish (1998) studied HPP applied to non-pasteurized Hamlin orange juice (pH 3.7). The target organism was *S. cerevisiae*. He calculated D-values of 4 to 76 s for ascospores treated at pressures between 500 and 350 MPa, respectively. For vegetative cells of *S. cerevisiae*, D-values were between 1 to 38 s. The native flora of the orange juice showed D-values ranging from 3 to 74 s. Surviving organisms in the orange juice after one to 300 s of HPP treatment were found to be yeasts, and gram-positive and gram-negative rods.

Shelf-life extension of fresh-cut pineapple was achieved by application of 340 MPa/15 min by Aleman and others (1994). D-values, as determined on plate count agar (PCA), were 3.0 min for processing at 4 °C, 3.1 min when treated at 21 °C, and >2.5 min at 38 °C. The post-treatment counts on PCA from pressure-treated pineapple were <50 cfu/g.

HPP inactivation of yeasts and vegetative bacteria in fruits is very effective because of their inherent low pH. In these products, the limiting parameter is usually the presence of browning enzymes. Blanching of the product or use of ascorbic acid in con-

junction with vacuum treatment to remove oxygen may help reduce enzymatic browning.

HPP application on non-heat pasteurized rice wine (Namazake) was examined by Hara and others (1990). No viable lactobacilli and yeasts could be recovered using a treatment at 294 MPa/10 min/25 °C. Processing at 392 MPa resulted in a shelf-stable product with a taste profile equivalent to the control due to the inactivation of problematic enzymes and microorganisms.

Lettuce and tomatoes were inoculated by Arroyo and others (1997) and pressurized at 20 °C for 10 min and 10 °C for 20 min. Microorganisms were not significantly affected at 100 and 200 MPa and gram-positive bacteria were not completely inactivated at 400 MPa (the highest pressure examined). Pressures of 300 and 350 MPa reduced populations of gram-negative bacteria, yeasts and molds by at least one log-cycle; however, in this range of pressures, skin loosened and peeled away in tomatoes, and lettuce browned. The authors noted that hurdle technology would be necessary to maintain the desired sensory quality of these vegetables while using HPP as a preservation treatment to lower populations of undesired microorganisms.

HPP was adapted for preservation of spreadable smoked salmon cream (pH 5.95; a_W 0.95) by Carpi and others (1995). Pressure-treated products were superior to heat-treated creams with regard to sensory quality. A 3-min exposure to 700 MPa extended shelf-life at both 3 and 8 °C from 60 to 180 d without changes in the sensory characteristics as compared to the product before treatment. In inoculated trials at 700 MPa/3 min (starting inocula 10^3 to 10^4 cfu/g), *L. monocytogenes*, *S. aureus*, *Salmonella* Typhimurium and lactic acid bacteria were completely inactivated while spores of sulfite-reducing clostridia were not affected and enterococci were only partially inactivated. Immediately after treatment the aerobic plate count was 3.0×10^3 cfu/g, but after 6 mo of refrigerated storage the level was $<10^2$ cfu/g. Aerobic plate counts were mostly comprised of spores of *Bacillus*.

Carlez and others (1994) worked with freshly minced meat that was pressure-processed for 20 min at 20 °C at 200 to 450 MPa and stored at 3 °C in air and under vacuum for up to 22 d. They found treatment at 200 and 300 MPa was somewhat effective in that microbial growth was delayed 2 to 6 d. As one would expect, treatments at 400 and 450 MPa were more effective, reducing total counts of the meat by 3- to 5-log cycles. At the higher levels of pressure treatment, pseudomonads were the most problematic organisms in the meat. Data suggested that approximately 0.01% of the pseudomonads survived exposures to these pressures with subsequent growth at 3 °C after a recovery period of 3 to 9 d. Lactobacilli also responded in a similar manner to such treatment. At the higher levels of pressure, changes to the color and texture of the minced meat were evident.

A "foie gras de canard" (fatty duck liver) was produced with incorporation of an HPP preservative treatment by El Moueffak and others (1995). Microbial analysis did not include an inoculated sample study, but instead foie gras stored 13-d post-slaughter was used to elevate the native microbial populations and allow estimation of the extent of inactivation. Compared to classical thermal pasteurization of this product, 400 MPa at 50 °C for 10 min stabilized the product as shown by reduction of the psychrotrophic microbiota, coliforms, and *S. aureus* below detectable levels with significant reduction of total mesophilic counts to approximately 10^2 cfu/g. Treatment at 300 MPa was found to be ineffective for foie gras.

Fujii and others (1994) monitored changes in sensory quality and bacterial levels in minced mackerel pressure-treated at 203 MPa for 60 min and stored at 5 °C. Growth of bacteria was delayed for approximately 4 d with populations of species of *Bacillus, Moraxella, Pseudomonas* and *Flavobacterium* no longer evident after pressurization. Coryneforms, *Staphylococcus* and *Micrococcus* dominated the flora during refrigerated storage. It was noted that fat rancidity was enhanced in the pressurized mackerel, becoming a leading factor in deterioration of the product.

In surimi, Miyao and others (1993) found that levels between 300 and 400 MPa were adequate to kill most of the fungi, gram-negative bacteria and gram-positive bacteria (in declining order). Notable pressure-resistant varieties were found and identified as *Moraxella* spp. (viable at 200 MPa), *Acinetobacter* spp. (viable at 300 MPa), *Streptococcus faecalis* (viable at 400 MPa), and *Corynebacterium* spp. (viable at 600 MPa). These pressure-treated isolates displayed significant lag time upon transfer to nutrient medium for batch culture. For example, following exposure to 400 MPa, growth of *S. faecalis* was delayed approximately 20 h more as compared to the control. The extracellular release of iron and magnesium ions, RNA and carbohydrates was detected after pressurization, suggesting that damage to the membrane occurred and that RNA degradation took place.

2.4.HPP in combination with other processing technologies

In the case of HPP, a hurdle approach (Leistner and Gorris 1995) is almost axiomatic for significant widespread use in commercial food processing. The inherent high resistances of bacterial endospores and food enzymes are the major challenges to the broad application of HPP.

A preservative method employing HPP (albeit at significantly reduced pressures) is the processing of food under pressure and carbon dioxide (Haas and others 1989). This method is often referred to as high pressure carbon dioxide processing, even though the pressure levels are normally <15 MPa. For example, Hong and others (1997) evaluated a CO_2-pressure process for the inactivation of lactobacilli in kimchi (fermented Korean vegetables, pH-4.2). The optimal process parameters that decreased populations of lactobacilli by 5-log cycles were a 200-min treatment at 30 °C under a CO_2 pressure of 6.9 MPa. Ballestra and others (1996) examined pressures of 1.2, 2.5 and 5 MPa at 25, 35 and 45 °C for the inactivation of *E. coli*. The higher treatment temperatures permitted a shortening of processing time to approximately 20 min for elimination of a cell suspension between 10^9 and 10^{10} cfu/mL in Ringer's solution when pressure was 1.2 MPa. At higher pressures, temperature had no effect on efficiency. Although the pressures are modest by HPP standards, the effectiveness is high due to the antimicrobial effect of carbon dioxide. The suggested lethal mechanism is a lowered intracellular pH caused by penetration of elevated levels of carbon dioxide into the cell, not by physical rupture of the cell walls or membrane due to the pressure of CO_2. Results were not as conclusive in studies by Wei and others (1991). These researchers used 13.7 MPa for 2 h at 35 °C to kill inoculated *Salmonalla* Typhimurium in chicken and egg yolk, and inoculated *L. monocytogenes* in shrimp, orange juice, and egg yolk. Levels of microbial reduction varied considerably depending on the nature of the food and treatment conditions. Bacterial reductions ranged from limited effect to 9-log cycles. Results were poor for whole egg formulations. Enomoto and others (1997) reduced spores of *Bacillus megaterium* by 10^7 cfu/mL 30-h exposures to 5.9 MPa and 60 °C, above this pressure spore inactivation was lessened. An obvious commercial limitation for pressurized carbon dioxide is the lengthy processing times necessary to allow for diffusion of carbon dioxide into microbial cells. Carlez and others (1992) investigated the effect of supercritical carbon dioxide on the inactivation rate of *Citrobacter freundii* at pressures of 230 MPa at 35 °C. This treatment did not affect the rate of inactivation. The pH of the meat did not drop below 5.7 and the concentration of carbon dioxide in the meat was calculated to be 6.5 g/kg. *Citrobacter freundii* was recommended as a surrogate for *Salmonella* spp.

Combination treatments of HPP and irradiation have been investigated by several laboratories. Paul and others (1997) targeted staphylococci in lamb meat. A population of approximately 10^4 staphylococci/g was reduced by only 1-log cycle by either treatment with gamma irradiation (1.0 kGy) or HPP (200 MPa for 30 min). When used in combination, no staphylococci were found immediately after completion of the tandem process. After 3 wk of storage at 0 to 3 °C, mannitol-negative staphylococci (presumably coagulase-negative as well) were detectable ($<10^3$ cfu/mL). Crawford and others (1996) were able to eliminate *C. sporogenes* in chicken breast using combinations of HPP and irradiation.

Raso and coworkers have combined heat, pressure and ultrasound. The pressures used in such combinations are significantly lower than the magnitudes traditionally used in HPP (for example, instead of MPa, kPa levels are used). Raso and others (1998a) found heat and ultrasound to act independently under pressure. To a large extent it appeared that the individual contributions of heat and ultrasound under pressure depended upon the temperature. Above 58 °C, any added inactivation caused by pressure disappeared. These results suggested that inactivation was not a simple additive reaction of the three treatment types. D-values recorded for *Y. enterocolitica* ATCC 9610 were 1.39 min at 59 °C, 1.5 min for the highest ultrasound setting (150 db at 20 kHz), and 0.28 min for a treatment of 300 kPa and 150 db (ultrasound) at 30 °C. In this study, *Y. enterocolitica* was suspended in citrate-phosphate buffer (pH 7.0) and the treatment chamber volume was 23 mL.

Raso and others (1998b) found that a 12-min treatment of 500 kPa and 117 db at 20 kHz killed approximately 99% of a spore suspension of *B. subtilis* ATCC 9372 in McIlvaine citrate-phosphate buffer (pH 7.0). The sporicidal effect depended upon the static pressure, amplitude of ultrasonic waves, and the treatment temperature. Above 500 kPa, additional increments of pressure did not increase the amount of spore inactivation. In the range of 70 to 90 °C, a combination with 20 kHz, 300 kPa, 117 db for 6 min had a synergistic effect on spore inactivation.

Many different antimicrobial compounds have been used in combination with HPP in a hurdle approach. Examples include HPP and lytic enzymes (lysozyme; Popper and Knorr 1990), HPP and antimicrobial chitosans (Papineau and others 1991), and HPP and bacteriocins. Use of nisin with pressure has been addressed by several laboratories. Roberts and Hoover (1996) examined the concurrent use of nisin with pressure treatment on *B. coagulans* 7050. While pressure alone (up to 400 MPa) had no effect in reducing the number of viable spores when treated at neutral pH and ambient temperature, the use of a 400 MPa/70 °C/30 min pressure treatment at pH 4.0 and 0.8 IU/mL nisin resulted in the sterilization of spore crops containing 2.5×10^6 cfu/mL.

Kalchayanand and others (1998) examined the effectiveness of the pediocin AcH in combination with HPP. The goal of this work was to identify those HPP/AcH treatments capable of inactivating within 5 min 10^7 to 10^8 cfu/mL of *S. aureus, L. monocytogenes, S.* Typhimurium, *E. coli* O157:H7, *Lactobacillus sake, Leuconostoc mesenteroides, Serratia liquefaciens* and *Pseudomonas fluorescens* in 0.1%-peptone water. This could not be accomplished using HPP treatments of 345 MPa/50 °C/5 min, unless 3,000 AU/mL of pediocin AcH were included in the peptone water. Of the gram-negative bacteria, *E. coli* O157:H7 strain 932 was the most pressure resistant, while for the gram-positive bacteria in the study, *L. sake* FM1 and *L. mesenteroides* Ly were the most barotolerant. In earlier work, Kalchayandand and others (1994) had evaluated the hurdle combination of electroporation with HPP and bacteriocins against various gram-negative and gram-positive bacteria.

The monoterpenes were investigated by Adegoke and others (1997) in combination with HPP versus *S. cerevisiae*. Alone, *S. cerevisiae* IFO 10149 was found to be resistant to exposure to 300 and 600 mg/L of α-terpinene, but sensitive to a concentration of 1,250 mg/L. When 150 mg/L of a-terpinene was combined with exposure to 177 MPa for 1 h at 25 °C a reduction of 6-log cycles was found. A 3-log cycle reduction was found with similar pressure parameters but replacement of the α-terpinene with 200-mg/L (+)-limonene.

Ishiguro and others (1993) examined the inactivation of *B. coagulans* in tomato juice with addition of the antimicrobial compounds polylysine, protamine, and an extract of etiolated seedlings of adlay. Polylysine and protamine were ineffective processing aids; in fact, these compounds conferred protection to *B. coagulans* in the tomato juice treated at 400 MPa. The adlay extract did demonstrate enhanced destruction of *B. coagulans*, improving inactivation by approximately 1 log cfu/mL after 100 min. The treatment temperature was not specified; regardless, treatment times of 100 min are not commercially practical.

2.5.HPP pulsed application

As described earlier in the report, use of pressure-pulsing or oscillatory pressure treatments has been shown to be generally more effective than equivalent single pulses or continuous pressurization of equal times (Hayakawa and others 1994a; 1994b). This enhanced inactivation has been found not only with spores, but also with yeasts and vegetative bacteria. The difference in effectiveness varies, and the measure of improved inactivation by pulsed pressurization must be weighed against the design capabilities of the pressure unit, added wear on the pressure unit, possible detrimental effect to the sensory quality of the product, and possible additional time required for cycling.

Aleman and others (1994; 1996) conducted studies on comparison of static versus pulsed pressure applications in the inactivation of *S. cerevisiae* in pineapple juice. They found that pulsed pressure treatments were more effective than static applications over comparable lengths of time. For example, it was shown that a total exposure time of 100 s with repetitive pulses of 0.66 s of on-pressure and 0.22 s off-pressure inactivated 4 log cfu/mL of *S. cerevisiae*. A comparable reduction using one static pulse at the same pressure required 5 to 15 min; however, they did discover that the pressure-pulse profile was critical for the inactivation of this yeast. Some ratios of pulsing negated any inactivation and fast sine wave forms allowed total survival of the yeast population.

Palou and others (1998) compared oscillatory application of HPP to single, continuous pressure treatments using *Z. bailii*. These workers found that cyclic applications improved inactivation of the yeast in sucrose-modified (a_w 0.98) Sabouraud glucose broth (pH 3.5). To detect a significant difference from a single pulse, however, at least two 5 min cycles were needed. Three cycles of 5-min each were necessary to generate a 1-log cfu/mL difference in plate counts at the 276-MPa level as compared to a continuous application of 15 min. Come-up time was approximately 2.7 min at 276 MPa and decompression was normally <15 s It was assumed that the greater rate of inactivation of the yeast due to oscillatory HPP was due to greater injury to the cellular membrane from rapid changes in intracellular/extracellular differences at the membrane interface.

Besides pressure-pulsing, another modification to pressurization mechanics is the use of very rapid pressure release (measured in milliseconds). Rapid decompression can be attained in pressure units designed with a "knuckle" (a quick-release joint in the connecting rod linked to the piston applying pressure to the chamber) that permits a very rapid but controlled release of high pressure. It is believed that rapid decompression invokes cavitations in the cells and spores that result in physical disruption and death. This approach is still quite novel and further information is presently quite limited.

2.6. Shape of inactivation curve

The shape of an inactivation curve resulting from the pressure treatment of a pure culture of microbes inoculated into a buffer may show a shoulder or an initial lag period, followed by first-order inactivation kinetics for the intermediate treatment period, and finally tailing as the surviving number of microbes approach <1000 cfu/g. In some cases plate counts of survivors will increase or decrease if measured after a significant time lag between HPP treatment and dilution and plating. Inactivation curves for natural flora in a food or for challenge microbes inoculated into a food can demonstrate a shoulder, a possible first-order inactivation period, and then tailing. Some food products may demonstrate extreme tailing due to spores normally present in the food; however, inactivation curves showing predominantly first-order kinetics are presented by Zook and others (1999), and Sonoike and others (1992), who have developed complete pressure-temperature D-value response surfaces for *Lactobacillus casei* and *E. coli* strains based on first-order inactivation kinetics.

A biphasic pressure inactivation is frequently encountered for both vegetative bacteria and endospores. At the attainment of pressure, an immediate consistent rate of inactivation is realized that within a few minutes of pressurization changes to a more reduced rate of inactivation. Such an inactivation curve indicates the residence of a small pressure-resistant sub-population. In such instances, two rates (or two D-values) can be calculated, often regardless of the type of microorganism evaluated. On occasion the reduced rate curve can flatten or level off, suggesting that additional time at pressure has no effect on further reducing the remaining microbial population. Microbiologists are increasingly capable of detecting vegetative pathogens damaged by non-thermal treatments at levels approaching one cfu/g. Furthermore, the capability of some vegetative pathogens to infect humans at concentrations below one cfu/g may require a zero tolerance for these microbes in foods. Tailing phenomena should be investigated carefully in challenge studies. The use of pathogens rather than surrogates for highly infective pathogens may be advised.

2.7. Summary of responses of microbes to HPP and commercial implications

Food processors who wish to use HPP to preserve foods would benefit from a specified limited number of pressure-time combinations. These combinations would be proven to inactivate 10^6 per gram of key food pathogens such as *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., *or Staphylococcus* spp. in acid foods held at room temperature and in low-acid refrigerated foods. At this time there are only a limited number of HPP-preserved products in the market place and thus only a limited amount of industrial experience is available upon which to base commercial processes. Specified pressure-time combinations would help equipment companies develop accurate process cost estimates for the HPP treatment of foods. Process costs are related to operating pressure, hold time at pressure, and operating costs for maintenance, power and labor. The capital cost of high pressure equipment increases exponentially with increasing operating pressure. Process costs are a direct function of process hold time and operating costs. Thus, a minimum HPP treatment cost per kilo of food based on a given yearly production rate will depend on the operating pressure and hold time specified for the process.

Current practical operating pressures for commercial HPP food treatment intensifiers and pressure vessels are in the range 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 one, 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 between the pH values of 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 (IT) in the range of 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 pasteurized by HPP that is rendered free of pathogens normally associated with the product; however, satisfactory guidelines for hold times at 580 MPa for low-acid food pasteurization have not emerged. For example, the post package-HPP 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 and the growing capability of detecting a single viable pathogen cell in a batch of treated product. Changes in product color and appearance may limit the usefulness of HPP treatment pressures above 200 to 300 MPa.

3. Mechanism of Inactivation

3.1. Culture maintenance history

Cells at exponential phase of growth are generally more susceptible to injury and inactivation than cells at stationary phase of growth. Cultures that are old tend to be more resistant to inactivation by most food processing methods; however, the physiological state of bacteria does not appear to be a significant factor if the HPP treatment has been developed to eliminate all microorganisms of safety concern. The physiological age of bacterial cells would seem to play a more important role in those instances where HPP is used as a pasteurization treatment to reduce the number of organisms of concern in a food to a level of acceptable risk. For example, the work of Berlin and others (1999) showed that cultures of *V. vulnificus* and *V. parahaemolyticus* that entered the dormant state of viable but non-culturable, were just slightly more pressure-resistant than control cultures. This enhanced resistance was so small it was deemed not to be a factor contributing an HPP process deviation.

Pagan and Mackey (1999) examined pressure-resistant (C9490) and pressure-sensitive (8003) strains of *E. coli* to determine the effect of growth phase on inactivation rate. Both strains were prepared for treatment using cells taken from exponential and stationary growth phases. Cells were exposed to pressures between 100 to 500 MPa at room temperature for 8 min. Viability was determined by plate counts. Membrane damage was determined by uptake of the fluorescent dye propidum iodide (PI) and loss of ability to plasmolyze in the presence of 0.75 NaCl. The stationary phase cells of the pressure-resistant strain, C9490, showed no loss of viability below 500 MPa while cells of 8003 showed some inactivation at 100 MPa. C9490 retained its ability to plasmolyze in a strong salt solution while 8003 lost some ability to plasmolyze. When PI was present during pressure treatment, both strains took up the dye at pressures above 100 MPa. The degree of staining was greater in the pressure-sensitive strain, 8003. PI added after compression was not taken up by either strain. Exponential growth phase cells of both strains showed no difference in pressure resistance. Loss of viability began for both strains with an 8 min pressure treatment at 100 MPa. At 200 MPa, viable numbers had been reduced by 6- to 7-

log cycles. There was post-compression uptake of PI and loss of ability to plasmolyze.

These results showed that exponential phase growth cells are pressure-sensitive and cannot reseal pressure-damaged membranes. Stationary phase cells appear to be able to reseal membranes damaged during pressure treatment after decompression. The degree of pressure resistance appears to be related to the cells' ability to repair leaks after decompression. Storage temperature after pressure treatment can affect the rate of leaky cell membrane repair. Refrigerated temperatures can prolong the time required to repair leaky membranes.

As noted earlier, Raso and others (1998d) presented data on the effect of sporulation conditions on pressure resistance for *B. cereus*. They found that *B. cereus* sporulated at a lower temperature (for example, 20 °C) was more pressure resistant than *B. cereus* sporulated at a higher temperature (for example, 37 °C).

3.2. Microbial enumeration conditions and methods

It is believed that nearly all microorganisms will respond in a general manner to conditions of stress and lethality in their environment. There has been no documentation to suggest that the response of a microorganism to hydrostatic pressure is a unique biological event that requires any revised technology for enumeration of viable cells. Most of the studies described earlier employ general plating media for enumeration of microorganisms. In some cases, specific types of media are used which are designed for the microorganism(s) of study. Since actual growth (viability) is of foremost concern in the assessment of HPP effectiveness, traditional plating methods have been used exclusively.

It has long been recognized that pressure will sublethally stress bacteria (Metrick and others 1989). The response is similar to that of heat injury. Therefore, when the extent of microbial inactivation due to HPP is being assessed in pure culture or food, microbiological media that allow for detection of all viable organisms of concern, both injured and non-injured, should be selected. Nutrient-rich environments are normally required for cellular repair. Under otherwise optimal conditions, psychrotrophic organisms have the potential to recover at refrigeration temperatures, while mesophiles require temperatures closer to body temperature to recover from pressure-induced injury and to resume replication.

3.3. HPP mechanisms of inactivation

The various effects of high hydrostatic pressure can be grouped into cell envelope-related effects, pressure-induced cellular changes, biochemical aspects, and effects on genetic mechanisms. It has been established that cellular morphology is altered by pressure, and that cell division slows with the application of increasing pressures. Hydrostatic pressures of 100 to 300 MPa can induce spore germination and resultant vegetative cells are more sensitive to environmental conditions (Gould and Sale 1970).

As a general rule (LeChatelier's Principle), pressure enhances reactions that lead to volume decrease, and reactions involving increases in volume are generally inhibited or reduced by pressure application (Johnson and Campbell 1945). The response of proteins to pressure varies largely because hydrophobic interactions act in a peculiar manner under pressure. Up to pressures of 100 MPa, hydrophobic interactions tend to result in a volume increase, but beyond this pressure range a volume decrease is associated with hydrophobic interactions and the pressure tends to stabilize these reactions (Suzuki and Taniguchi 1972). Consequently the extent of hydrophobicity of a protein will determine, to a large degree, the extent of protein denaturation at any given pressure (Jaenicke 1981). Among proteins, important sites for pressure inactivation of microbial cells are enzymes, especially membrane-bound ATPases (Mackey and others 1995; Marquis and Bender 1987). Enzymes vary in their sensitivities to denaturation. It is assumed that in some organisms denaturation of key enzymes by pressure plays an important role in pressure-induced death and injury. Factors that induce enzyme inactivation are the alteration of intramolecular structures and conformational changes at the active site (Suzuki and Suzuki 1962). Enzyme inactivation under pressure is also affected by pH, substrate concentration, and subunit structure of the enzyme (Laidler 1951).

Pressurized membranes normally show altered permeabilities. A reduction in volume occurs along with a reduction in the cross-sectional area per phospholipid molecule. It is generally felt that for microorganisms the primary site of pressure damage is the cell membrane (Paul and Morita 1971). Pressure-induced membrane malfunctions cause inhibition of amino acid uptake probably due to membrane protein denaturation. Numerous studies have shown loss of intracellular constituents from microorganisms after pressure treatment. Leakages of these components from the cells indicate damage to the cellular membrane, and the higher the amount lost from cells correlates with a greater degree of death and injury.

Bacteria with a relatively high content of diphosphatidylglycerol (shown to cause rigidity in membranes in the presence of calcium) are more susceptible to inactivation by HPP (Smelt and others 1994). Conversely, those compounds that enhance membrane fluidity tend to impart resistance of the organism to pressure (Russell and others 1995). Yano and others (1998) isolated two taxonomically unidentifiable bacteria, strains 16C1 (facultatively barophilic) and 2D2 (obligately barophilic) from the intestinal contents of deep-sea fish retrieved from depths of 3,100 and 6,100 m, respectively. In these bacteria there was a general trend from saturated to unsaturated fatty acids (especially docosahexaenoicacid, DHA, 22:6n-3) in the membrane with exposure to increasing magnitudes of pressure with growth. Their results suggested that DHA is an important factor in maintaining membrane fluidity under pressure. Furthermore, this same compositional change in the membrane was evident in strain 16C1 with growth at low temperatures.

At pressures greater than 500 MPa, it is not uncommon to view physical disruption to the surface of intact cells using scanning electron microscopy. At levels <500 MPa, it is possible to observe internal cellular damage using transmission electron microscopy. Perrier-Cornet and others (1995) measured cell volume during high pressure application with an image analysis system connected to a light microscope. For *Saccharomyces* spp., 250 MPa generated an observed compression rate of 25% with partial irreversibility of cell compression (10%) upon return to atmospheric pressure. The occurrence of mass transfer implied cell disruption or increase in membrane permeability.

Iwashasi and others (1993) suggested that the damage caused by HPP was essentially equivalent to the damage caused by high temperature and oxidative stress in yeast. The cellular membrane was noted as the primary lesion site. Their conclusion was based on observations of the tolerance of strains of *S. cerevisiae* (including HPP-resistant strains) to different applications of heat shock and recovery and to different growth phases. Comparable effects were found with HPP, heat treatment, and exposure to oxidative stress. It was suggested that plasma-membrane ATPase may be the key component in tolerance of many environmental stresses in *Saccharomyces* spp.

3.4. Mathematical models for microbial inactivation by heat and pressure

When foods are subjected to high pressure the compression is instantly transmitted through the hydrostatic media to the microbes in the food. Compression appears to affect microbial inactivation by altering the proteins responsible for replication, in-

tegrity, and metabolism. High pressure will not break covalent bonds, but will alter hydrogen and ionic bonds responsible for holding proteins in their biologically active form. Thus, observed microbial inactivation kinetics can be postulated to be the result of the irreversible denaturation of one or more critical proteins in the microbe. Since the ease or difficulty of irreversible protein denaturation is a function of protein structure, a wide range of pressure resistances must be expected among vegetative microbes. Smelt (1998) showed a six-fold range in D-values among 100 strains of *L. monocytogenes*.

Also cell repair can take place after pressure or mild heat treatment. This indicates that a critical protein was denatured, but repair proteins possibly were not damaged so that the critical protein could be repaired. Repair can be affected by food composition. Acids in foods may inhibit repair of damaged cell proteins and thus appear to make a microbe more sensitive to pressure or heat.

3.4.1. Absolute reaction rate theory

Research on the effects of pressure on proteins shows a close parallel between heat and pressure activation and reversible and irreversible inactivation of proteins. Kinetic models for activation and irreversible inactivation of proteins by heat and pressure have been proposed by Johnson and Eyring (1974). The theory of absolute reaction rates is based on the formation of an unstable intermediate complex which decomposes at a rate which is fixed by the temperature of the system. Thus the rate of the reaction, whether it is an enzyme-catalyzed reaction or an irreversible protein denaturation reaction, will be controlled by the rate of formation of the activated complex. This rate (at 0.15 MPa) is a function of the "Gibbs free energy change in going from the normal to the activated state" (Johnson and Eyring 1974).

The effect of a temperature change on the rate of a biological reaction is given by the Arrhenius equation:

$$k = Ae^{-E/RT} \quad (1)$$

where A, a constant, and k, the reaction velocity, are experimentally determined. This equation (Eq. 2) can be written to determine E, the activation energy, if the rates of the reaction, k_{T1} and k_{T0}, are known at two temperatures, T_1 and T_0. Pressure is constant.

$$E = R \times 2.3 [\log k_{T1} - \log k_{T0}] / [(1/T_0) - (1/T_1)] \quad (2)$$

where R is 2 (calories/ °C-mole), 2.3 converts from natural to common logarithms, and temperature is in degrees Kelvin. A similar equation (Eq. 3) can be written for the effect of pressure on a reaction at constant temperature (that is, 0 °C). In this case the volume change of activation ΔV^* is the change in the volume between the activated complex and the reactants. For proteins this would be the change in volume between the activated protein and its irreversibly denatured protein form.

$$\Delta V^* = 2.3RT [\log k_{p1} - \log k_{p0}] / [p_1 - p_0] \quad (3)$$

where the pressure is in atmospheres, R = 82 (cm^3 /mole), and the temperature is in degrees Kelvin.

The process of pressure treating a food always results in a temperature increase due to the work of compression. By contrast, the warming of a food by heat transfer (at 0.15 MPa) does not result in a pressure increase in the food. For this reason care must be taken in keeping a food sample at constant temperature during pressure treatment or determining the temperature of the food during compression and decompression. Most food researchers working on pressure treatment of foods do not control the temperature during pressure treatment. Temperature control would be necessary to obtain meaningful microbial or enzymatic inactivation kinetics.

It is recommended that high pressure microbial and enzyme inactivation kinetic data obtained by the pressure treatment of foods be obtained at temperatures in the range of 0 °C. This temperature may be considered as a base temperature. Biologically active proteins of interest in food preservation and processing show minimal activity. Zook and others (1999) illustrate this approach in determining the pressure inactivation of yeast ascospores in orange and apple juice. Lüdemann (1992) shows curves for the true density of water as a function of temperature and pressure.

3.4.2. Importance of temperature

Proteins show a critical temperature T_C at which heat denaturation begins at 0.15 MPa. The rate of irreversible protein denaturation appears to increase according to Eq. (2) as the temperature is increased above the critical temperature. Proteins also show a critical pressure P_C at which irreversible protein denaturation starts at a temperature of 0 °C. The rate of protein denaturation with increased pressure above P_C should be described by Eq. (3). Thus there is a need for experimental data comparing the inactivation of microbes or the denaturation of enzymes by pressure at 0 °C so that the results can be compared with heat treatments carried out at 0.15 MPa. It is suspected that the change in the activation volume ΔV^* of a critical protein in a microbe, or of an enzyme undergoing irreversible pressure denaturation, is very sensitive to small changes in temperature above 0 °C. This may explain why microbial inactivation kinetic data, taken at room temperature, with no temperature control during compression, is so difficult to interpret with the mathematical models used in heat inactivation kinetics. Protein denaturation by pressure appears to be a far more subtle process than heat denaturation. Much more research appears to be needed before the effects of pressure on irreversible protein denaturation can be predicted at temperatures above 0 °C (Smelt and Hellemons 1998).

The best that can be done at this time is to define a process using the parameters of initial temperature, compression time, product temperature, process pressure, and process hold time at pressure, and reproduce these conditions for every batch of food treated. This requires careful monitoring of food composition including pH and water activity. These critical process factors are discussed in the next section.

4. Validation/ Critical Process Factors

4.1. Critical process factors

4.1.1. Type of microorganism

Gram-positive bacteria are usually more pressure resistant than gram-negative bacteria (although there are notable exceptions). The more developed (evolutionarily) the life form, the more sensitive it is to pressure.

4.1.2. Culturing or growth conditions and age of the microorganisms

In general, cells in the exponential growth phase are more pressure-sensitive than cells in the stationary phase. Incomplete inactivation of microorganisms by pressure will result in injured cells capable of recovery under optimal growth conditions.

4.1.3. Composition, pH, and water activity of the food

Pressure inactivation rates will be enhanced by exposure to acidic pH. Low water activities appear to prevent inactivation. Compression of foods may shift the pH of the food as a function of imposed pressure. Heremans (1995) indicates a lowering of pH in apple juice by 0.5 units per 100 MPa increase in pressure.

The direction of pH shift and its magnitude must be determined for each food treatment process. As pH is lowered most microbes become more susceptible to HPP inactivation, and recovery of sublethally injured cells is reduced. Ionic bonds, such as those responsible for the folding of proteins, are influenced by pH and also can be disrupted by pressure. Instrumentation for routine measurement of pH between 100 and 800 MPa must be developed.

The magnitude and direction of the shift, if any, of water activity as a function of pressure has not been reported. Oxen and Knorr (1993) showed that a reduction of water activity from 0.98 – 1.0 to 0.94 – 0.96 resulted in a marked reduction in inactivation rates for microbes suspended in a food. Reducing the water activity appears to protect microbes against inactivation by HPP. On the other hand, it is to be expected that microbial cells may be sublethally injured by pressure, and recovery of sublethally injured cells can be inhibited by low water activity. Consequently, the net effect of water activity may be difficult to predict. Foods are more pressure-protective for microorganisms than buffers or microbiological media.

4.4.4. Temperature, pressure magnitude, rate of compression, and holding time at pressure

Increasing the pressure magnitude, time, or temperature of the pressure process will increase the number of microorganisms inactivated (with bacterial endospores the exception). An increase in food temperature above room temperature and to a lesser extent a decrease below room temperature has been found to increase the inactivation rate of microorganisms during HPP treatment. Temperatures in the range of 45 to 50 °C appear to increase the rate of inactivation of food pathogens and spoilage microbes and thus merit the development of processes which incorporate a uniform initial food temperature in this range. Process temperatures in the range of 90 – 110 °C in conjunction with pressures of 500 – 700 MPa have been used to inactivate spore-forming bacteria such as *C. botulinum*. The use of elevated temperatures as part of a specified HPP process will require monitoring the food temperature during the process to insure every element of the food is at or above the specifieD-value. The effect of temperature in the rate of inactivation of microbes and enzymes subjected to pressure treatment is discussed more fully in the section on mechanisms of inactivation.

There is a minimum critical pressure below which microbial inactivation by pressure will not take place regardless of process time. Important items of information not to be overlooked in HPP are the come-up times (period necessary to reach treatment pressure), pressure-release times, and changes in temperature due to compression. Obviously, long come-up times will add appreciably to the total process time and affect the product throughout, but these periods will also affect inactivation kinetics of microorganisms; therefore, consistency and awareness of these times are important in the process development of HPP. Temperature increases due to compression can be 3°C or more per 100 MPa. Zook and others (1999) were able to get first-order inactivation curves with no induction period or tailing with *S. cerevisiae* ascospores in orange, apple, and a model juice system, using pressures ranging from 300 to 500 MPa. They took care to eliminate any significant temperature increase in their samples due to compression by cooling their samples and the equipment used to compress them.

4.1.5. Secondary factors

Other factors influence the effectiveness of HPP. For example, the redox potential of the pressure menstruum may also play a role in the inactivation for some microorganisms (Hoover 1993). Addition of bacteriocins may influence the inactivation of microorganisms by pressure, as discussed in section 2.5. Other secondary factors are unknown at this time of writing.

4.2. Measurement of critical process factors

Currently, HPP is a batch or semi-continuous operation, since volume containment is necessary to generate the high pressure used in food treatment. Batch and semi-continuous treatment makes testing of samples before and after treatment necessary. It is also necessary to have a hard copy record of the pressure in the process vessel for each batch and for each treatment cycle in the pressure vessel of a semi-continuous unit. If temperature is specified as an integral part of the preservation process, the internal temperature of the pressure vessel must be recorded in hard copy at a point representing the temperature distribution in the vessel used to develop the process. The initial temperature (IT) of the food must be controlled as a critical process factor. Key measurements to be taken and recorded over the course of treatment would be pressure, time, and temperature. Product composition and pH should not change at the initial and termination points of the process. Package integrity should also be monitored.

4.3. Microbial surrogates/indicators for HPP validation

Because some types of spores of *C. botulinum* (for example strains 17B and CAP 9B) are capable of surviving even the most extreme pressures and temperatures of HPP, there is no absolute microbial indicator for sterility. To date, no nonpathogenic spore-forming bacterium has been found that can endure the high pressures and temperatures observed with *C. botulinum* strains 17B and CAP 9B.

For vegetative types of bacteria, nonpathogenic *L. innocua* has become a favorite surrogate for the foodborne pathogen, *L. monocytogenes*. *Listeria* are very hardy, gram-positive bacteria that have demonstrated reasonable resistance to HPP. These bacteria are also commonly found in a range of raw foods, both animal and plant derived. Hence, *L. innocua* is a logical test organism for HPP validation.

Patterson and coworkers have examined a clinical isolate of *E. coli* O157:H7 that can endure exposures to HPP almost equivalent to that for spores of *Bacillus* and *Clostridium*. A nonpathogenic strain of *Bacillus* may be useful, since spore suspensions are more easily stored and contained than vegetative bacteria. Section 6.2 lists a number of pathogens, spoilage, and possible surrogate microbes.

5. Process Deviations

PROCESS DEVIATIONS ARE EXPECTED IN ANY MANUFACTURING process. Once critical process factors are identified and their values established, limit values for each critical process factor must be established. In addition, a plan needs to be prepared to determine corrective actions in case a process deviation occurs. The corrective action would depend on the magnitude that the critical process factor deviates from the established limit(s).

This section identifies critical process factors considered in high pressure food preservation. The critical process factor identified must be monitored by appropriate transducers. Types of transducers are described along with their accuracy and precision, and appropriate location in the pressure vessel. This section also discusses methods for periodic calibration of instrumentation. Process control systems are identified for generating permanent process records, identifying process deviations, and for determining when and which type of corrective action should be taken based on the magnitude of the deviation. The control of a high pressure process used for the preservation of food requires transducers for the measurement of pressure, time, and

temperature of the process. The following is a list of transducers, their precision and accuracy, and their location in the vessel.

Measuring Transducer	Accuracy/ Precision	Location in Pressure Vessel
Pressure Gauge (Electronic)	+/− 1/2% / 3.4 MPa	Any place in high pressure system
Pressure Gauge (Dial Display)	+/− 1% / 6.8 MPa	Any place in high pressure system
Temperature (Thermocouple)	+/− 1/2% / 0.5 °C	Vessel cold point or its equivalent
Time (Recorder)	+/− 1% / one second	(not applicable)

Pressure and temperature transducers used in validating and controlling a process should be calibrated periodically against traceable instruments. The frequency of calibration will be a function of the number of pressure cycles and must be determined by testing.

5.1. Detection methods

It is recommended that the critical process factors of an HPP process be monitored and recorded in the form of a hard copy record. The recording system used must contain instrumentation that will signal process deviations outside the limits developed in the validation of the process. The critical process factors and parameters to be controlled for batch, semi-continuous, and pulsed HPP, including processes where temperature is specified, are as follows.

Batch Systems

Critical Process Factor	Detection and Method for Deviation Determination
Product initial temperature	Periodic temperature measurement of product temperature and plotting of values on a control chart showing lower limits.
Time to bring vessel to pressure	Printed record of pressure against time with controls set to indicate a deviation if pressure is not achieved within a specified time period.
Pressure of vessel during process	Printed record of pressure against time with controls set to indicate a deviation if pressure drops below a minimum value.
Process time at pressure	Printed record of pressure against time with controls set to indicate a deviation if process hold time drops below a set minimum value.
Process temperature	Printed record of temperature with controls set to indicate a deviation if process temperature drops below a set minimum.
Decompression time	Printed record of pressure with controls set to indicate a deviation from a time developed in the validation of the process.

5.1.2. Semi-continuous systems

Semi-continuous systems are used to treat liquids and pastes that can be pumped. The actual pressure treatment cycle is equal to a batch process. Thus, the critical process factors to be controlled are those shown in 5.1.1. Process temperatures can be monitored in a continuous manner by placing a thermocouple in the inlet and outlet pipe. A diversionary valve can be included in the outlet pipe to recycle product, produced during any process deviation, back to the inlet pipe for reprocessing.

5.1.3. Pulsed systems

Pulsed systems can be semi-continuous or batch. The actual pressure treatment cycle is equal to a batch process with more than one pressure cycle used to treat the food. Thus the critical process factors to be controlled are those shown in 5.1.1. for batch systems and 5.1.2. for semi-continuous systems.

5.2. Methods to assess deviation severity

The development of a valid HPP process must include information on the limits of critical process factors. These limits must include appropriate corrective actions as a function of the severity of each deviation. A cumulative estimate of deviation severity should show several critical process factors deviations simultaneously. Corrective action will depend on the severity of the deviation incident. For example, complete loss of process pressure before the process is complete could require reprocessing. A 10% loss of process pressure, for a known time, could be corrected by adding additional holding time on the process at the specified pressure, provided the pressure could be returned to the specifieD-value immediately after the deviation.

5.3. Corrective actions

Corrective actions will reflect the cumulative severity of the process deviations identified. It is recommended that the validation of a process for the high pressure preservation of food include studies on the effect of process deviations of various magnitudes. For example, it is known that decompression can be accompanied by product cooling. For processes specifying a process temperature, a pressure drop can mean both a pressure and temperature deviation. The cumulative effect of the deviation must be determined during the validation of the process if corrective action for this deviation is proposed during the process.

5.4. Sample deviation

It is proposed that a single sample be considered a lot and be equal to a batch treated during one pressure cycle in a batch system. Since pressure acts uniformly throughout the pressure vessel, each package will be exposed to the same pressure and temperature deviation during the process, provided the walls of the pressure vessel are kept at the final process temperature of the process. Any process deviation will require the treatment of the lot as a whole by the appropriate corrective action.

6. Research Needs

FURTHER RESEARCH IS PARTICULARLY NEEDED TO VALIDATE HPP as a food preservation technology. Pressing needs include:

- Conduct additional modeling research, using data generated by multiple-cell pressure units that allow for similar come-up times. Although HPP-derived semi-logarithmic survival curves appear nonlinear (for example, sigmoidal or biphasic), in HPP predictive microbiology, a logarithmic order of reduction is normally assumed. This assumption carries the danger of underestimating the subpopulation of pressure-resistant organisms.
- Investigate the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that kinetic parameters are quantified. In this way, critical process factors can be evaluated for survival of pathogens or surrogates in a statistical manner and microbial inactivation could be predicted.
- Evaluate synergistic effects among pressure, temperature and other variables.

Glossary

A COMPLETE LIST OF DEFINITIONS REGARDING ALL THE TECHnologies is located at the end of this document.

Adiabatic compression. Compression or decompression processes occurring without heat transfer.

Batch treatment. Treatment of a static mass of food in bulk or packaged.

Compression time. Recorded time to bring a mass of food from 0.1 MPa to process pressure (s)

Continuous HPP treatment. Treatment of liquiform products using a hold chamber designed to insure every food element receives a specified residence time at process pressure (and temperature) with subsequent means for the product to do work during decompression followed by aseptic or clean filling of packages.

Critical process factor. Any specified process condition and specified limit (see process deviation) required to achieve a desired/specified residual level of activity of a specified pathogen. Critical process factors can include, but not be limited to, process pressure, product IT, process temperature, pH, a_w product composition, compression time, and process pressure hold time.

Decompression time. Recorded time to bring a mass of food from process pressure to 37% of process pressure. If decompression time is 0.5% or less of process pressure hold time, it may be neglected in process determination calculations. (s)

High pressure processing (HPP). Adiabatic compression, hold, and decompression of foods at pressures in the range of 100 to 800 MPa for hold times of 0.001 to 1200 s or longer.

Intensifier. Device for delivering high pressure process liquid generally by using a large-diameter, low-pressure piston to drive a small-diameter, high-pressure piston. The ratio of intensification is directly proportional to the ratio of the area of the large-diameter piston divided by the area of the small-diameter piston. A 20:1 intensification ratio is common. The pressure in the low-pressure cylinder may be used to estimate the pressure of the high pressure process liquid. Intensifiers may be operated as single or multiple stroke devices. Single stroke intensifiers may be used to control the decompression rate of an HPP system.

Process deviation. Any critical HPP process factor which lies outside of specifieD-value and limit, lower limit, or range limit during the treatment and subsequent handling of an HPP-treated food. Examples include pH, water activity, initial temperature (IT), process temperature, process pressure, process pressure hold time, number of pulses, compression time (pulsed HPP treatment).

Process pressure (PP). Constant holding pressure for any HPP treatment. Process pressure should be controlled to +/− 0.5% and recorded to the same level of accuracy. (+/− 500 psi at 100,000 psi) or (+/− 3.4 MPa at 680 MPa). (MPa) (psi) (see pressure conversion factors at the end of the glossary).

Process pressure hold time. Recorded time from end of compression to beginning of decompression (s).

Product a_w. Value of water activity measured at product IT at atmospheric pressure. (a_w units)

Product composition. Specified percent by weight and range limit of stated product ingredients (%).

Product initial temperature (IT). Product IT can be specified as a critical process factor. IT must be not less than 0.5 °C below specifieD-value in all food locations from start of compression time to end of compression time (°C).

Product pH. Value of pH measured at product initial temperature at atmospheric pressure.

Product process temperature. Foods will increase in temperature as a function of the imposed pressure and their composition. Final product temperature at process pressure is independent of compression rate as long as heat transfer is negligible. Initial temperature (IT) and process temperature at all points in the process vessel must be monitored if it is an integral condition for microbial inactivation. (°C)

Pulsed HPP treatment. Treatment of a food using more than one treatment cycle consisting of elements of compression time, pressure hold time, decompression time, and specified pressure hold time between cycles such that each cycle element is accurately and precisely reproduced until a specified number is achieved. Cycle elements may display a square, ramp, sinusoidal, or other wave form when recorded.

Semi-continuous HPP. Treatment of liquiform products using one or more chambers fitted with a free piston to allow compression, hold, and decompression with discharge under clean or sterile conditions.

Surrogate microbe. A non-pathogenic species and strain responding to HPP treatment in a manner equivalent to a pathogenic species and strain. The surrogate allows biological verification of an HPP treatment without introducing pathogens into a food processing area. For example, PA 3679 is used as a surrogate microbe for *C. botulinum* in thermal process validation. *Listeria innocua* is a possible surrogate for *L. monocytogenes* in HPP process validation.

z(T) value. The increase in number of degrees centigrade to reduce the D-value by a factor of 10. For example, when an increase of 7 °C centigrade in the process temperature changes the D-value from 30 to 3 min, the Z_T value is 7 °C.

z(P) value. The increase in number of MPa to reduce the D-value by a factor of 10. For example, when an increase of 150 MPa in the process pressure changes the D-value from 30 to 3 min, the Z_p value is 150 MPa. (MPa)

Pressure Units Conversion Factors (To convert from the units shown across the top of the table to the units shown in the left side column, multiply by the values shown.)

	Atmospheres (ATM)	Bars[1]	Kg/cm²	Megapascals MPa	Pounds/inch (PSI)
ATM	1	0.987	0.968	9.901	0.068
BARS	1.013	1.00	0.981	10.000	0.069
Kg/cm²	1.033	1.021	1	10.228	0.070
MPa	0.101	0.1	0.098	1	0.00689
PSI	14.696	14.504	14.223	145.038	1

Suggestions for standardized microbial cultures to be used in HPP process development, challenge work, and process validation

(P) = Pathogens, (S) = Spoilage/Surrogate

Listeria monocytogenes Scott A (NCTC 11994) (P) - Dairy, Meat, Seafood, Vegetables
Clostridium botulinum 62A,17B or Beluga (P) - Meat, Seafood
Escherichia coli O157:H7 * NCTC 12079 (P) - Meat
Staphylococcus aureus NCTC 10652 (enterotoxin A producer) (P) - Poultry Products
Salmonella Typhimurium DT 104 (P) - Poultry Products
Salmonella Enteritidis (P)- Poultry products
Bacillus cereus T (P) - Poultry, Meat
Clostridium sporogenes PA 3679 (S) - Meat
Lactobacillus fructivorans (S) - Fruit Products
Leuconostoc mesenteroides (S) - Vegetable Products
Lactobacillus sake (S) - Acidified Products
Zygosaccharomyces bailii (S) - Fruit Juices
Campylobacter sp. (P)
Clostridium perfringens (P)
Yersinia enterocolitica (P)
Vibro parahaemolyticus (P)
Listeria innocua (S)

• Since this list was prepared, two strains of *E. coli* O157:H7 of greater pressure resistance than NCTC 12079 have been identified. Strain C490 (Benito and others 1999) and strain 30-2C4 (clinical isolate from dry cured salami).

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Pulsed Electric Fields

GUSTAVO V. BARBOSA-CANOVAS, MERLE D. PIERSON, Q. HOWARD ZHANG, AND DONALD W. SCHAFFNER

Scope of Deliverables: This section discusses current knowledge in the application of pulsed electric fields as a method of non-thermal food preservation. It includes mechanisms of inactivation, studies on microbial inactivation, critical process factors, and future research needs. Detailed descriptions of pilot and laboratory-scale equipment and their use in food preservation are also covered.

1. Introduction

1.1. Definition, Description and Applications

1.1.1 Definition

High intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20 - 80 kV/cm) to foods placed between 2 electrodes. PEF treatment is conducted at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s, and energy loss due to heating of foods is minimized. For food quality attributes, PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods (Quass 1997). Although some studies have concluded that PEF preserves the nutritional components of the food, effects of PEF on the chemical and nutritional aspects of foods must be better understood before it is used in food processing (Qin and others 1995b).

Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature, and the design of electrodes that minimize the effect of electrolysis. The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a DC power supply, which is then discharged in the form of high voltage pulses (Zhang and others 1995). Studies on energy requirements have concluded that PEF is an energy-efficient process compared to thermal pasteurization, particularly when a continuous system is used (Qin and others 1995a).

1.1.2. Description of pulsed waveforms

PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses. An exponential decay voltage wave is a unidirectional voltage that rises rapidly to a maximum value and decays slowly to zero. The circuit in Fig. 1 may be used to generate an exponential decay waveform. A DC power supply charges a capacitor bank connected in series with a charging resistor (R_s). When a trigger signal is applied, the charge stored in the capacitor flows though the food in the treatment chamber.

Square pulse waveforms are more lethal and more energy efficient than exponential decaying pulses. A square waveform can be obtained by using a pulse-forming network (PFN) consisting of an array of capacitors and inductors and solid state switching devices (Fig. 2).

The instant-charge-reversal pulses are characterized by a $+v_e$ part and $-v_e$ part (Fig. 3) with various widths and peak field strengths. An instant-charge-reversal pulse width with charge-reversal at the end of the pulse is considerably different from a standard bipolar pulse. In the latter, the polarity of the pulses is reversed alternately with relaxation time between pulses. Even with a high frequency pulser (for example, 1000 Hz), the dielectric relaxation time at zero voltage between 4 μs square wave pulses is 0.996 μs (Quass 1997). Instant-charge-reversal pulses can drastically reduce energy requirements to as low as 1.3 J/ml (EPRI 1998).

Oscillatory decay pulses are the least efficient, because they prevent the cell from being continuously exposed to a high intensity electric field for an extended period of time, thus preventing the cell membrane from irreversible breakdown over a large area (Jeyamkondan and others 1999).

1.1.3. Treatment chambers and equipment

Currently, there are only 2 commercial systems available (one by PurePulse Technologies, Inc. and one by Thomson-CSF). Different laboratory- and pilot-scale treatment chambers have been designed and used for PEF treatment of foods. They are classified as static (U-shaped polystyrene and glass coil static chambers) or continuous (chambers with ion conductive membrane, chambers with baffles, enhanced electric field treatment chambers, and coaxial chambers). These chambers are described in Appendix 1. A continuous flow diagram for PEF processing of foods is illustrated in Fig. 4. The test apparatus consists of 5 major components: a high-voltage power supply, an energy storage capacitor, a treatment chamber(s), a pump to con-

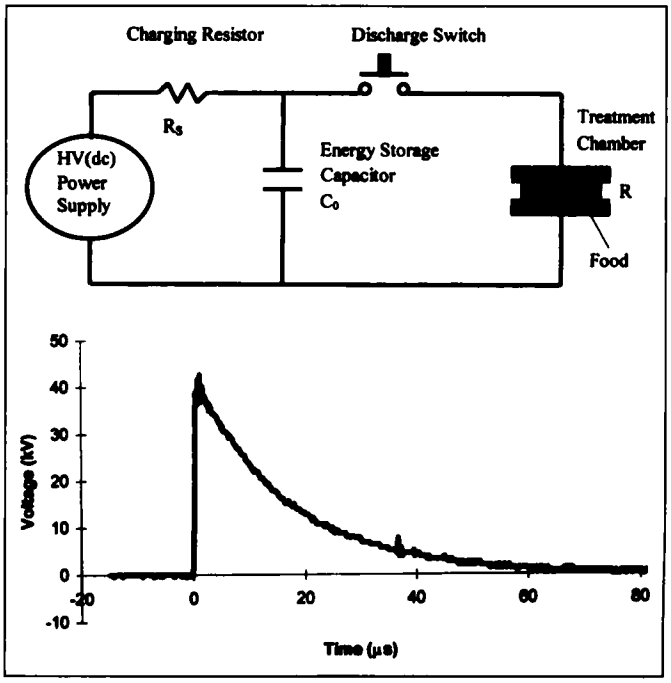

Fig. 1—Electrical circuit for the production of exponential decay waveforms

duct food though the treatment chamber(s), a cooling device, voltage, current, temperature measurement devices, and a computer to control operations.

1.2. Applications of PEF Technology in Food Preservation

To date, PEF has been mainly applied to preserve the quality of foods, such as to improve the shelf-life of bread, milk, orange juice, liquid eggs, and apple juice, and the fermentation properties of brewer's yeast.

1.2.1. Processing of apple juice

Simpson and others (1995) reported that apple juice from concentrate treated with PEF at 50 kV/cm, 10 pulses, pulse width of 2 μs and maximum processing temperature of 45 °C had a shelf-life of 28 d compared to a shelf-life of 21 d of fresh-squeezed apple juice. There were no physical or chemical changes in ascorbic acid or sugars in the PEF-treated apple juice and a sensory panel found no significant differences between untreated and electric field treated juices. Vega Mercado and others (1997) reported that PEF extended the shelf-life at 22–25 °C of fresh apple juice and apple juice from concentrate to more than 56 d or 32 d, respectively. There was no apparent change in its physicochemical and sensory properties.

1.2.2. Processing of orange juice

Sitzmann (1995) reported on the effectiveness of the ELSTER-IL continuous process developed by the food engineers at Krupp Maachinentechnik GmbH in Hamburg, in association with the University of Hamburg, Germany. They reported the reduction of the native microbial flora of freshly squeezed orange juice by 3-log cycles with an applied electric field of 15 kV/cm without significantly affecting its quality.

Zhang and others (1997) evaluated the shelf-life of reconstituted orange juice treated with an integrated PEF pilot plant system. The PEF system consisted of a series of co-field chambers. Temperatures were maintained near ambient with cooling devices between chambers. Three waveshape pulses were used to compare the effectiveness of the processing conditions. Their results confirmed that the square wave is the most effective pulse shape. In addition, the authors reported that total aerobic counts were reduced by 3- to 4-log cycles under 32 kV/cm. When stored at 4 °C, both heat- and PEF-treated juices had a shelf-life of more than 5 mo. Vitamin C losses were lower and color was generally better preserved in PEF-treated juices compared to the heat-treated ones up to 90 d (storage temperature of 4 °C or 22 °C) or 15 d (storage temperature of 37 °C) after processing.

1.2.3. Processing of milk

Dunn and Pearlman (1987) conducted a challenge test and shelf-life study with homogenized milk inoculated with *Salmonella* Dublin and treated with 36.7 kV/cm and 40 pulses over a 25-min time period. *Salmonella* Dublin was not detected after PEF treatment or after storage at 7–9 °C for 8 d. The naturally occurring milk bacterial population increased to 10^7 cfu/ml in the untreated milk, whereas the treated milk showed approximately 4×10^2 cfu/ml. Further studies by Dunn (1996) indicated less flavor degradation and no chemical or physical changes in milk quality attributes for cheesemaking. When *Escherichia coli* was used as the challenge bacteria, a 3-log reduction was achieved immediately after the treatment.

Fernandez-Molina and others (1999) studied the shelf-life of raw skim milk (0.2% milk fat), treated with PEF at 40 kV/cm, 30 pulses, and treatment time of 2 μs using exponential decaying pulses. The shelf-life of the milk was 2 wk stored at 4 °C; however, treatment of raw skim milk with 80 °C for 6 s followed by PEF treatment at 30 kV/cm, 30 pulses, and pulse width of 2 μs increased the shelf-life up to 22 d, with a total aerobic plate count of 3.6-log cfu/ml and no coliform. The processing temperature did not exceed 28 °C during PEF treatment of the raw skim milk.

Qin and others (1995b) reported that milk (2% milk fat) subjected to 2 steps of 7 pulses each and 1 step of 6 pulses with an electric field of 40 kV/cm achieved a shelf-life of 2 wk at refrigeration temperature. There was no apparent change in its physical and chemical properties and no significant differences in sensory attributes between heat pasteurized and PEF treated milk

Calderon-Miranda (1998) studied the PEF inactivation of *Listeria innocua* suspended in skim milk and its subsequent sensitization to nisin. The microbial population of *L. innocua* was reduced by 2.5-log after PEF treatments at 30, 40 or 50 kV/cm. The same PEF intensities and subsequent exposure to 10 IU nisin/ml achieved 2-, 2.7- or 3.4-log reduction cycles of *L. innocua*. It appears that there may be an additional inactivation effect as a re-

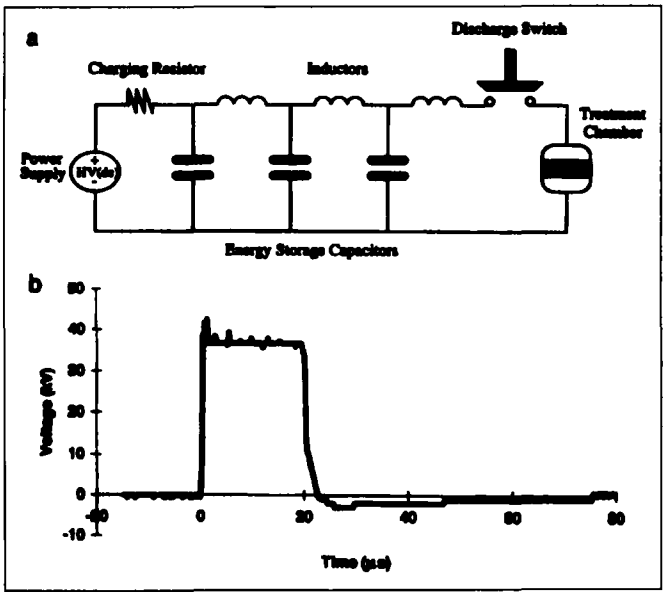

Fig. 2—Square pulse generator using a pulse-forming network of 3 capacitors inductor units and a voltage trace across the treatment chamber

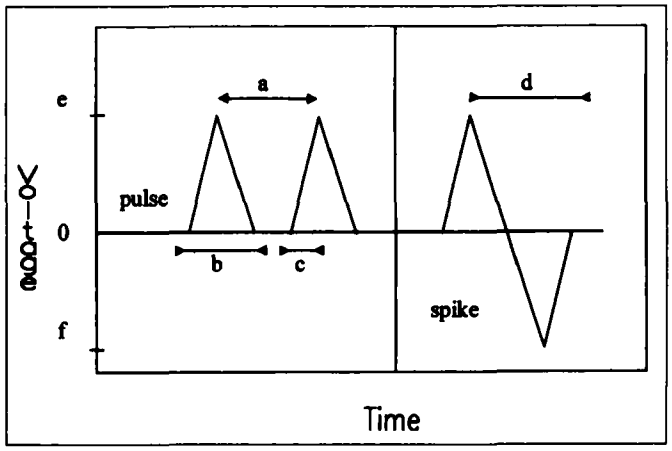

Fig. 3—A voltage (V) trace of an instant-charge-reversal pulse where a is pulse period (s), b is pulse width (μs), c is a pulse rise time(s) to reach e (kV), d is a spike width(s), e is a peak voltage (kV), and f is a spike voltage (kV) (Ho and others 1995)

sult of exposure to nisin after PEF. Reina and others (1998) studied the inactivation of *Listeria monocytogenes* Scott A in pasteurized whole, 2%, and skim milk with PEF. *Listeria monocytogenes* was reduced 1- to 3-log cycles at 25 °C and 4-log cycles at 50 °C, with no significant differences being found among the 3 milks. The lethal effect of PEF was a function of the field intensity and treatment time.

1.2.4. Processing of eggs

Some of the earliest studies in egg products were conducted by Dunn and Pearlman (1987) in a static parallel electrode treatment chamber with 2-cm gap using 25 exponentially decaying pulses with peak voltages of around 36 kV. Tests were carried out on liquid eggs, on heat-pasteurized liquid egg products, and on egg products with potassium sorbate and citric acid added as preservatives. Comparisons were made with regular heat-pasteurized egg products with and without the addition of food preservatives when the eggs were stored at low (4 °C) and high (10 °C) refrigeration temperatures. The study showed the importance of the hurdle approach in shelf-life extension. Its effectiveness was even more evident during storage at low temperatures, where egg products with a final count around 2.7 log cfu/ml stored at 10 °C and 4 °C maintained a low count for 4 and 10 d, respectively, versus a few hours for the heat pasteurized samples.

Other studies on liquid whole eggs (LWE) treated with PEF conducted by Qin and others (1995) and Ma and others (1997) showed that PEF treatment decreased the viscosity but increased the color (in terms of β-carotene concentration) of liquid whole eggs compared to fresh eggs. After sensory panel evaluation with a triangle test, Qin and others (1995b) found no differences between scrambled eggs prepared from fresh eggs and electric field-treated eggs; the latter were preferred over a commercial brand.

In addition to color analysis of eggs products, Ma and others (1997) evaluated the density of fresh and PEF-treated LWE (indicator of egg protein-foaming ability), as well as the strength of sponge cake baked with PEF-treated eggs. The stepwise process used by Ma and others (1997) did not cause any difference in density or whiteness between the PEF-treated and fresh LWE. The strength of the sponge cakes prepared with PEF-treated eggs was greater than the cake made with non-processed eggs. This difference in strength was attributed to the lower volume obtained after baking with PEF-treated eggs. The statistical analysis of the sensory evaluation revealed no differences between cakes prepared from PEF processed and fresh LWE.

1.2.5. Processing of green pea soup

Vega-Mercado and others (1996a) exposed pea soup to 2 steps of 16 pulses at 35 kV/cm to prevent an increase in temperature beyond 55 °C during treatment. The shelf-life of the PEF-treated pea soup stored at refrigeration temperature exceeded 4 wk, while 22 or 32 °C were found inappropriate to store the product. There were no apparent changes in the physical and chemical properties or sensory attributes of the pea soup directly after PEF processing or during the 4 wk of storage at refrigeration temperatures.

1.3. Current Limitations

Some of the most important current technical drawbacks or limitations of the PEF technology are:

a) The availability of commercial units, which is limited to one by PurePulse Technologies, Inc., and one by Thomson-CSF. Many pulse-power suppliers are capable of designing and constructing reliable pulsers, but except for these 2 mentioned, the complete PEF systems must be assembled independently. The systems (including treatment chambers and power supply equipments) need to be scaled up to commercial systems.

b) The presence of bubbles, which may lead to non-uniform treatment as well as operational and safety problems. When the applied electric field exceeds the dielectric strength of the gas bubbles, partial discharges take place inside the bubbles that can volatize the liquid and therefore increase the volume of the bubbles. The bubbles may become big enough to bridge the gap between the 2 electrodes and may produce a spark. Therefore, air bubbles in the food must be removed, particularly with batch systems. Vacuum degassing or pressurizing the treatment media during processing, using positive back pressure, can minimize the presence of gas. In general, however, the PEF method is not suitable for most of the solid food products containing air bubbles when placed in the treatment chamber.

c) Limited application, which is restricted to food products that can withstand high electric fields. The dielectric property of a food is closely related to its physical structure and chemical composition. Homogeneous liquids with low electrical conductivity provide ideal conditions for continuous treatment with the PEF method. Food products without the addition of salt have conductivity in the range of 0.1 to 0.5 S/m. Products with high electrical conductivity reduce the resistance of the chamber and consequently require more energy to achieve a specific electrical field. Therefore, when processing high salt products, the salt should be added after processing.

d) The particle size of the liquid food in both static and flow treatment modes. The maximum particle size in the liquid must be smaller than the gap of the treatment region in the chamber in order to maintain a proper processing operation.

e) The lack of methods to accurately measure treatment delivery. The number and diversity in equipment, limits the validity of conclusions that can be drawn about the effectiveness of particular process conditions. A method to measure treatment delivery would prevent inconsistent results due to variations in PEF systems. Such a method is not available yet.

1.4. Summary of Mechanisms of Microbial Inactivation

The application of electrical fields to biological cells in a medium (for example, water) causes buildup of electrical charges at the cell membrane (Schoenbach and others 1997). Membrane disruption occurs when the induced membrane potential exceeds a critical value of 1 V in many cellular systems, which, for example, corresponds to an external electric field of about 10 kV/cm for *E. coli* (Castro and others 1993). Several theories have been proposed to explain microbial inactivation by PEF. Among them, the most studied are electrical breakdown and electroporation or disruption of cell membranes (Zimermmann and Benz

Fig. 4—Continuous PEF flow diagram

1980; Zimermmann 1986; Castro and others 1993; Sale and Hamilton 1967; Vega-Mercado and others 1996a; 1996b). These theories will be explained in greater detail in Section 3.

1.5. Summary of Microbial Inactivation Kinetics

The development of mathematical models to express the inactivation kinetics of PEF is an area of research that needs extensive further work. Nevertheless, some models have been proposed and need further evaluation (see Section 3.2).

1.6. Summary of Critical Process Factors

Three types of factors that affect the microbial inactivation with PEF have been identified: factors depending on (1) the process (electric field intensity, pulse width, treatment time and temperature, and pulse waveshapes), (2) microbial entity (type, concentration, and growth stage of microorganism), and (3) treatment media (pH, antimicrobials, and ionic compounds, conductivity, and medium ionic strength).

2. Critical Process Factors and How they Impact Microbial Inactivation

2.1. Analysis of Critical Factors

2.1.1. Process factors

a) Electric field intensity. Electric field intensity is one of the main factors that influences microbial inactivation (Hüshelguer and Niemann 1980; Dunne and others 1996). The microbial inactivation increases with an increase in the electric field intensity, above the critical transmembrane potential (Qin and others 1998). This is consistent with the electroporation theory, in which the induced potential difference across the cell membrane is proportional to the applied electric field (Section 3.1.2.). Some empirical mathematical models (that is, Tables 4 and 5) have been proposed to describe the relationship between the electric field intensity and microbial inactivation. The critical electric field E_c (electric field intensity below which inactivation does not occur) increases with the transmembrane potential of the cell. Transmembrane potentials, and consequently E_c, are larger for larger cells (Jeyamkondan and others 1999). Pulse width also influences the critical electric field; for instance, with pulse widths greater than 50 μs, E_c is 4.9 kV/cm. With pulse widths less than 2 μs, E_c is 40 kV/cm (Schoenbach and others 1997).

The model of Peleg (Table 5) was used to relate the electric field intensity and applied number of pulses required to inactivate 50% of the cells (Peleg 1995).

b) Treatment time. Treatment time is defined as the product of the number pulses and the pulse duration. An increase in any of these variables increases microbial inactivation (Sale and Hamilton 1967). As noted above, pulse width influences microbial reduction by affecting E_c. Longer widths decrease E_c, which results in higher inactivation; however, an increase in pulse duration may also result in an undesirable food temperature increase. Optimum processing conditions should therefore be established to obtain the highest inactivation rate with the lowest heating effect. Hülsheger and others (1981) proposed an inactivation kinetic model that relates microbial survival fraction (S) with PEF treatment time (t). The inactivation of microorganisms increases with an increase in treatment time (Table 4; Hülsheger and others 1983). In certain cases, though, the number of pulses increasing inactivation reaches saturation. Such is the case of *Saccharomyces cerevisiae* inactivation by PEF that reaches saturation with 10 pulses of an electric field at 25 kV/cm (Barbosa-Cánovas and others 1999).

Critical treatment time also depends on the electric field intensity applied. Above the critical electric field, critical treatment time decreases with higher electric fields. Barbosa-Cánovas and others (1999) reported that for an electric field strength 1.5 times higher than E_c, the critical treatment time would remain constant.

c) Pulse waveshape. Electric field pulses may be applied in the form of exponential decaying, square-wave, oscillatory, bipolar, or instant reverse charges. Oscillatory pulses are the least efficient for microbial inactivation, and square wave pulses are more energy and lethally efficient than exponential decaying pulses. Bipolar pulses are more lethal than monopolar pulses because a PEF causes movement of charged molecules in the cell membranes of microorganisms, and reversal in the orientation or polarity of the electric field causes a corresponding change in the direction of charged molecules (Ho and others 1995; Qin and others 1994). This difference was reported in *Bacillus* spp. spores (Ho and Mittal 1997) and *E. coli* (Qin and others 1994). With bipolar pulses, the alternating changes in the movement of charged molecules cause a stress in the cell membrane and enhance its electric breakdown. Bipolar pulses also offer the advantages of minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis (Barbosa-Cánovas and others 1999).

As mentioned earlier in this report, the instant-charge-reversal pulse can be described as partially positive at first and partially negative immediately thereafter. This characteristic of the waveshape is influenced by the electrical conductivity of the treated food. In this regard, an increase in conductivity decreases the duration of the positive part of the pulse as well as the span of the negative part, which in turn increases the overall peak/voltage ratio.

The difference between a bipolar and instant charge reverse pulse is the relaxation time between pulses, which is only present in the former. The inactivation effect of an instant-reversal-charge is believed to be due to a significant alternating stress on the microbial cell that causes structural fatigue. Ho and Mittal (1997) reported that instant-reversal-charge may reduce the critical electric field strength required for electroporation of the microbial cell. The effectiveness of this waveform to inactivate microorganisms compared to other pulse waveforms can save up to 1/5 or 1/6 of total energy and equipment cost. Further work is required to verify the effect of reversal-charge pulses on the inactivation ratio. The inactivation of *Bacillus subtilis* and *Bacillus cereus* spores suspended in NaCl solutions has been reported to be higher when instant reverse pulses and a polarity of electric field chambers with high pulse frequencies are used. Instant reverse charge has been reported to be effective in inactivation of 5-log cycles of *Bacillus* spp. spores. These researchers established that the survival fraction is not only a function of the temporal pulse area but that even when both bipolar (alternating exponential) and exponential waves had the same area per pulse, bipolar waves yielded a higher inactivation ratio (Ho and Mittal 1997).

A study conducted by Zhang and others (1997) showed the effect of square wave, exponentially decaying, and instant-charge-reversal pulses on the shelf-life of orange juice. Three waveshape pulses were used: (a) square waves with peak electric field of 35 kV/cm, an effective pulse width of 37.22 μs, and a pulse rise time of 60 ns; (b) exponential decaying waves with a peak electric field of 62.5 kV/cm, an effective pulse width of 0.57 μs and a pulse rise time of 40 ns; and (c) charge-reversal waves with a peak electric field of 37 kV/cm, an effective pulse width of 0.96 μs, and a pulse rise of 400 ns. Square wave pulses were more effective, yielding products with longer shelf-lives than those products treated with exponentially decaying and charge reverse pulses. In agreement with this study, Love (1998) quantitatively demonstrated the stronger inactivation effect of square wave pulses over other wave shapes.

Qin and others (1994) studied the inactivation of *S. cerevisiae* using square and exponential decay waveforms and a peak electric field of 12 kV/cm and 60 J/pulse for both waveforms. The results of this investigation suggested that both waveforms were effective in the microbial inactivation, with square wave pulse waveform being the most effective.

d) Treatment temperature. Experimental results have demonstrated that, in challenge tests, both treatment temperatures and process temperatures impact microbial survival and recovery.

PEF treatments at moderate temperatures (about 50 to 60 °C) have been shown to exhibit synergistic effects on the inactivation of microorganisms (Jayaram and others 1992; Dunn and Pearlman 1987). With constant electric field strength, inactivation increases with an increase in temperature. Because the application of electric field intensity does cause some increase in the temperature of the foods, proper cooling is necessary to maintain food temperatures far below those generated by thermal pasteurization.

The effect of temperature was observed when *E. coli* reduction increased from 1 to 6.5-log reduction cycles with a temperature change from 32 to 55 °C (Vega-Mercado and others 1996a). A higher lethal effect of PEF treatment is accomplished by increasing the process temperature to 25 °C, from 5 or 10 °C. This may be due to the increase in the electrical conductivity of the solution at the higher temperature (Marquez and others 1997). The authors suggested that the leakage of mobile ions in decoated spores may increase as the temperature is raised due to an increase in average kinetic energy of the ions. A higher temperature also increases the motion of the solvent molecules in both the surrounding cortex and the core so that the molecules could migrate from one electrode to the other.

Additional effects of high treatment temperatures are changes in cell membrane fluidity and permeability, which increases the susceptibility of the to cell to mechanical disruption (Hulsheger and others 1981). Also, a low transmembrane potential decreases E_c and therefore increases inactivation (Jeyamkondan 1999).

2.1.2. Product factors

a) Conductivity, pH, and ionic strength. The electrical conductivity of a medium (σ, Siems/m), which is defined as the ability to conduct electric current, is an important variable in PEF. Electrical conductivity is the inverse of the resistivity, which is defined by the letter r and measured in ohm-meters (Ω.m). Foods with large electrical conductivities generate smaller peak electric fields across the treatment chamber and therefore are not feasible for PEF treatment (Barbosa-Cánovas and others 1999). Inactivation of *Lactobacillus brevis* with PEF showed that as the conductivity of the fluid increased, the resistance of the treatment chamber was reduced (Jayaram and others 1992), which in turn reduced the pulse width and decreased the rate of inactivation. Because an increase in conductivity results from increases the ionic strength of a liquid, an increase in the ionic strength of a food results in a decrease in the inactivation rate. Furthermore, an increase in the difference between the conductivity of a medium and microbial cytoplasm weakens the membrane structure due to an increased flow ionic substance across the membrane. Thus, the inactivation rate of microorganisms increases with decreasing conductivity even with an application of equal input energy (Jayaram and others 1992). Another study by Dunne and others (1996) with a model system showed resistivity had no effect on PEF effectiveness on *E. coli* and *L. innocua*. These apparent controversial results may be due to the microorganisms or media used.

Vega-Mercado and others (1996b) studied the effect of pH and ionic strength of the medium (SMFU) during PEF treatment. The inactivation ratio increases from not detectable to 2.5-log cycles when ionic strength solutions were adjusted from 168 to 28mM. At 55 kV/cm (8 pulses), as the pH was reduced from 6.8 to 5.7, the inactivation ratio increased from 1.45- to 2.22-log cycles. The PEF treatment and ionic strength were responsible for electroporation and compression of the cell membrane, whereas the pH of the medium affected the cytoplasm when the electroporation was complete. Dunne and others (1996) reported that, depending on the microorganism, acidic pH enhanced microbial inactivation. No mention was made of what microorganisms were affected or what range of pH was used.

b) Particulate foods. Inactivation of microorganisms in liquid-particulate systems has been studied by Dunne et at (1996). *E. coli, L. innocua, Staphyloccocus aureus,* and *Lactobacillus acidophilus* were suspended in a 2 mm diameter alginate beads model, and the effect of variables in PEF microbial inactivation was tested. The researchers concluded that the process was effective in killing microorganisms embedded in particulates. However, to achieve more than a 5-log cycle reduction, high energy inputs were needed (70 - 100 J/ml, depending on the bacteria). With those high PEF intensities, the possibility of dielectric breakdown exists- a limitation still to be overcome. Qin and others (1995c) reported that dielectric breakdown occurs when air or liquid vapor is present in the food because of the difference in dielectric constant between liquid and gas. Likewise, dielectric breakdown may occur at a particle- to -liquid interface due to differences in electric constants.

c) Hurdle approach. In general, the combination of factors (hurdles) such as pH, ionic strength and antimicrobial compounds during PEF treatment would be an effective means to aid in the inactivation of microorganisms with PEF.

2.1.3. Microbial factors

a) Type of microorganisms. Among bacteria, those that are gram-positive are more resistant to PEF than those that are gram-negative (Hülsheger and others 1983). In general, yeasts are more sensitive to electric fields than bacteria due to their larger size, although at low electric fields they seem to be more resistant than gram-negative cells (Sale and Hamilton 1967; Qin and others 1995a). A comparison between the inactivation of 2 yeast spp. of different sizes showed that the field intensity needed to achieve the same inactivation level was inversely proportional to cell size. Those results are logical but inconsistent with results by Hülsheger and others (1983). Studies need to continue in this area to better understand the effect of the type of microorganism on microbial inactivation.

b) Concentration of microorganisms. The number of microorganisms in food may have an effect on their inactivation with electric fields. Barbosa-Cánovas and others (1999) reported that inactivation of *E. coli* in a model food system of simulated milk ultrafiltrate (SMUF) was not affected when the concentration of microorganisms was varied from 10^3 to 10^8 cfu/ml after being subjected to 70 kV/cm, 16 pulses, and a pulse width of 2 μs. Increasing the number of *S. cerevisiae* in apple juice resulted in slightly lower inactivation (25 kV/cm, 1 pulse, and pulse width of 25 μs). The effect of microbial concentration on inactivation may be related to cluster formation of yeast cells or possibly concealed microorganisms in low electric field regions.

c) Growth stage of microorganisms. In general, logarithmic phase cells are more sensitive to stress than lag and stationary phase cells. Microbial growth in logarithmic phase is characterized by a high proportion of cells undergoing division, during which the cell membrane is more susceptible to the applied electric field. Hülsheger and others (1983) concluded that cells from logarithm growth phase are more sensitive to PEF than from the

stationary growth phase. Likewise, *E. coli* cells in the logarithmic phase were more sensitive to PEF treatment when compared to cells in the stationary and lag phases (Pothakamury and others 1996). Studies with *S. cerevisiae* have shown that the susceptibility of actively growing cells to PEF also occurs with yeast cells (Jacob and others 1981; Gaskova and others 1996). For instance, Gaskova and others (1996) reported that the killing effect of PEF in the logarithmic phase is 30% greater than of those in stationary phase of growth.

2.2. Data from Microbial Inactivation Studies

Numerous publications on inactivation present data on vegetative cells, the majority of them from a few genera. Tables 1, 2, and 3 summarize research on the inactivation of microorganisms and enzymes. Table 1 lists the published papers on microorganisms and enzymes, except for *E. coli* and *S. cerevisiae*. Tables 2 and 3 list inactivation data collected from *S. cerevisiae* and *E. coli*, respectively. The tables include, when available, information on the treatment vessel, process conditions (treatment time, temperature, electric field intensity, number of pulses, and waveshape), media, and data on the log reduction achieved.

Various inactivation levels of *S. cerevisiae* have been achieved in food models and foods using a variety of PEF chambers and experimental conditions (Mizuno and Hori 1991; Zhang and others 1994a, 1994b; Qin and others 1994, 1995a). Other yeasts of importance in food spoilage have also been reduced, suggesting PEF's potential to prevente or delay yeast-related food spoilage.

Fernandez-Molina and others (1999) reported 2.6- and 2.7-log reductions for different microorganisms such as *L. innoccua* and *Pseudomonas fluorescens* with 2 μs 100 pulses at 50 kV/cm at ambient temperature. The influence of the food composition was shown by Calderon-Miranda (1998) studies where *L. innoccua* was reduced by 2.4- and 3.4-log cycle reductions in raw skim milk and liquid whole egg, respectively, under the same experimental conditions.

Hülsheger and others (1983) tested PEF inactivation effectiveness of a variety of microorganisms in phosphate buffer, under the same conditions. The results from these studies suggested that *L. monocytogenes* (2-log reduction) is more resistant to PEF than *Pseudomonas auruginosa or S. aureus* (3- to 3.5-log reduction cycles), and that *Candida albicans* was the most sensitive microorganism among them (4.5-log reduction cycle). For these experiments 30 pulses of 36 μs duration of 20 kV/cm were applied.

Grahl and others (1992) reported the influence of pulse number in microbial inactivation of *E. coli*. They were able to reduce populations of *E. coli* in UHT milk by 1-, 2-, and 3-log cycles when 5, 10, and 15 pulses (22 kV/cm) were applied. Qin and others (1998) achieved more than a 6-log cycle reduction in *E. coli* suspended in simulated milk ultrafiltrate (SMUF) using electric field intensity of 36 kV/cm with a 5-step (50 pulses) PEF treatment. The temperature in the chamber was maintained below 40 °C during the PEF treatment, which is lower than the temperature of commercial pasteurization (70 to 90 °C) for milk. Hülsheger and others (1983) reported a 4-log reduction of *E. coli* in an electric field intensity of 40 kV/cm accompanied with a long treatment time of 1080 μs. A PEF method suitable to inactivate up to 7-log cycles of *E. coli* with fewer pulses (20 versus 70) is stepwise recirculation whereby the product is processed several consecutive times (Barbosa-Cánovas and others 1999). Liu and others (1997) reported that PEF and organic acids (benzoic and sorbic) achieved 5.6- and 4.2-log cycle reductions, compared to a 1-log cycle reduction when PEF was used alone, suggesting enhanced effects with the combination of PEF and organic acids.

The higher efficiency of bipolar pulses versus monopolar pulses was suggested by Qin and others (1994). Cells of *B. subtilis* were reduced to 3- and <2-log cycles when bipolar and monopolar pulses were applied, respectively.

Inactivation studies on the effects of PEF on bacterial spores are scarce and results vary. Early studies (Sale and Hamilton 1967) reported that Bacillus spp. spores were resistant to exponential wave PEF with strength fields up to 30 kV/cm. Only after germination did they become sensitive to PEF. Simpson and others (1995) confirmed the high resistance of *B. subtilis* spores to PEF, and subsequently studied a hurdle approach with heat-shock, lysozyme, EDTA, and pH. Only a combination of 80 °C heat-shock, lysozyme, followed by PEF at 60 °C was able to achieve a 2- to 4-log cycle reduction of spores. The resistance of spores to PEF was shown by Pothakamury (1995). They reported only 3- to 4-log reduction cycles for *B. subtilis* ATCC 9372 spores that were subjected to 60 pulses of 16 kV/cm electric field intensity and 200 - 300 μs pulse widths. Pagán and others (1998) found that spores of *B. subtilis* were not inactivated when PEF (60 kV/cm, 75 pulses) was used in combination with high hydrostatic pressure (HHP) (1500 atm, 30 min, 40 °C). These treatments, however, induced the germination of the spores of *B. subtilis* by more than 5-log cycles, making them sensitive to subsequent pasteurization heat treatment. Thus, combinations of HHP, PEF, and heat treatments constitute an alternative to the stabilization of food products by heat to inactivate spores. Marquez and others (1997) successfully inactivated 3.4- and 5-log cycles of *B. subtilis* and *B. cereus* spores at room temperature, an electric field of 50 kV/cm, and 30 and 50 instant-charge-reversal pulses, respectively.

As Tables 1, 2, and 3 show, many researches 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 critical process factors effects on specific pathogen reductions cannot be made. Research that provides conclusive data on the PEF inactivation of pathogens of concern is clearly needed.

3. Mechanisms of Microbial Inactivation

3.1. Analysis of Microbial Inactivation Mechanism (s)

Two mechanisms have been proposed as the mode of action of PEF on microorganisms: electrical breakdown and electroporation.

3.1.1. Electrical breakdown

Zimmermann (1986), as shown in Fig. 5, explains what electrical breakdown of cell membrane entails. The membrane can be considered as a capacitor filled with a dielectric (Fig. 5a). The normal resisting potential difference across the membrane V'm is 10 mV and leads to the build-up of a membrane potential difference V due to charge separation across the membrane. V is pro-

Table 1 Inactivation kinetics of microorganisms and enzymes by pulsed electric fields (PEF)

Source	Microorganisms	Suspension media	Log reduction (max)	Treatment Vessel[a]	Process conditions[b]
Fernandez-Molina and others (1999)	*Listeria innocua*	Raw skim milk (0.2% milkfat)	2.6	C, coaxial, 29 ml, d = 0.63,	15 to 28 °C, 0.5 l/min 100 pulses, 50 kV/cm 0.5 μF, 2μs, 3.5 Hz Exponential decay

Fernandez-Molina and others (1999)	*Pseudomonas fluorescens*	Raw skim milk (0.2% milkfat)	2.7	C,coaxial, 29 ml, d = 0.63,	15 to 28 °C, 0.5 l/min 30 pulses, 50 kV/cm 0.5 μF, 2μs, 4.0 Hz Exponential decay
Reina and others (1998)	*Listeria . monocytogenes* (scott A)	Pasteurized whole milk (3.5% milkfat) 2% milk (2% milkfat) skim milk (0.2%)	3.0-4.0	C, cofield flow, 20 ml,	10 to 50 °C, 0.07l/s 30 kV/cm 1.5μs, 1,700 Hz bipolar pulses t = 600μs
Calderon-Miranda (1998)	*L. innocua*	Raw Skim milk	2.4	C, continuous, 29 ml d = 0.6 cm	22 to 34 °C, 0.5 l/min 2 μs, 3.5 Hz 32 pulses, 50 kV/cm Exponential decay
Calderon-Miranda (1998)	*L. innocua*	Liquid whole egg (LWE)	3.4	C, continuous, 29 ml,2 d = 0.6 cm	6 to 36 °C, 0.5 l/min 32 pulses, 50 kV/cm 2μs, 3.5 Hz Exponential decay
Hulsheger and others (1983)	*Klebsiella pneumoniae* ATCC 27736	Phosphate buffer	3.0	B, 4 ml, d = 0.5 cm, parallel plates	2.o V/μm, 36 μ sec, 30 pulses, exponential decay, t= 1080μs
Sensoy and others (1997)	*Salmonella* Dublin	Skim milk	3.0	C, continuous, cofield	10 to 50 °C, 15-40 kv/cm, 12-127 μs
Lubicki and Jayaram (1997)	*Yersinia enterocolitica*	NaCl solution pH = 7.0	6.0-7.0	B, Parallel electrodes	2 to 3 °C, 75 kV, 150-200 pulses 500-1300 ns
Hulsheger and others (1983)	*Pseudomonas* aeruginosa	Phosphate buffer	3.5	B, 4 ml, d = 0.5 cm	2.o V/μm, 36 m sec, 30 pulses, exponential decay, t= 1080μs
Hulsheger and others (1983)	Staphylococcus *aureus* (ATCC 25923)	Phosphate buffer	3.0	B, 4 ml, d = 0.5 cm	2.o V/μm, 36 m sec, 30 pulses, exponential decay, t= 1080μs
Hulsheger and others (1983)	*Listeria monocytogenes*	Phosphate buffer	2.0	B, 4 ml, d = 0.5 cm	2.o V/μm, 36 m sec, 30 pulses, exponential decay, t= 1080μs
Hulsheger and others (1983)	*Candida albicans*	Phosphate buffer	4.5	B, 4 ml, d = 0.5 cm	2.o V/μm, 36 m sec, 30 pulses, exponential decay, t= 1080μs
Dunn and Pearlman (1987)	*Salmonella* Dublin	Milk	4.0	B, parallel plates	63 °C, 3.67 V/μm, 36μs, 40 pulses
Dunn and Pearlman (1987)	*Lactobacillus brevis*	Yogurt	2.0	B, parallel plates	50 °C, 1.8 V/μm
Gupta and Murray (1989)	*Salmonella* Typhimurium	NaCl	5.0	B, d= 6.35 mm	1μs, 20 pulses, exponential, 83 kV/cm
Gupta and (1989)	*Pseudomonas fragi*	Milk	4.5	B, d= 6.35 mm	9.0 V/μm, 1μs, 10 of Murray 6.8 V/μm + 1 of 7.5 V/μm + 1 of 8.3 V/μm + 5 of 9.0 V/μm
Jayaram and others (1992)	L. *brevis*	NaH_2PO_4 / $Na_2HPO_4H_2O$	9.0	B, parallel plate, 0.5ml, d = 0.2cm	60 °C, 2.5 V/μm, 46μs, 200 pulses, t= 10,000μs
Pothakamury (1995)	*Lactobacillus delbrueckii* ATCC 11842	SMUF	4.0-5.0	B,1 ml, d= 0.1cm	<30 °C, 1.6V/μm, 200-300μs 40 pules exponential decay, t=10,000μs
Pothakamury (1995)	Bacillus *subtilis* spores ATCC 9372	SMUF	4.0-5.0	B, parallel plate, 1 ml, d=0.1cm	<30 °C, 1.6 V/μm, 200-300μs 50 pulses, exponential decay, t= 12,500μs
Pothakamury and others (1995)	S. *aureus*	SMUF	3.0-4.0	B, parallel plate, 1 ml, d=0.1cm	<30 °C, 1.6 V/μm, 200-300μs 60 pulses, exponential decay
Vega-Mercado and others (1996a)	B. *subtilis* spores ATCC 9372	Pea soup	5.3	C., coaxil, 0.51 / min	<5.5 °C, 3.3 V/μm, 2μs, 0.5 mF, 4.3 Hz, 30 pulses, exponential decay
Ho and others (1995)	P. *fluorescens*	Distilled water, 10-35% sucrose 0.1 and 0.5% xanthan 0.1 and 0.5 % sodium chloride	> 6.0	B, 49.5, 99.1, 148.6 ml d = 0.3	20 °C, 2.5 V/μm, 2μec, 10-20 pulses, t = 2sec, reverse polarity
Qin and others (1994)	B. *subtilis*	SMUF	4.5	B. parallel plate, 100 μl, d=0.1cm	1.6 V/μm, monopolar, 180μs, 13 pulses
Qin and others (1994)	B. *subtilis*	SMUF	5.5	B. parallel plate, 100 μl, d=0.1cm	1.6 V/μm, bipolar, 180μs, 13 pulses
Keith and others (1997)	Aerobic Plate Count	Basil, dill, onion	0.30	B, chamber, 10 ml d = 5 mm, 200 ml d = 9 mm	10-25 kV/cm, 1-10180μs,sec, 200-320 ms, bipolar pulses
Castro (1994)	Alkaline Phosphatase	Raw milk, 2% milk Non-fat milk, SMUF	65%	B, Cuvette, d = 0.1 cm	22 to 49 °C, 18 to 22 kV/cm, 70 pulses, 0.7-0.8μs
Vega-Mercado and others (1995)	Plasmin	SMUF	90%	C, parallel plate	150 °C, 30-40 kV/cm, 50 pulses, 0.1 Hz, 2μs
Ho and others (1997)	Lipase, glucose Oxidase, α-amylase Peroxidase, Phenol oxidase	Buffer solutions	70-85% 30-40%	B, circular chamber, 148 ml	13-87 kV/cm, 30 instant charge reversal pulses, 2μs, 2 sec, 0.12 μF

[a] B, batch, C, continuous, d, gap between electrodes
[b] Temperature, peak electric field, pulse width, number of pulses and shape, and t, total treatment time (sec).

Table 2—Summary of *Saccharomyces cerevisiae* inactivation with PEF

Source	Suspension media	Log reduction (max)	Treatment Vessel[a]	Process conditions[b]
Jacob and others (1981)	0.9% NaCl	1.3	B, 3 ml, d= 0.5 cm	3.5 V/μm, 20μs, 4 pulses
Dunn and Pearlman (1987)	Yogurt	3	B	55 °C, 1.8 V/μm
Hulsheger and others (1983)	Phosphate buffer, PH 7.0	3 stationary cells, 4 Logarithmic cells	B, 4ml, d= o.5 cm	2.0 V/μm, 36μs, 30 pulses t= 1080μs
Mizuno and Hori (1988)	Deionized water	6	0.77 cal/cm^3/pulse, B, Parallel plate, 0.5 cm^3, d= 0.8 cm	2.0 V/μm, 160μs, 175 pulses exponential decay
Matsumoto and others (1991)	Phosphate buffer	5	B	3.0 V/μm
Yonemoto and others (1993)	0.85%	2	B, parallel plate, 2 ml, d= 0.55 cm	0.54 V/μm, 90μs, 10 pulses
Zhang and others (1994b)	Potato dextrose agar	5.5	62 J/ml, B, 14 ml	15 ± 1 °C, 4.0 V/μm, 3μs, 16 Pulses
Qin and others (1994)	Apple juice	4	270 J/pulse, B, parallel plate	<30 °C, 1.2 V/μm, 20 pulses, Exponential decay
Qin and others (1994)	Apple juice	4.2	270 J/pulse, B, parallel plate	<30 °C, 1.2 V/μm, 20 pulses, Square wave
Zhang and others (1994a)	Apple juice	4	260 J/pulse, B, parallel plate,	4-10 °C, 1.2 V/μm, 90μs, 6 pulses, exponential decay
Zhang and others (1994a)	Apple juice	3.5	260 J/pulse, B, Parallel plate, 25 ml, d= 0.95 cm	4-10 °C, 1.2 V/μm, 60μs, 6 pulses, square wave
Zhang and others (1994a)	Apple juice	3-4	558 J/pulse, B, Parallel plate, 25.7 ml, d= 0.95 cm	<25 °C, 2.5 V/μm, 5 pulses
Qin and others (1995a)	Apple juice	7	C, coaxial, 29 ml, d= 0.6 cm, 0.2 μF, 1 Hz	<30 °C, 2.5 V/μm, 2-20μs, ± 150 pulses, exponential decay
Qin and others (1995a)	Apple juice	6	28 J/ml, C, coaxial, 30 ml, 2-10 1/min	22-29.6 °C, 5.0 V/μm, 2.5μs, 2 pulses
Grahl and others (1992); Grahl and Markl (1996)	Orange juice	5	B, 25 ml, d= 0.5 cm, E_c = 4.7	0.675 V/μm, 5 pulses

[a] From Barbosa-Canovas and others (1999).
[b] B, batch; C, continuous.
[c] Temperature, peak electric field, pulse width, number and shape, and total treatment time (t).

portional to the field strength E and radius of the cell. The increase in the membrane potential leads to reduction in the cell membrane thickness. Breakdown of the membrane occurs if the critical breakdown voltage Vc (on the order of 1 V) is reached by a further increase in the external field strength (Fig. 5c). It is assumed that breakdown causes the formation of transmembrane pores (filled with conductive solution), which leads to an immediate discharge at the membrane and thus decomposition of the membrane. Breakdown is reversible if the product pores are small in relation to the total membrane surface. Above critical field strengths and with long exposure times, larger areas of the membrane are subjected to breakdown (Fig. 5d). If the size and number of pores become large in relation to the total membrane surface, reversible breakdown turns into irreversible breakdown, which is associated with mechanical destruction of the cell membrane.

The corresponding electric field is $E_{critical} = V_{critical} / f_a$, where a is the radius of the cell and f is a form that depends on the shape of the cell (Schoenbach and others 1997). For a spherical cell, f is 1.5; for cylindrical cells of length l and hemispheres of diameter d at each end, the form factor is $f = l(l - d)/3$. Typical values of $V_{critical}$ required for the lysing of *E. coli* are on the order of 1 V. The critical field strength for the lysing of bacteria with a dimension of approximately 1 mm and critical voltage of 1 V across the cell membrane is therefore on the order of 10 kV/cm for pulses of 10 microsecond to millisecond duration (Schoenbach and others 1997).

3.1.2. Electroporation

Electroporation is the phenomenon in which a cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes (Castro and others 1993). The plasma membranes of cells become permeable to small molecules after being exposed to an electric field, and permeation then causes swelling and eventual rupture of the cell membrane (Fig. 6) (Vega-Mercado 1996b). The main effect of an electric field on a microorganism cell is to increase membrane permeability due to membrane compression and poration (Vega-Mercado and others 1996b). Kinosita and Tsong (1977; 1979) demonstrated that an electric field of 2.2 kV/cm induced pores in human erythrocytes of approximately 1 nm in diameter. Kinosita and Tsong (1977) suggested a 2-step mechanism for pore formation in which the initial perforation is a response to an electrical suprathreshold potential followed by a time-dependent expansion of the pore size (Fig. 6). Large pores are obtained by increasing the intensity of the electric field and pulse duration or reducing the ionic strength of the medium.

In a lipid model vesicle (liposome), the electrophoretic movement of ions and water dipoles through the spontaneous hydrophobic pores is postulated to be the first event of electroporation, after which lipid molecules rearrange to form more stable hydrophilic pores. This could also take place in a cell membrane. In addition, protein channels, pores, and pumps in these membranes are extremely sensitive to transmembrane electric field and become initiation sites for the electropores (Tsong 1990). In the cell membrane charges to electric dipoles of lipids, proteins, carbohydrates, and ions and the polarizability of these molecules make up the electric field. Therefore, electroporation occurs both in the liposomes and cell membranes, but the molecules affected by the applied field are not necessarily the same in these 2 systems (Tsong 1990). The gating potentials to the channel constituted by the proteins are in the 50 - mV range (Castro and others 1993).

Miller and others (1988) found that electroporation permits the uptake of DNA by mammalian cells and plant protoplasts

Table 3—Summary of *Escherichia coli* inactivation with PEF[a]

Source	Suspension media	Log reduction (max)	Treatment Vessel[b]	Process conditions[c]
Sale and Hamilton (1967)	0.1% NaCl	2	B	20 °C, 1.95 V/μm, 20μs, 10 Pulses
Hulsheger and Nieman (1980)	17.1 mM saline, $Na_2S_2O_3$, NaH_2PO_4/Na_2HPO, PH 7.0	3-4	B, 4 ml, d= 0.5 cm	<30 °C, 2.0 V/μm, 30μs, 10 pulses, t= 300μs
Hulsheger and others (1983)	Phosphate buffer, pH 7.0	3 stationary Cells, 4 Logarithmic Cells	B, 4ml, d= o.5 cm t= 1080 μs	2.0 V/μm, 36μs, 30 pulses
Dunn and Pearlman (1987)	Milk	3	B	43 °C, 3.3 V/μm, 35 pulses
Matsumoto and others (1991)	Phosphate buffer	5	B	4.0 V/μm, 4-10 μs, Exponential decay
Grahl and others (1992); Grahl and Markl (1996)	Sodium alginate	4-5	B, 25 ml, d= 0.5 cm	<45-50 °C, 2.5 V/μm, 5 pulses
Grahl and others (1992); Grahl and Markl (1996)	UHT milk (1.5% fat)	1	B, 25 ml, d= 0.5 cm	< 45-50 °C, 2.24 V/μm, 5 pulses 5.0 μF
Grahl and others (1992); Grahl and Markl (1996)	UHT milk (1.5% fat)	2	B, 25 ml, d= 0.5 cm	< 45-50 °C, 2.24 V/μm, 10 pulses 5.0 μF
Grahl and others (1992); Grahl and Markl (1996)	UHT milk (1.5% fat)	3	B, 25 ml, d= 0.5 cm	< 45-50 °C, 2.24 V/μm, 15 pulses 5.0 μF
Grahl and others (1992); Grahl and Markl (1996)	UHT milk (1.5% fat)	4	B, 25 ml, d= 0.5 cm	< 45-50 °C, 2.24 V/μm, 20 pulses 5.0 μF
Zhang and others (1994b)	Potato dextrose agar	3	B, 14 ml	15 ± 1°C, 4.0 V/μm, 3μs, 16 Pulses
Zhang and others (1994b)	Potato dextrose agar	6	B, 14 ml	15 ± 1°C, 4.0 V/μm, 3μs, 64 Pulses
Zhang and others (1994b)	Skim milk	0.5	B	15 ± 1°C, 4.0 V/μm, 3μs, 16 Pulses
Zhang and others (1994b)	Skim milk	3	B	15 ± 1°C, 4.0 V/μm, 3μs, 64 Pulses
Zhang and others (1994a)	SMUF	3	604 J, B, parallel plate, 25.7 ml, d= 0.95 cm	< 25 °C, 2.5 V/μm, 20 pulses
Pothakamury and others (1995)	SMUF	4	B, parallel plate, 1 ml, d= 0.1 cm	< 30 °C, 1.6 (1.2, 1.4, 1.6 tested) V/μm, 200-300 μs, 60 (20, 30, 40, 50, 60) pulses
Qin and others (1994)	SMUF	1.5	80 J/pulse, B, Parallel plate	< 30 °C, 4.0 V/μm, 8 pulses, oscillatory decay
Qin and others (1994)	SMUF	3	80 J/pulse, B, Parallel plate	< 30 °C, 4.0 V/μm, 8 pulses, oscillatory decay
Qin and others (1994)	SMUF	3	60 J/pulse, B, Parallel plate	< 30 °C, 4.0 V/μm, 4 pulses, monopolar
Qin and others (1994)	SMUF	3	60 J/pulse, B, Parallel plate	< 30 °C, 4.0 V/μm, 4 pulses, bipolar
Qin and others (1995c)	Skim milk	2.5	B, parallel plate, 14 ml	< 30 °C, 5.0 V/μm, 2μs, 62 pulses, square wave
Qin and others (1995c)	Skim milk	3.5	C, parallel plate	< 30 °C, 5.0 V/μm, 2μs, 48 pulses, square wave
Qin and others (1995c)	SMUF	3.6	C, parallel plate 8 cm^3 d= 0.51 cm	< 30 °C, 5.0 V/μm, 2μs, 48 pulses, square wave
Qin and others (1995a)	SMUF	7	C, coaxial, 29 ml, d= 0.6 cm, 0.2μF, 1 Hz	< 30 °C, 2.5 V/μm, ± 300 pulses, exponential decay pulse width 20 μs
Martin-Belloso and others (1997b)	Skim milk diluted with water (1:2:3)	Nearly 3	B, parallel plate, 13.8 ml, 0.51 cm	15 °C; 4.0 V/ μm; 6μs
Martin-Belloso and others (1997b)	Skim milk	2	C, parallel plate with flow-through Capability, 45 ml/sec, v= 8ml	15 °C; 4.5 V/ μm; 1.8μs 64 pulses
Martin-Belloso and others (1997a)	Liquid egg	6	C, coaxial, 11.9 ml, d= 0.6 cm, 0.5 1/min	<37 °C; 2.6 V/ μm; 4μs 100 pulses, color changes
Vega-Mercado and others (1996a)	Pea soup	6.5	C, coaxial, 0.5 1/min	>53 °C; 3.3 V/ μm; 2μs 30 pulses
Zhang and others (1995a)	Modified SMUF	9	B, parallel plate, 14 ml, d= 0.51 cm	
Pothakamury and others (1995)	SMUF	4	B, parallel plate, 12.5 ml, d = 0.5 cm	<30 °C; 16 V/ μm; 200-300μs Exponential decay
Pothakamury and others (1995)	SMUF	5	C, parallel plate	

[a] From Barbosa-Canovas and others (1999).
[b] B, batch; C, continuous.
[c] Temperature, peak electric field, pulse width, number of pulses and shape, and total treatment time (t).

because it induces transient permeability to the cell membrane. These researchers demonstrated the utility of high-voltage electroporation for the genetic transformation of intact bacterial cells by using the enteric pathogen *Campylobacter jejuni* as a model system. The method involved the exposure of a *C. jejuni* cell suspension to a high-voltage potential decay discharge of 5 - 13 kV/cm with a short treatment time ranging

between 2.4 - 2.6 μs in the presence of plasmid DNA. Electrical transformation of *C. jejuni* resulted in frequencies as high as 1.2×10^6 transformats per μg of DNA.

3.2. Inactivation Models

Hülsheger and Niemann (1980) were the first to propose a mathematical model for inactivation of microorganisms with PEF. Their model was based on the dependence of the survival ratio S (N/N_0 or the ratio of living cell count before and after PEF treatment) on the electric field intensity E according to the following expression:

$$\ln(S) = -b_E(E-E_c) \quad (1)$$

where b_E is the regression coefficient, E is the applied electric field, and E_c is the critical electric field obtained by the extrapolated value of E for 100% survival. The regression coefficient describes the gradient of the straight survival curves and is a microorganism-media constant. The critical electric field (E_c) has been found to be a function of the cell size (much lower for large cells) and pulse width (that is, with pulse width > 50 μs, E_c = 4.9 kV/cm; pulse width > 2 μs, E_c = 40 kV/cm). Hülsheger and others (1981) proposed an inactivation kinetic model that relates microbial survival fraction (S) with PEF treatment time (t) in the form of

$$\ln(S) = -b_t \ln(t/t_c) \quad (2)$$

where b_t is the regression coefficient, t is the treatment time, and t_c is the extrapolate value of t for 100% survival, or critical treatment time. The model can also be expressed as

$$S = \left(\frac{t}{t_c}\right)^{\frac{-(E-E_c)}{K}} \quad (3)$$

where t is treatment time, tc is critical treatment time, *Ec* is critical electric field intensity, and K is the kinetic constant. Table 4 shows K values calculated by fitting experimental data for the cited microorganisms (Hülsheger 1983). A small value for the kinetic constant [K] indicates a wide span in the inactivation rate curve and lower sensitivity to PEF, whereas a large value implies a steep decline or higher susceptibility to PEF. Lower E_c values would indicate less resistance to the PEF treatment.

Table 4 shows that E_c for gram-negative bacteria is lower than that for gram-positive, in accordance with the smaller PEF resistance of the former. The kinetic constant for the yeast *C. albicans* is smaller than for gram-negative and gram-positive bacteria, implying that yeast are more resistant to inactivation with PEF than bacteria. This result is inconsistent with results from other studies. The table also shows that *E. coli* cells in the log stage of growth have lower t_c and E_c and higher K than cells, which is in accordance with other studies. Correlation coefficients of the lines where high, indicating the model may have some future use.

A second model proposed by Peleg (1995) describes a sigmoid shape of the survival curves generated by the microbial inactivation with PEF. The model (equation 4) represents the percentage of surviving organisms as a function of the elec-

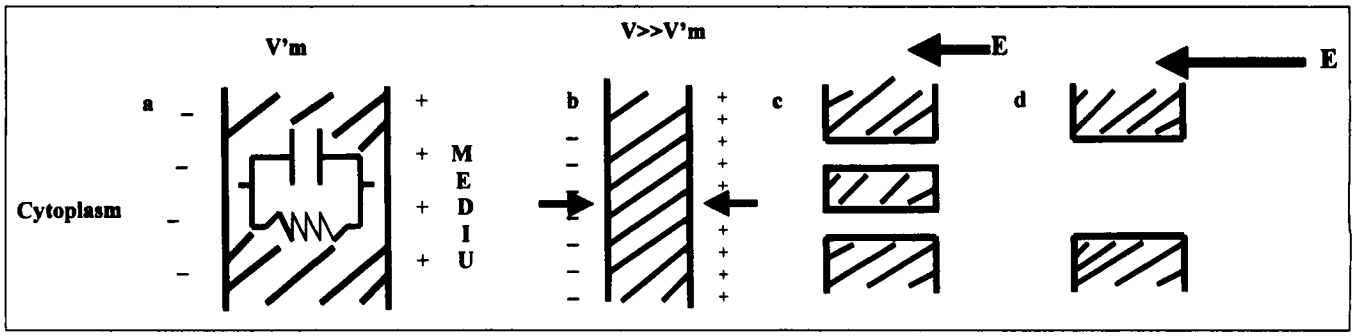

Fig. 5—Schematic diagram of reversible and irreversible breakdown. (a) cell membrane with potential V'm, (b) membrane compression, (c) pore formation with reversible breakdown, (d) large area of the membrane subjected to irreversible breakdown with large pores (Zimmermann, 1986)

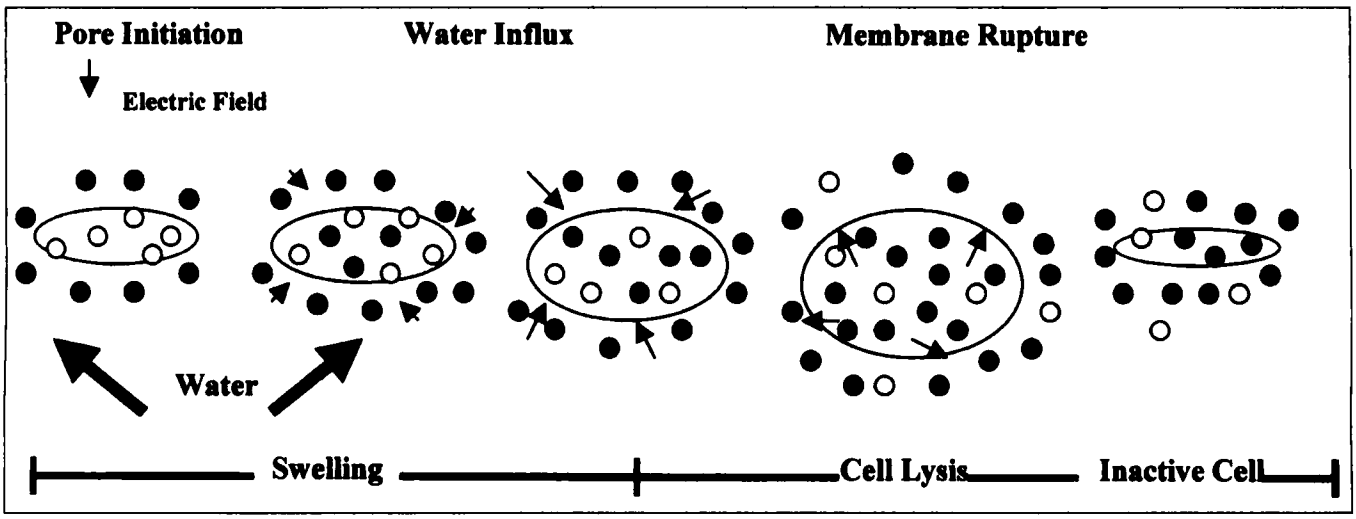

Fig. 6—Electroporation of a cell membrane (Vega-Mercado, 1996b)

tric fields and number of pulses applied. This model is defined by a critical electric field intensity that corresponds to 50% survival (E_d) and a kinetic constant (K_n, a function of the number of pulses) that represents the steepness of the sigmoid curve:

$$S = \frac{1}{1 + e^{\frac{E - E_d(n)}{K(n)}}} \quad (4)$$

Mathematically, about 90% inactivation is achieved within the critical electric field plus 3 times the kinetic constant. In this generalized model, E_d(n) and K(n) are algebraic functions that not only depend on the electric field but also on the number of pulses or treatment time. The

$$S = \frac{1}{1 + e^{\frac{E - E_d}{K}}} \quad (5)$$

model can be simplified by not considering the relationship between the electric field and the number of pulses:

A small value for the kinetic constant [K (n) or K] indicates a wide span in the inactivation rate curve and lower sensitivity to PEF, whereas a large value implies a steep decline or higher susceptibility to PEF. Lower E_d values would indicate less resistance to the PEF treatment.

Table 5 shows the kinetic constant for various microorganisms calculated using Peleg's equation. Experimental data was compiled from various published studies performed with those microorganisms and were fitted to the Peleg's model (Peleg 1995). Results indicate that the higher the number of pulses, the lower the E_d and kinetic constant K. The high regression coefficients for all the studies show the model has potential use to predict microbial inactivation.

Table 4—Kinetic constants of Hülsheiger's model for different microorganisms suspended in a Na_2HPO_4/KH_2PO_4 buffer with pH of 7.0.

Microorganism	E (kV/cm)	t (μs)	E_c (kV/cm)	t_c (μs)	K (kV/cm)	r (%)
Escherichia coli (4 h)[1]	4 - 20	0.07 - 1.1	0.7	11	8.1	97.7
E.coli (30 h)[1]	10 - 20	0.07 - 1.1	8.3	18	6.3	97.6
Klebsiella pneumonia	8 - 20	0.07 - 1.1	7.2	29	6.6	95.7
Pseudomonas auriginosa	8 - 20	0.07 – 1.1	6.0	35	6.3	98.4
Staphylococcus aureus	14 - 20	0.07 - 1.1	13.0	58	2.6	97.7
Listeria monocytogenes I	12 - 20	0.07 - 1.1	10.0	63	6.5	97.2
L. monocytogenes II	10 - 20	0.07 - 1.1	8.7	36	6.4	98.5
Candida albicans	10 - 20	0.14 - 1.1	8.4	110	2.2	96.6

(From Hulsheger and others 1983)
E, electric field; t, treatment time; E_c, critical electric field; t_c, critical time; K, kinetic constant; r, correlation coefficient of regression line; [1]Incubation time.

Table 5—Kinetic Constants of Peleg's model

Organism	Number of Pulses	E_d (kV/cm)	K (kV/cm)	r^2
Lactobacillus brevis	—	11.4	1.6	0.973
Saccaromyces cerevisiae	—	13.2	2.3	0.994
Staphylococcus aureus	—	14.1	2.0	0.991
Candida albicans	2	21.2	3.1	0.999
	4	15.3	3.1	0.993
	10	10.1	1.3	0.997
	30	7.5	1.2	0.999
Listeria monocytogenes	2	14.9	2.8	0.981
	4	12.7	2.0	0.994
	10	10.3	2.4	0.990
	30	8.5	2.0	0.999
Pseudomonas aeruginosa	2	12.9	2.6	0.982
	4	10.6	2.4	0.994
	10	8.3	2.1	0.990
	30	6.7	1.8	0.999

(from Peleg 1995)
E_d, electrical field when 50% of population is reduced; K, kinetic constant; r^2, regression coefficient

4. Validation/Critical Process Factors

4.1. Summary of Critical Process Factors

Extensive microbial inactivation tests have been conducted to validate the concept of PEF as a non-thermal food pasteurization process (Zhang and others 1994a, 1994b; Zhang and others 1995a, 1995b; Pothakamury and others 1995; Keith and others 1996, Marquez and others 1997; Qin and others 1995a, 1995b. 1995c; Vega-Mercado and others 1996a, 1996b; Qin and others 1998; Castro and others 1993).

High intensive pulsed electric field treatments produce a series of degradative changes in blood, algae, bacteria and yeast cells (Castro and others 1993). The changes include electroporation and disruption of semipermeable membranes which lead to cell swelling and/or shrinking, and finally to lysis of the cell. The mechanisms for the inactivation of microorganisms include electric breakdown, ionic punch-through effect, and electroporation of cell membranes (Qin and others 1994). The inactivation of microorganisms is caused mainly by an increase in their membrane permeability due to compression and poration (Vega-Mercado and others 1996b).

Castro and others (1993) reported a 5-log reduction in bacteria, yeast, and mold counts suspended in milk, yogurt, orange juice and liquid egg treated with PEF. Zhang and others (1995a) achieved a 9-log reduction in *E. coli* suspended in simulated milk ultrafiltrate (SMUF) and treated with PEF by applying a converged electric field strength of 70 KV/cm and a short treatment time of 160 μs. This processing condition is adequate for commercial food pasteurization that requires 6- to 7-log reduction cycles (Zhang and others 1995a).

In conclusion, numerous critical process factors exist. Carefully designed studies need to be performed to better understand how these factors affect populations of pathogens of concern.

4.2. Methods to Measure Critical Process Factors

PEF critical process factors may be monitored as follows:

- Pulse voltage waveform. The average electric field strength is calculated by dividing the peak voltage by the gap distance between the electrodes. A voltage probe and an oscilloscope make such measurement. Data logging is necessary to keep this critical process variable.
- Pulse current waveform. Pulse current should have a waveform very similar to that of the voltage waveform, different by a ratio, the load resistance. In the case of a partial breakdown, the ratio changes. A shunt resistor or a current monitor, such as a Pearson Coil, together with an oscilloscope may be used to measure the current waveform.
- Pulse duration time is determined from the voltage waveform.
- Pulse repetition rate.
- Voltage waveform, current waveform, duration time, and repetition rate may be logged by a computerized oscilloscope system.
- Temperatures at the inlet and outlet of each treatment chamber should be monitored. A Resistive Temperature Device (RTD) may be used on-line for such monitors. Temperature data may be used to estimate the energy delivery to the PEF chamber.
- Flow rate should be monitored because it determines the resident time within a treatment chamber, allowing the number of pulses applied to be determined.

In some continuous PEF processing systems, pressure should

also be monitored. An on-line pressure transmittor may be used for this purpose.

4.3. Microbial Surrogates

Currently, there is no 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. In PEF, as with other inactivation methods, the potential for injury and recovery exists. Experts should consider this possibility and choose the appropriate microbial enumeration methods. 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 Overarching Principles.

S. cerevisiae and *Candida* spp. are 2 microorganisms of particular relevance in spoilage of foods. Although their inactivation has been proven in many food models and foods, their susceptibility to PEF may prevent their use as a surrogate.

5. Process Deviations

5.1. Methods for Determining Process Deviations

Continuous monitoring of storage temperatures, pH, color, and acidity of PEF-treated and -untreated products will indicate any deviation of products from their standardized conditions. A data acquisition system is needed to monitor the number of pulses and the frequency applied to the food products. A digital oscilloscope is required to monitor the wave shape and the peak electric field. To ensure desirable temperature during PEF processing of foods, digital thermocouples or fiber optic probes must be used to record the temperature the entrance and exit of the PEF treatment chamber.

5.2. Methods to Assess Deviation Severity

5.2.1.Temperature sensors

Temperature sensors such as thermocouples are connected from the tubing at the entrance and exit of the PEF treatment chamber. A continuous recording of temperature will avoid undesirable temperature increases caused by overheating treatment electrodes inside the chamber.

5.2.2. Data acquisition system

A computer with data acquisition systems will monitor the entire system. Continuous recording of the number of pulses and frequencies will correct such deviations caused by malfunction of the high voltage power supply, which may lead to underprocessed product.

5.2.3. Automatic shut down

Aborting the pulser automatically from the computer will avoid damage to the chamber and electrode due to arcing. If there is no product leakage, the equipment can be restarted and the product can be reprocessed. Otherwise, it has to be discarded.

5.2.4. Sample deviation

Milk is a fluid containing proteins and minerals, such as calcium, iron, and magnesium, that are very likely to cause fouling on the electrode surface during PEF treatment. If the milk has a high level of microorganisms, this film may serve as a good substrate for microorganisms to reproduce and form a biofilm in the treatment chamber. Therefore, the efficiency of the pulser is lower and the milk will receive fewer pulses due to the clotting on the electrodes. To resolve this situation, and in order to attain the required processing conditions, optimization of the process has to be performed.

6. Research Needs

Despite significant developments in PEF technologies in the 1990s several areas need further research before the technology is applied commercially. These include:

- Confirming the mechanisms of microbial and enzyme inactivation.
- Identification of the pathogens of concern most resistant to PEF.
- Identification of surrogate microorganisms for the pathogens of concern.
- Development of validation methods to ensure microbiological effectiveness.
- Development and evaluation of kinetic models that take into consideration the critical factors influencing inactivation.
- Studies to optimize and control critical process factors.
- Standardization and development of effective methods for monitoring consistent delivery of a specified treatment.
- Treatment chamber design uniformity and processing capacity.
- Identification and application of electrode materials for longer operation time and lower metal migration.
- Process system design, evaluation, and cost reduction.

Glossary

A COMPLETE LIST OF DEFINITIONS REGARDING ALL THE TECHnologies is located at the end of this document.

Batch or static chamber. Chamber that treats a static mass of food in bulk or packaged. A chamber that processes a limited volume of food at one time.

Breakdown. Rupture of bacterial cell membranes with the application of an electric field

Capacitor bank. Network of 2 or more capacitors used to store the energy supply from a DC power source.

Co field flow. One possible configuration for a PEF continuous chamber

DC power supply. Electric device to deliver direct current to the capacitor bank.

Continuous chamber. Opposite to batch chamber, it processes liquid foods that are pumped between pulsing electrodes.

Electric field intensity or strength Average voltage (kV) divided by the distance between 2 electrodes (cm).

Electrical breakdown. An abrupt rise in electric current in the presence of a small increase in voltage. As a consequence, rupture of bacterial cell membranes may occur with the application of an electric field. This effect is more pronounced in pulsed electric field treatment. In microwaves, this can happen if operating at very low pressures, as in freeze-drying.

Electrical conductivity. Physical property of a food material that determines its ability to conduct electricity, expressed in Siemens per cm (S/cm).

Electroporation. Destabilization of the lipid bilayer and proteins of cell membranes, as well as the formation of pores induced when a microbial cell is temporarily exposed to high voltage electric field pulses.

Electrode gap. Distance (cm) between the inner and outer electrode inside PEF treatment chambers.

Input voltage. Voltage (kV) supplied from a DC power source.

Irreversible breakdown. Irreversible generation of pores in the bacterial cell membranes.

Peak voltage. Maximum voltage (kV) delivered by PEF system.

Pulse width or time constant. Duration of the pulse. For an

exponential decaying pulse, the resistance of the food times the capacitor capacitance gives a measure of the pulse width.

Pulse rate. Number of pulses per s or input frequency (1/s).

Reversible breakdown. Formation of reversible pores in the bacterial cell membranes.

Treatment time. The product of the number of pulses and the duration of the pulses, usually expressed in microseconds (μs).

Waveform/Waveshape. Type of electric pulses generated by the high-voltage pulser.

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Appendix

PEF Treatment Chambers

Static Chambers

a) U-shaped polystyrene. This model consists of 2 carbon electrodes supported on brass blocks placed in a U-shape polystyrene spacer (Fig.7). Different spacers regulate the electrode area and amount of food to be treated. The brass blocks are provided with jackets for water recirculation and controlling temperature of the food during PEF treatment. This chamber could support a maximum electric field of 30 kV/cm. A second chamber model designed by Dunn and Pearlman (1987) consists of 2 stainless steel electrodes and a cylindrical nylon spacer. The chamber is 2-cm high with an inner diameter of 10 cm, electrode area of 78 cm^2 and stainless steel electrodes polished to mirror surfaces (Fig. 8). Another model (Barbosa-Cánovas and others 1999) consists of 2 round-edged, disk-shaped stainless steel electrodes (Fig. 9). Polysulfone or Plexiglas was used as insulation material. The effective electrode area is 27 cm^2 and the gap between electrodes can be selected at either 0.95 or 0.5 cm. The chamber can support 70 kV/cm. Circulating water at pre-selected temperatures though jackets built into electrodes provides cooling of the chamber.

b) Glass coil static chamber. A model proposed by Lubicki and Jayaram (1997) uses a glass coil surrounding the anode (Fig. 10). The volume of the chamber was 20 cm^3, which requires a filling liquid with high conductivity and similar permittivity to the sample (media NaCl solution, s = 0.8 to 1.3 S/m, filling liquid water ~ 10^{-3} S/m) used because there is no inactivation with a non-conductive medium (that is, transformer silicon oil).

Continuous PEF Chambers

Continuous PEF treatment chambers are suitable for large-scale operations and are more efficient than static chambers.

a) Continuous chamber with ion conductive membrane. One design by Dunn and Pearlman (1987) consists of 2 parallel plate electrodes and a dielectric spacer insulator (Fig. 11). The electrodes are separated from the food by conductive membranes made of sulfonated polystyrene and acrylic acid copolymers. An electrolyte is used to facilitate electrical conduction between electrodes and ion permeable membranes. Another continuous chamber described by Dunn and Pearlman (1987) is composed of electrode reservoir zones instead of electrode plates (Fig. 12). Dielectric spacer insulators that have slot-like openings (orifices)

Fig. 9—Cross-section of a PEF static treatment chamber

Fig. 12—Continuous treatment chamber with electrode reservoir zones

between which the electric field concentrates and liquid food are introduced under high pressure. The average residence time in each of these 2 reservoirs is less than 1 min.

b) Continuous PEF chamber with baffles. This design consists of 2 stainless steel disk-shaped electrodes separated from the chamber by a polysulfone spacer (Fig. 13). The operating conditions of this chamber are: chamber volume, 20 or 8 ml; electrode gap, 0.95 or 0.51 cm; flow rate, 1200 or 6 ml/min (Barbosa-Cánovas and others 1999).

c) Enhanced electric continuous field treatment chambers. Yin and others (1997) applied the concept of enhanced electric fields in the treatment zones by development of a continuous co-field flow PEF chamber (Fig. 14) with conical insulator shapes to eliminate gas deposits within the treatment volume. The conical regions were designed so that the voltage across the treatment zone could be almost equal to the supplied voltage. Other configurations with enhanced electric fields are illustrated in Fig. 15 and 16. In these designs the flow chamber can have several cross-section geometries that may be uniform or non-uniform. In this type of chamber configuration, the first electrode flow chamber, the insulator flow chamber, the second electrode flow chamber, the conducting insert members, and the insulating insert members are formed and configured such that the electrode flow chamber and insulator flow chamber form a single tubular flow chamber though the PEF treatment device (Barbosa-Cánovas and others 1999).

d) Coaxial continuous PEF chambers. Coaxial chambers are basically composed of an inner cylinder surrounded by an outer annular cylindrical electrode that allows food to flow between them. Fig. 17 illustrates such a coaxial chamber. A protruded outer electrode surface enhances the electric field within the treatment zones and reduces the field intensity in the remaining portion of the chamber. The electrode configuration was obtained by optimizing the electrode design with a numerical electric field computation. Using the optimized electrode shape, a prescribed field distribution along the fluid path without an electric field enhancement point was determined. This treatment chamber has been used successfully in the inactivation of pathogenic and non-pathogenic bacteria, molds, yeasts, and enzymes present in liquid foods such as fruit juices, milk, and liquid whole eggs (Barbosa-Cánovas and others 1999).

Fig. 16—Treatment chamber with different electrode geometries and enhanced electric fields in the insulator channel

Fig. 17—Cross-sectional view of a coaxial treatment chamber

High Voltage Arc Discharge

GUSTAVO V. BARBOSA-CANOVAS, Q. HOWARD ZHANG, MERLE D. PIERSON, AND DONALD W. SCHAFFNER

Scope of Deliverables: This section covers early applications of electricity to pasteurize fluids. The use of arc discharge for liquid foods is unsuitable largely because electrolysis and the formation of highly reactive chemicals occur during the discharge. More recent designs show some promise for this technology, although this should be confirmed by independent researchers.

1. Definition, Description and Applications

HIGH VOLTAGE ARC DISCHARGE IS A METHOD TO PASTEURIZE liquid foods by applying rapid discharge voltages through an electrode gap below the surface of aqueous suspensions of microorganisms. Inactivation of microorganisms and enzymes contained in food products by electrical discharges began in the 1920s with the electropure process for milk. This process consisted of passing an electrical current through carbon electrodes and heating milk to 70 °C to inactivate *Mycobacterium tuberculosis* and *Escherichia coli* (Barbosa-Cánovas and others 1999). It was one of the first electrical techniques used by the food industry to pasteurize milk (Palaniappan and others 1990). When rapid high voltages are discharged through liquids, a multitude of physical effects (intense waves) and chemical compounds (electrolysis) are generated, referred to as electrohydraulic shock, which inactivate the microorganisms (Edebo and Selin 1968). Enzymes are also inactivated by high voltage arc discharges. Inactivation is attributed to oxidation reactions mediated by free radicals and atomic oxygen. There is no significant temperature increase during treatment by arc discharge. The major drawbacks of this electrical method, however, are contamination of the treated food by chemical products of electrolysis and disintegration of food particles by shock waves (Barbosa-Cánovas and others 1999). The method based on continuous high voltage arc discharges was not considered suitable for use in the food industry (Dunn and Pearlman 1987; Jayaram and others 1991). More recent designs show some potential; however, chemical reaction products need to be identified and results validated.

2. Inactivation of Microorganisms

ALLEN (1969) DISCLOSED AN ELECTROHYDRAULIC PROCESS for producing microbial antigens. In this U.S. patent, he tested his electrohydraulic process with *E. coli* ATCC 11229 suspended in 0.01M phosphate buffer of pH 7.2. After 200 shocks of 182 J/discharge to a spark gap of 1.6 mm at 4.5 kV, a 6-log reduction was observed in a 1.2-liter volume of the static treatment chamber.

Another study provided critical operational conditions such as the stored electrical energy for each discharge in the range of 2500 to 22000 J; electric current of at least 1500 A; and time of discharge of less than 1 millisecond, preferably of 50 to 300 microseconds (Wesley 1971). The study did not provide microbial test data.

Gilliland and Speck (1967a) found electrohydraulic treatment to be effective in inactivating at least 95% of the vegetative cells of *E. coli, Enterococcus faecalis, Micrococcus radiodurans, Bacillus subtilis,* and its spores. High voltage electrical impulses were discharged at a rate of 1/s. *E. faecalis* and *E. coli* were less resistant, whereas *M. radiodurans* and *B. subtilis* were more resistant to electrohydraulic shock. Beattie and Lewis (1925) demonstrated a lethal effect of electrical discharges on microorganisms suspended in milk when applied voltage used to treat food was increased from 3000 V to 4000 V.

Gilliland and Speck (1967b) used a double tank system to study the mechanical and thermal effects of shock waves separately. The high voltage discharge occurred in the lower chamber and was separated from the bacterial suspension in the upper chamber by a rubber diaphragm. Both chambers were completely filled to obtain the full effect of pressure pulses. No significant inactivation or metabolic injury was observed, indicating that mechanical action alone was not responsible for the bactericidal action. No significant amount of cell breakage was observed by phase microscopy and cell wall stain preparations. Other studies by the same authors (Gilliland and Speck 1967a) suggest that there are no thermal effects, since more than 90% of the population was killed within 10 discharges, when the increase in temperature was only 0.5 °C. Sytnik and Sytnik (1976) reported inactivation of the yeasts *Candida utilis, C. guilliermondii,* and *Saccharomyces cerevisiae* in foods using a high voltage DC by employing 40 kV and 50 electrical discharges. They concluded that the chemical reactions were the major contributors to bacterial inactivation by electrohydraulic shock, and that thermal effects were insignificant, since the temperature rise reported in many studies was only a few degrees.

According to FABCO Technologies (FABCO Technologies 1998), submerged electrical-arc technology may be an alternative to heat pasteurization of liquid foods, but these claims need to be validated independently. Recent experiments were reported to achieve a 5- to 7-log reduction of pathogenic bacteria in inoculated orange juice without affecting taste or color (FABCO Technologies 1998). This company reported that their arc discharge commercial-scale system reduced total microbial plate counts in fresh-squeezed grapefruit juice by more than 50 percent, boosting refrigeration shelf-life and fresh flavor to more than 100 d. Endotoxins and target microorganisms such as *Listeria monocytogenes, Clostridium sporogenes, Salmonella* Typhimurium, *Lactobacillus lactis, E. coli* O157:H7, *Aspergillus niger,* and *Penicillum digitatum* were tested in citrus juices using the electric pulsed power, reportedly achieving 5- to 7-log microbial and endotoxin reductions (FABCO Technologies 1998). The process was also reported to achieved 6- to 7-log reductions of pathogenic bacteria in milk. Their process consumes little energy compared to thermal pasteurization of juices and was reportedly to be more energy efficient than other non-thermal processes (that is, high pressure and pulse electrical fields) with juice-processing potential. The process was reported to have the potential to disinfect process water. It must be noted that there has been no other publication on the effectiveness of this process. Results reported by FABCO Technologies should be independently validated by other researchers.

3. Mechanism of Microbial Inactivation

In the early literature (Allen 1969; Wesley 1971; Sytnik and Sytnik 1976) the inactivation of microorganisms by high voltage arc discharge was reported to be related to the hydraulic shock wave generated by an electrical arc. More recent literature concluded that arc discharge prompted the formation of highly reactive free radicals from chemical species in foods, such as oxygen (Gilland and Speck 1967b; Vega-Mercado and others 1999). These free radicals are toxic compounds that serve to inactivate certain intracellular components required for cellular metabolism. Gilliland and Speck (1967b) found that lactic dehydrogenase, trypsin, and proteinases of *B. subtilis* were inactivated by electrohydraulic shock. They concluded that the enzyme inactivation was due to free radical oxidation reactions.

Palaniappan and Sastry (1990) presented an extensive literature review on the effect of electrohydraulic shock on the inactivation of microorganisms. They reported that bacterial inactivation was not due to heating, but mainly to irreversible loss of membrane function as a semipermeable barrier between the bacterial cell and the environment and to the formation of toxic compounds (oxygen radicals and other oxidizing compounds). In their review, it was concluded that chemical action is a complex effect and depends not only on the voltage applied but also on the type of microorganism, initial concentration of cells, volume of the medium used, distribution of chemical radicals, and electrode material (Palaniappan and Sastry 1990). Membrane damage was demonstrated by the lysis of protoplasts, leakage of intracellular contents, the loss of the ability of *E. coli* to plasmolyze in a hypertonic medium, and the release of galactosidase activity in a permease-negative mutant of *E. coli* (Sale and Hamilton 1967). Along with this, the number of cells of *Staphylococcus aureus* destroyed by high voltage discharges correlated with the numbers that could not be converted to spheroplasts. This result led to the conclusion that cell death was due to membrane damage.

4. Validation/Critical Process Factors

There is not enough information in the literature to determine critical process factors and to devise ways to handle deviations. In this regard, the following is a list of process variables that should be considered:

- Discharge field should be higher than 25 kV/cm to initiate breakdown of the gas phase.
- Discharge energy should be high enough to generate a sufficient quantity of ozone and/or UV irradiation for microbial inactivation to occur.
- Discharge repetition rate should be high enough to maintain a continuous ionization in the gas phase.
- Product should be sufficiently aerated to maintain a continuous gas bubble phase and ionization.

5. Research Needs

Early literature attempted to explain the phenomenon of microbial inactivation, and it was concluded that the hydraulic wave, that is, pressure, did not contribute to the bactericidal effect. A more recent development (FABCO Technologies 1998) focuses on the delivery of the treatment to the product while flowing through an arc plasma chamber. Oxygenation is a critical part of the process since the submerged arc discharge actually takes place within the gas bubbles. This partial breakdown of gas causes ionization, resulting in reactive ozone and UV radiation. In this regard, some research needs include:

- Understanding how delivery of highly reactive ozone and UV radiation by electric arc discharge inactivates microorganisms.
- Quantifying the inactivation kinetics and mechanisms.
- Identifying reaction process products generated during the submerged arc discharge process due to the highly reactive nature of ozone and UV radiation.
- Defining maximum allowable dose, in a manner similar to food irradiation.

Glossary

Electrohydraulic treatment. A rapid discharge of high voltage electricity across an electrode gap below the surface of aqueous suspensions.

High voltage electrical impulse. Application of high voltage discharges to a liquid medium in a very short time.

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Pulsed Light Technology

GUSTAVO V. BARBOSA-CANOVAS, DONALD W. SCHAFFNER, MERLE D. PIERSON, AND Q. HOWARD ZHANG

Scope of Deliverables: Processes designed for the bacterial inactivation of foods and packages with pulsed light are described here. Possible mechanisms of bacterial inactivation are also discussed as well as critical factors that influence the efficiency of the process. Also, the need for future research is addressed. Much of the information comes from industry sources; therefore, independently conducted research is needed to validate the effectiveness of pulsed light processes for food preservation.

1. Definition, Description and Applications

PULSED LIGHT IS A METHOD OF FOOD PRESERVATION THAT INvolves the use of intense and short duration pulses of broad-spectrum "white light". The spectrum of light for pulsed light treatment includes wavelengths in the ultraviolet (UV) to the near infrared region. The material to be treated is exposed to a least 1 pulse of light having an energy density in the range of about 0.01 to 50 J/cm^2 at the surface. A wavelength distribution such that at least 70% of the electromagnetic energy is within the range from 170 to 2600 nm is used. The material to be sterilized is exposed to at least 1 pulse of light (typically 1 to 20 flashes per s) with a duration range from 1 μs to 0.1 s (Dunn and others 1991). For most applications, a few flashes applied in a fraction of a s provide a high level of microbial inactivation.

Pulsed light is produced using engineering technologies that multiply power many fold. Accumulating electrical energy in an energy storage capacitor over relatively long times (a fraction of a s) and releasing this storage energy to do work in a much shorter time (millionths or thousandths of a s) magnifies the power applied. The result is a very high power during the duty cycle, with the expenditure of only moderate power consumption (Dunn and others 1995).

The technology for using light pulses is applicable mainly in sterilizing or reducing the microbial population on the surface of packaging materials, transparent pharmaceutical products, or other surfaces (Dunn 1996). Usually, the packaging material used in aseptic processing is sterilized with hydrogen peroxide, which may leave highly undesirable residues in the food or package (Barbosa-Cánovas and others 1997). Light pulses may be used to reduce or eliminate the need for chemical disinfectants and preservatives. Pulsed light may also be used to extend the shelf-life or improve the quality of produce.

MacGregor and others (1998) described the power light source suitable for inactivation of microorganisms. The test assembly used for experiments consisted of a rectangular PVC housing, a pulsed generator and associated switching and controlled circuitry as illustrated in Fig. 1. Internally, the light source was mounted 4-5 cm above the 2 sample holders, which were set 45 degrees to the horizontal. This arrangement permitted 2 petri dish samples to be located at right angles and irradiated simultaneously, with each sample receiving the same average exposure. The light source employed was a Hearaeus Noble light XAP Series that was constructed from a clear fused quartz tube filled with Xenon to pressure of 450 torr. The dimension of the tube was 3 mm dia and 7.5 cm arc length. The tube had an enriched light trigger along the length of the envelope and was capable of being operated with an average power of about 100 W. The pulsed generator was a single stage, inverting PFN Marx generator, which was charged to 30 kV dc. The generator was fired using a trigatron via a high voltage auto transformer. The source capacitance of the generator was 6.4 nF and the source impedance, when fired, was 6.25 Ohms.

The pulsed light process developed by PurePulse Technologies Inc. utilizes flashes of intense broad-spectrum pulsed light (BSPL) to sterilize pharmaceuticals, medical devices, packaging, and water. The spectrum closely mimics the spectrum of sunlight at sea level with an important difference: The system delivers a spectrum 20,000 times more intense than sunlight at the earth's surface during the pulse. The intense flashes of light are less than 1 millisecond in duration. Several pulses can be delivered per s, creating sterilized material at a greater speed than conventional processes. The efficacy of the process has been tested against a broad range of microorganisms, including bacteria (vegetative cells and spores), fungi, viruses, and protozoa (PurePulse Technologies Inc. 1999).

Figure 2 illustrates the treatment system. The basic electrical components are shown schematically in the upper portion of the Figure. In the lower portion of the Figure, energy per unit time diagrams are shown below the system components to emphasize the power magnification available through pulsed energy processing.

The lamp unit consists of 1 or more inert gas lamps arranged to illuminate the desired treatment area. A high voltage cable connects the lamps to the power unit. To flash the lamp, a high-voltage, high-current pulse of electricity is applied. The high current passing through the gas in the lamp causes it to emit an intense pulse of light that lasts a few hundred microseconds. The frequency of flashing is adjustable, allowing optimization of the process in conjunction with any particular processing speed. Systems are normally designed for each application to provide flexibility in the number of lamps, the flashing configuration, and the

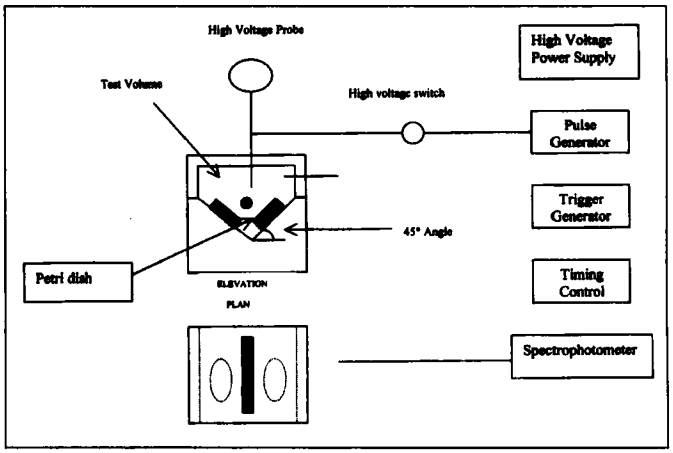

Fig. 1—Experimental facility for bacterial inactivation using a pulsed light generator

flash rate. Lamps can be flashed simultaneously or sequentially.

A different system using pulsed UV light for microbial control has been patented in the United States (Lagunas-Solar and Pyne 1994), with patent pending in Chile. This technology uses monochromatic excimer lamps at a wavelength of 247 nm rather than the xenon flash tubes described above. This technology is in commercial production in Chile and is being used on grapes exported to the United States.

2. Inactivation of Microorganisms

PULSED LIGHT PROVIDES SHELF-LIFE EXTENSION AND PRESERvation when used with a variety of foods. Because pulsed light applications are limited to the surfaces of products, most studies have tested the effectiveness of pulsed light on food or packaging surfaces. For instance, the process was reported to be effective to inactivate molds in a variety of baked goods and to extend their shelf-lives (Dunn and others 1995). Similarly, shrimp treated with pulsed light and stored under refrigeration for 7 d remained edible, while untreated shrimp showed extensive microbial degradation and were discolored, foul smelling, and not edible (Dunn and others 1995). More than 7-log cycles of *Aspergillus niger* spore inactivation resulted with a minimal number of pulsed light flashes with 1 J/cm^2 (Dunn and others 1991). A variety of microorganisms including *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Saccharomyces cerevisiae* have been inactivated by using 1 to 35 pulses of light with an intensity ranging from 1-2 J/cm^2 as illustrated in Fig. 3, 4, and 5. *Salmonella* serovars were reduced by 2-log cycles on chicken wings in samples inoculated with either 5 or 2 log/cm^2. *Listeria innocua* was reported to be reduced by 2-log cycles on hot dogs (inoculated with 3 or 5 log/wiener) after pulsed light treatment (Dunn and others 1995).

Curds of commercially dry cottage cheese inoculated with *Pseudomonas* spp. and treated with pulsed light with an energy density of 16 J/cm^2 and pulse duration of 0.5 ms reduced the microbial population by 1.5-log cycles after 2 flashes. The temperature at the surface of the curd increased by 5 °C (Dunn and others 1991). Sensory evaluation with trained panelists showed no effects of the treatment on the taste of the cheese. A combination of high-pressure wash and exposure to pulsed light was reported to reduce the psychrotroph and coliform population on the surface of fish tissue by 3-log cycles. The sensory attributes of the fish remained acceptable after 15 d of refrigerated storage (Dunn and others 1988).

Pulsed light was reported to be effective in eliminating microbial contamination from the surface of eggshells. Up to an 8-log reduction of *Salmonella* Enteriditis was achieved for commercial or raw eggs, which were treated with 0.5 J/cm^2 for 8 flashes (Dunn 1995). On the surface of different packaging materials inoculated with 10-1000 cfu/cm^2, a single light pulse inactivated *S. aureus* with an intensity as small as 1.25 J/cm^2, while *B. cereus* and *Aspergillus* spp. spores were inactivated with intensities greater than 2 J/cm^2 (Dunn and others 1991).

The inactivation of microorganisms on dry surfaces by pulsed light has been demonstrated using *Salmonella* Enteriditis ATCC 13076, *E. coli* O157:H7, *Salmonella* Typhimirium ATCC 13312, *Listeria monocytogenes* ATCC 15314, and *S. aureus* ATCC 6539, among other microorganisms. Information of the initial inoculation levels, though, is not available. The challenge studies are initiated by first inoculating the surface to be treated with a uniform layer of the test organism. This is accomplished by a spray inoculation method developed for this application by PurePulse Technologies Inc. (PurePulse Technologies Inc. 1999). Once the microbial layer is allowed to dry, it is exposed to treatment. Since the exposure is less than 1 s, treatment is quantified by the lamp output expressed as "fluence", the time integrated light energy per unit surface.

The ability of pulsed light technology to sterilize liquids after filling in containers has been tested against a broad range of microorganisms (Table 1). At a Japanese Congress (PurePulse Technologies Inc. 1999), Nissin Pharmaceutical presented a case study on the effectiveness of pulsed light technology for achieving sterility in liquids. Low-density polyethylene (LDPE) containers were filled with 20% (w/v) glucose, physiological saline, or water for injection using blow/fill/seal technology (Table 1). Subsequent to filling, samples were aseptically inoculated with the

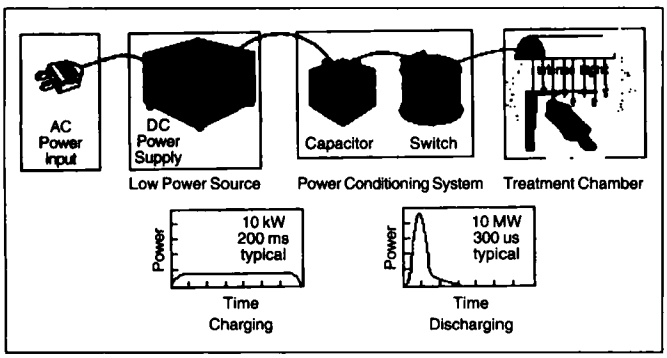

Fig. 2—Schematic diagram of a pulsed light treatment system

Fig. 3—Inactivation of (a) *Escherichia coli* vegetative cells using filtered spectrum-light and (b) *Bacillus subtilis* spores using full-spectrum light. (Dunn and others 1988)

Table 1—Treatment of Blow-Fill Seal Packaging using pulsed light

Organism	Inoculation (in 20 mL)
Bacillus subtilis var. niger spores	4.8×10^6
Bacillus pomilus spores	3.0×10^6
Bacillus stearothermophilus spores	4.4×10^6
Clostridium sporogenes spores	2.4×10^6
Aspergillus niger spores	1.4×10^6
Candida albicans	3.0×10^6
Deinococcus radiodurans	2.2×10^6
Staphylococcus aureus	5.6×10^6
Enterococcus faecalis	2.0×10^6
Escherichia coli	3.4×10^6
Salmonella choleraesuis	1.8×10^6
Pseudomonas aeruginosa	2.8×10^6

challenge microorganisms and treated with pulsed light under sterilizing conditions. Each set of 20 containers was tested for sterility by the membrane filter technique described in the Japanese Pharmacopoeia. Pulsed light was applied in 2 modes. In 1 mode, containers were treated using a lamp reflector that illuminated each container from above (single lamp/reflector or SLR mode). For SRL mode, 20 flashes at 1 J/cm^2/flash were used (Dunn and others 1991). In the second treatment mode, the containers were treated in a reflective cavity containing a single lamp, which was flashed 10 times (cavity treatment or CT mode). For the sterility test, 0.5, 5, and 15 ml samples were assayed directly for the presence of survivors using 20, 40, and 60 ml pour plates, respectively. In addition, 120 ml samples were assayed by filtration through a 0.45 mm filter.

Dunn and others (1991) reported that in the SLR mode, 36 out of 40 *A. niger* samples were sterile, and all 40 *Bacillus pumilus, B. subtilis,* and *Bacillus stearothermophilus* spore inoculated samples were sterile. No viable organisms were recovered from any of the 160 samples treated using the CT mode.

3. Mechanisms of Microbial Inactivation

THE LETHALITY OF THE LIGHT PULSES IS DIFFERENT AT DIFFERENT wavelengths. Therefore, the full spectrum or selected wavelength may be used to treat the foods. Wavelengths known to produce undesirable products in foods are eliminated by filtering through glass or liquid filters. Light pulses induce photochemical or photothermal reactions in foods. The UV-rich light causes photochemical changes, while visual and infrared lights cause photothermal changes. UV light has been shown to inactivate pathogens and indicator organisms (Chang and others 1985). The antimicrobial effects of these wavelengths are primarily mediated through absorption by highly conjugated carbon-to-carbon double-bond systems in proteins and nucleic acids (Jay 1992).

The mode of action of the pulsed light process is attributed to unique effects of the high peak power and the broad-spectrum of the flash. A primary cellular target is nucleic acids. Inactivation occurs by several mechanisms, including chemical modifications and cleavage of the DNA. The impact of pulsed light on proteins, membranes, and other cellular material probably occurs concurrently with the nucleic acid destruction. For example, the motility of *E. coli* ceases immediately after exposure to pulsed light. In additional studies, loss of motility of protozoan sporozoites was observed after pulsed light treatment of oocysts. As with any lethal physical agent, it is difficult to determine the actual sequence of events due to the possible "domino effect" (PurePulse Technologies Inc. 1999).

Experience suggests that shorter wavelengths in the UV range of 200-320 nm are more efficient inactivation agents than the longer wavelengths due to their higher energy levels. Because DNA is a target molecule for these UV wavelengths, it is thought that the one primary cause of killing microorganisms is through DNA structural changes (Farkas 1997). Conventional UV treatment primarily affects DNA by mechanisms that are reversible under certain experimental conditions. Cell repair systems are classified as either "dark enzymatic repair" or "light enzymatic repair" (PurePulse Technologies Inc. 1999). Experiments designed to test enzymatic repair of DNA using pulsed light have shown that this repair does not occur after pulsed light treatment. The magnitude of the damage caused by pulsed light may also be too massive for the repair mechanisms to be effective. It is conceivable the DNA repair system itself is inactivated as well as other enzymatic functions. In summary, the high energy and intensity of pulsed light are thought to amplify the known mechanisms of destruction of cellular components caused by individual wavelengths of light. The sum of the damage caused by the broad-spectrum light is thought to produce extensive irreversible damage to DNA, proteins, and other macromolecules.

4. Validation/Critical Process Factors

4.1. Critical Process Factors

Due to failure of light to penetrate opaque and irregular surfaces, there is generally less microbial inactivation with pulsed

Fig. 4—Inactivation of *Aspergillus niger* spores using full-spectrum light. (Dunn 1988)

Fig. 5—Inactivation of *Aspergillus niger* spores using filtered-spectrum light (Dunn and others 1988)

light, compared to other technologies. Light characteristics (wavelength, intensity, duration and number of the pulses), packaging and food attributes (type, transparency and color) are considered to be critical process factors. In the case of a fluid food, transparency and depth of the fluid column become critical factors.

Despite its minimal effectiveness with opaque foods, pulsed light has been reported to have limited ability to reduce microbial counts (about 1- to 4-log cycles) on eggs, including organisms inoculated onto the surface of eggs and then drawn into egg air pores by a temperature differential (Dunn and others 1995).

The lethality of the pulsed light increases with increasing light intensity or fluence (PurePulse Technologies Inc. 1999), although formulation of a model for dose-response is not currently possible. Data presented in Table 2 summarizes experiments examining the effect of pulsed light on spores from 3 different strains of *Bacillus* spp. and *A. niger*. The spores were exposed to 3 flashes from a single lamp with flashes of 0.5 J/cm^2, 0.75 J/cm^2, and 1.5 J/cm^2 per flash (PurePulse Technologies Inc. 1999). Microbial reduction increased with increasing light intensities.

Table 2—The effects of fluence on different microorganisms subjected to pulsed light treatment

	Recovery cfu/ml			
	Bacillus stearothermophilus	*Bacillus subtilis*	*Bacillus pumilus*	*Aspergillus niger*
0 J/cm^2	1.4 x 10^6	2.6 x 10^6	3.1 x 10^6	ND
0 J/cm^2	177	436	570	ND
0 J/cm^2	63	43	90	ND
0 J/cm^2	ND	ND	ND	ND

ND, not detectable

5. Process Deviations

IN THIS APPLICATION, EACH PULSED LIGHT SYSTEM IS DESIGNED with monitoring devices that capture lamp output (fluence) and the lamp current. The silicon photo-diode detectors measure fluence in the UV range wavelengths. The UV range is monitored because it is the most sensitive to variations in both the lamp drive and the optical characteristics of the lamp units. Due to the nature of the lamps and the lamp unit optics, a change in the UV output of the system will always be detected before any measurable changes occur in the full spectrum output. Therefore, monitoring the UV output is the best measure of overall system performance, even though all the wavelengths contribute to the microbiocidal effect. Should a flash fall below the validated minimum threshold, the pulsed light system can be programmed to automatically shut down to avoid underprocessing. Monitors are available to measure the full spectrum of light or specific wavelengths of interest. The lamp current determines the light intensity and spectrum. Monitors record the lamp current for each flash and have the capability of halting operations if an abnormal signal is detected.

6. Research Needs

A GREAT DEAL OF RESEARCH REMAINS TO BE DONE BEFORE pulsed light technology will be suitable for commercial use. Most results presented in this report should be confirmed by independent researchers. The following is a list of areas where relevant information is lacking:

- Identification of critical process factors and their effect on microbial inactivation.
- Suitability of the technology for solid foods and non-clear liquids where penetration depth is critical.
- Potential formation of unpalatable and toxic by-products.
- Resistance of common pathogens or surrogate organisms to pulsed light treatments.
- Differences between this technology and that of the more conventional UV (254 nm) light treatment.
- Mechanisms of microbial inactivation to determine whether they are significantly different from those proposed for UV light.
- Understanding of the mechanism and quantification of the benefit attributed to the pulse effect.

Glossary

A COMPLETE LIST OF DEFINITIONS REGARDING ALL THE TECHnologies is located at the end of this document.

Broad-spectrum light. For pulsed light technology, the ultraviolet, visible and infrared light wavelengths.

Energy density. See light fluence.

Light fluence. Energy delivered from a light source per unit area (Joules/ cm^2).

High voltage switch. Device used to trigger the delivery of high intensity light pulses to foods or packaging materials.

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Oscillating Magnetic Fields

GUSTAVO V. BARBOSA-CANOVAS, DONALD W. SCHAFFNER, MERLE D. PIERSON, AND Q. HOWARD ZHANG

Scope of Deliverables: This section reports the effects of magnetic fields on microbial populations. Mechanisms of inactivation and critical process factors are described. Results of microbial testing experiments are controversial. Consistent results concerning the efficacy of this method are needed before its potential use as a food preservation method is assessed.

1. Definition, Description and Applications

STATIC (SMF) AND OSCILLATING (OMF) MAGNETIC FIELDS HAVE been explored for their potential as microbial inactivation methods. For SMF, the magnetic field intensity is constant with time, while an OMF is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. The magnetic field may be homogeneous or heterogeneous. In a homogeneous magnetic field, the field intensity B is uniform in the area enclosed by the magnetic field coil, while in a heterogeneous field, B is nonuniform, with the intensities decreasing as distances from the center of the coil increases. OMF applied in the form of pulses reverses the charge for each pulse, and the intensity of each pulse decreases with time to about 10% of the initial intensity (Pothakamury and others 1993).

Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperatures in the range of 0 to 50 °C for a total exposure time ranging from 25 to 100 μs. Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material (Barbosa-Cánovas and others1998). Magnetic field treatments are carried out at atmospheric pressure and at moderate temperatures. The temperature of the food increases 2–5 °C. According to Hoffman (1985) exposure to magnetic fields causes inhibition in the growth and reproduction of microorganisms. OMF of intensity of 5 to 50 telsa (T) and frequency of 5 to 500 kHz was applied and reduced the number of microorganisms by at least 2-log cycles. Within the magnetic field of 5–50 T, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} to 10^{-3} eV (Hoffman 1985). OMF of this intensity can be generated using: (1) superconducting coils; (2) coils which produce DC fields; or (3) coils energized by the discharge of energy stored in a capacitor (Gersdof and others 1983). Inhibition or stimulation of the growth of microorganisms exposed to magnetic fields may be a result of the magnetic fields themselves or the induced electric fields. The latter is measured in terms of induced electric field strength and induced current density. To differentiate between electric field and magnetic field effects, a cylindrical enclosure containing cells and a medium that can be adapted to in vitro studies employing uniform, single-phase, extremely low frequency (ELF) magnetic fields is recommended.

2. Inactivation of Microorganisms

YOSHIMURA (1989) CLASSIFIED THE EFFECTS OF MAGNETIC fields on microbial growth and reproduction as (1) inhibitory, (2) stimulatory and (3) none observable. Pothakamury and others (1993) summarized the effect of magnetic fields on microorganisms as shown in Table 1.

Hoffman (1985) reported on the inactivation of microorganisms with OMF in milk, yogurt, orange juice, and bread roll dough. According to Hoffman (1985) only 1 pulse of OMF was adequate to reduce the bacterial population between 10^2 and 10^3 cfu/g. The intensity of the magnetic field required to achieve these effects varied between 2–25 T and a frequency range from 5–500 Hz.

A review of the literature shows that inconsistent results have been obtained on the effect of OMF on microbial growth (Table 1). In some cases OMF stimulated or inhibited microbial growth and, in others, it had no effect on microbial growth. The results presented in Table 1 show that, although not well understood, the effect of magnetic fields on the microbial population of foods may depend on the magnetic field intensity, number of pulses, frequency and property of the food (that is, resistivity, electrical conductivity, and thickness of the foodstuff).

3. Mechanisms of Microbial Inactivation

SMF OR OMF MAY HAVE SOME POTENTIAL TO INACTIVATE MICROorganisms in food. Pothakamury and others (1993) reported 2 theories to explain the inactivation mechanisms for cells placed in SMF or OMF. The first theory stated that a "weak" OMF could loosen the bonds between ions and proteins. Many proteins vital to the cell metabolism contain ions. In the presence of a steady background magnetic field such as that of the earth, the biological effects of OMF are more pronounced around particular frequencies, the cyclotron resonance frequency of ions (Coughlan and Hall 1990).

An ion entering a magnetic field B at velocity v experiences a force F given by:

$$F = q\vec{v} \times \vec{B} \quad (1)$$

Figure 1 shows the movement of a charged particle in a magnetic field. When v and B are parallel, F is zero. When v is normal to B, the ion moves in a circular path (Fig. 2). For other orientations between n and B, the ions move in a helical path (Fig. 3). The frequency at which the ions revolve in the magnetic field is known as the ion's gyrofrequency n, which depends on the charge/mass ratio of the ion and the magnetic field intensity:

$$n = q\,B / (2\pi m) \quad (2)$$

where q is the charge and m is the mass of the ion. Cyclotron resonance occurs when n is equal to the frequency of the magnetic field. At 50 μT, the resonance frequency of Na^+ and Ca^+ is 33.33 and 38.7 Hz, respectively. At cyclotron resonance, energy is transferred selectively from the magnetic field to the ions with n equivalent to frequency of the magnetic field. The interaction site of the magnetic field is the ions in the cell, and they transmit the effects of magnetic fields from the interaction site to other cells, tissues, and organs.

A second theory considers the effect of SMF and OMF on calcium ions bound in calcium-binding proteins, such as calmodulin.

Table 1 Effect of magnetic fields on microorganisms

Microorganism	Type of Magnetic field[a]	Field Strength (T)	Frequencyof pulse (Hz)	Effect	Reference
Wine yeast cell	Heterogeneous Smagnetic field	0.04	0	Growth inhibited when exposed for 5, 20, 25, 60, 120, or 150 min; no inhibition for 10, 15, 17 min exposure	Kimball (1937)
Wine yeast cell	Heterogeneous Smagnetic field	1.1	0	No effect for 5, 10, 20, 40 or 80 min exposure	Kimball (1937)
Serratia marcescens	Heterogeneous Smagnetic field	1.5	—	Growth rate remains same as in controls up to 6 h; growth rate decreases between 6 and 7 h and again increases between 8 and 10 h; at 10 h cell population same as in controls	Gerenscer and others (1962)
Staphylococcus aureus	Heterogeneous Smagnetic field	1.5	0	Growth rate increases between 3 and 6 h; then decreases between 6 and 7 h; cell population at 7 h is same as controls	Gerenscer and others (1962)
Saccharomyces cerevisiae	Heterogeneous Smagnetic field	0.465	0	Rate of reproduction reduced, incubated for 24, 48 or 72 h	Van Nostrand and others (1967)
Escherichia coli	Smagnetic field	0.3	0	Growth simulated	Moore (1979)
Halobacterium halobium, Bacillus subtilis	Smagnetic field	0.015 0.03 0.06	0	Growth inhibited	Moore (1979)
Pseudomonas aeruginosa, Candida albicans	Omagnetic field	0.015 0.03 0.06	0.1-0.3	Growth simulated; stimulation increases with increase in frequency	Moore (1979)
E. coli	Omagnetic field	0.15	0.05	Inactivation of cells when concentration was 100 cells/mL	Moore (1979)
Streptococcus themophilus in milk	Omagnetic field	12.0	6,000 (1 pulse)	Cell population reduced from 25,000 cells/ml to 970	Moore (1979)
Saccharomyces in yogurt	Omagnetic field	40.0	416,000 (10 pulses)	Cell population reduced from 3,500 cells/ml to 25	Hofmann (1985)
Saccharomyces in orange juice	Omagnetic field	40.0	416,000 (1 pulse)	Cell population reduced from 25,000 cells/ml to 6	Hofmann (1985)
Mold spores	Omagnetic field	7.5	8,500 (1 pulse)	Population reduced from 3,000 spores/ml to 1	Hofmann (1985)
Saccharomyces cerevisiae	Smagnetic field	0.56	0	Decreased growth rate; interaction between temperature and magnetic field only during the logarithmic phase	Van Nostrand and others (1967)

[a]Smagnetic field = static magnetic field; Omagnetic field = oscillating magnetic field

The calcium ions continually vibrate about an equilibrium position in the binding site of calmodulin. A steady magnetic field to calmodulin causes the plane of vibration to rotate, or proceed in the direction of magnetic field at a frequency that is exactly ½ of the cyclotron frequency of the bound calcium. Adding a "wobbling" magnetic field at the cyclotron frequency disturbs the precision to such an extent that it loosens the bond between the calcium ion and the calmodulin (Pothakamury and others 1993).

Hoffman (1985) suggested that the inactivation of microorganisms may be based on the theory that the OMF may couple energy into the magnetically active parts of large critical molecules such as DNA. Within 5–50 T range, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} to 10^{-3} eV. Several oscillations and collective assembly of enough local activation may result in the breakdown of covalent bonds in the DNA molecule and inhibition of the growth of microorganisms (Pothakamury and others 1993).

The work of San-Martin and others (1999) shows that an externally applied electromagnetic signal at frequencies close to a given resonance and parallel to an SMF (Fig. 4) may couple to the corresponding ionic species in such a way as to selectively transfer energy to these ions and thus indirectly to the metabolic ac-

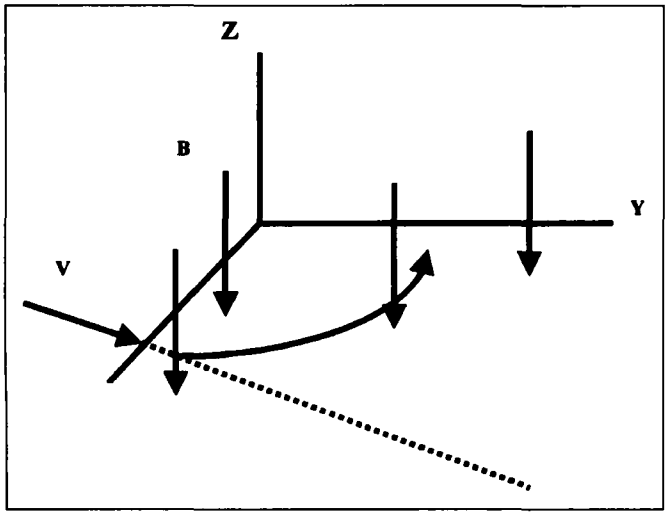

Fig. 1—Charged particle in a magnetic field

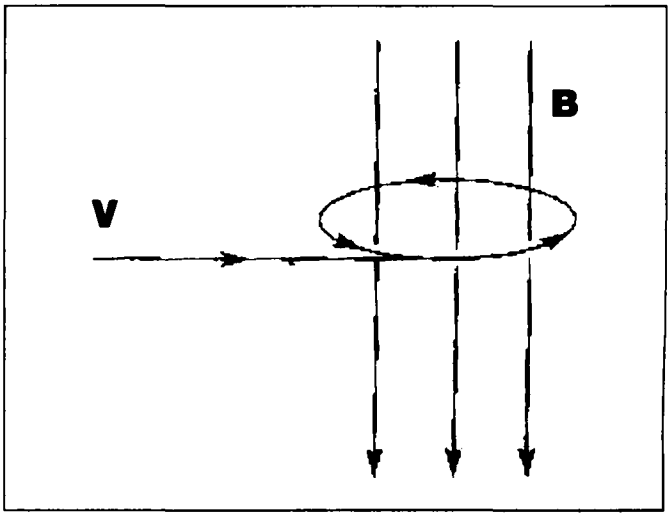

Fig. 2—Charged particle in a magnetic field when V is normal to B

tivities in which they are involved. The earth's total field ranges from 25 to 70 μT. Most of the slightly and double charged ions of biological interest have corresponding gyrofrequencies in the ELF range 10 to 100 Hz for this field strength.

4. Validation/Critical Process Factors

THE CRITICAL PROCESS FACTORS AFFECTING THE INACTIVATION of microbial populations by magnetic fields are not completely understood. Some factors believed to influence microbial inactivation include magnetic field intensity, electrical resistivity, and microbial growth stage.

4.1. Magnetic Field

Exposure to a magnetic field may stimulate or inhibit the growth and reproduction of microorganisms. A single pulse of intensity of 5 to 50 T and frequency of 5 to 500 kHz generally reduces the number of microorganisms by at least 2-log cycles (Hoffman 1985). High intensity magnetic fields can affect membrane fluidity and other properties of cells (Frankel and Liburdy 1995). Inconsistent results of other inactivation studies (see Table 1), however, make it impossible to clearly state the microbial inactivation efficiency of magnetic field or to make any predictions about its effects on microbial populations.

4.2. Electrical Resistivity

For microorganisms to be inactivated by OMF, foods need to have a high electrical resistivity (greater than 10 to 25 ohms-cm). The applied magnetic field intensity depends on the electrical resistivities and thickness of the food being magnetized, with larger magnetic fields intensities used with products with large resistivity and thickness.

4.3. Microbial Growth Stage

Tsuchiya and others (1996), working with homogeneous (7 T) and inhomogeneous (5.2 to 6.1 T and 3.2 to 6.7 T) magnetic fields, found a growth stage dependent response of *Escherichia coli* bacterial cultures. The ratio of cells under magnetic field to cells under geomagnetic field was less than 1 during the first 6 h of treatment and greater than 1 after 24 h. These authors also found that cell survival was greater under inhomogeneous compared with homogeneous fields. Based on the assumption that magnetic fields could act as a stress factor, cells collected after 30 min of incubation under magnetic field treatment (lag or early lag growth phase) or in the stationary phase after long-term magnetic field treatment were heated to 54 °C. No differences were observed between the treated and control samples. Little else is known about the effect of microbial growth stage on susceptibility to magnetic fields.

5. Process Deviations

DATA ACQUISITION SYSTEMS MUST BE INSTALLED IN THE PROCESSing area to monitor and control the power source, number of pulses, and frequencies applied to the food. Food composition, temperature, size of unit, among other factors also would require control and monitoring to assure constant treatments. Any deviation from the specified conditions such as temperature changes must be continuously recorded and appropriate responses taken. If the system shuts down or fails to deliver the described treatment during processing, the food must be reprocessed to assure quality and safety.

6. Research Needs

THERE IS A SIGNIFICANT LACK OF INFORMATION ON THE ABILITY of OMF treatment to inactivate pathogenic microorganisms and surrogates. A main area that needs to be elucidated is the confirmation that magnetic field treatment is an effective process to inactivate microbes. Once this is established, significant data gaps still must be closed before this technology can be safely and practically applied to food preservation. Some of the more significant research needs are:

- Identify key resistant pathogens.
- Establish the effects of magnetic fields on microbial inactivation.
- Elucidate the destruction kinetics of magnetic fields.
- Determine the mechanism of action of magnetic fields.
- Determine critical process factors and effects on microbial inactivation.
- Validate the process and evaluate indicator organisms and appropriate surrogates.
- Identify process deviations and determine ways to address them.

Glossary

A COMPLETE LIST OF DEFINITIONS REGARDING ALL THE TECHnologies is located at the end of this document.

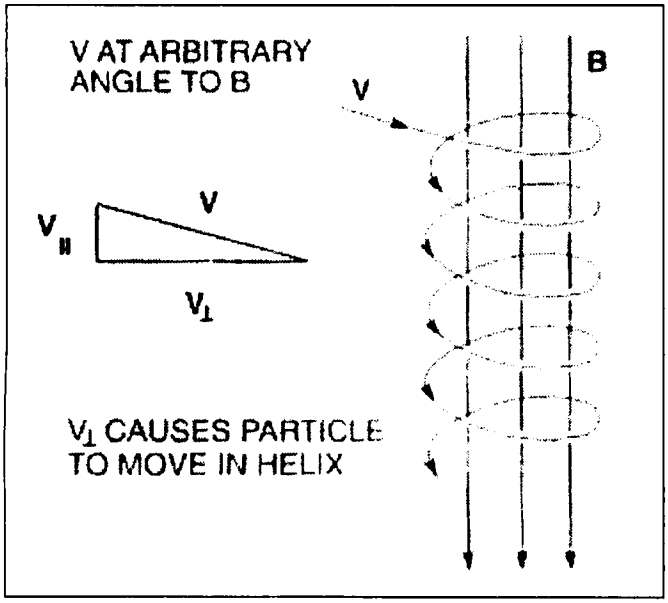

Fig. 3—Charged particle in a magnetic field when V makes an arbitrary angle with B

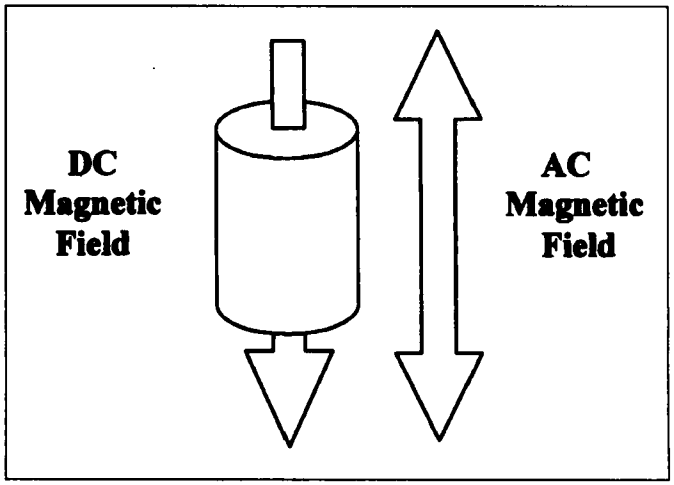

Fig. 4—Required AC and DC magnetic field orientation to achieve ion cyclotron

Cyclotron resonance. Phenomenon that occurs when the frequency of revolving ions induced by a specific magnetic field intensity is similar to the frequency of that magnetic field and parallel to it. In these instances, energy may be transferred to the ions, affecting cell metabolic activities.

Cyclotron. An accelerator in which particles move in spiral paths in a constant.

Dipole. For oscillating magnetic fields, a magnetic particle that contains a *north* and *south* magnetic pole.

Gyrofrequency. Frequency at which the ions revolve in a magnetic field.

Heterogeneous magnetic field. Magnetic field that exhibits a gradient depending on the nature of the magnet.

Homogeneous magnetic field. Magnetic field with a constant strength over space.

Magnetic flux density. Force that an electromagnetic source exerts on charged particles. Magnetic flux density is measured in Telsa (1 Telsa = 104 gauss).

Oscillating magnetic field. Fields generated with electromagnets of alternating current. The intensity varies periodically according to the frequency and type of wave in the magnet.

Sinusoidal Wave. A mode of propagation of the magnetic field.

Static magnetic field. Magnetic fields with a constant strength over time.

Telsa. Unit to express magnetic flux density (B). 1 Telsa (T) = 104 gauss.

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Ultraviolet Light

SUDHIR K. SASTRY, ASHIM K. DATTA, AND RANDY W. WOROBO

Scope of Deliverables: This section covers information, where available, on microbial kinetics and relevant safety considerations when ultraviolet (UV) radiation is used for the purposes of food preservation. The section pays particular attention to the growing interest in using UV light to treat fruit juices, especially apple juice and cider. It does not include pulsed UV, which is being actively investigated by Dr. Lagunas-Solar and co-workers at the University of Calif., Davis, nor does it address combinations of other alternative processing technologies with UV processing.

1. Definition, Description and Applications

ULTRAVIOLET PROCESSING INVOLVES THE USE OF RADIATION from the ultraviolet region of the electromagnetic spectrum for purposes of disinfection. Typically, the wavelength for UV processing ranges from 100 to 400 nm. This range may be further subdivided (Bolton 1999) into UVA (315 to 400 nm) normally responsible for changes in human skin that lead to tanning; UVB (280 to 315 nm) that can cause skin burning and eventually lead to skin cancer; UVC, (200 to 280 nm) called the germicidal range since it effectively inactivates bacteria and viruses, and the vacuum UV range (100 to 200 nm) that can be absorbed by almost all substances and thus can be transmitted only in a vacuum. The germicidal properties of UV irradiation are mainly due to DNA mutations induced through absorption of UV light by DNA molecules. This mechanism of inactivation results in a sigmoidal curve of microbial population reduction.

To achieve microbial inactivation, the UV radiant exposure must be at least 400 J/m^2 in all parts of the product. Critical factors include the transmissivity of the product, the geometric configuration of the reactor, the power, wavelength and physical arrangement of the UV source(s), the product flow profile, and the radiation path length. UV may be used in combination with other alternative processing technologies, including various powerful oxidizing agents such as ozone and hydrogen peroxide, among others.

Applications include disinfection of water supplies and food contact surfaces. Recently, interest has increased in using UV to reduce microbial counts in juices.

2. Pathogens of Public Health Concern

2.1. Pathogens of Concern in Apple Juice and Cider

Escherichia coli O157:H7 is a nonsporeforming bacterium that has been implicated in numerous foodborne illness outbreaks of contaminated, raw apple cider. *Cryptosporidium parvum* is a protozoan parasite that has the capability of forming oocysts and has also been implicated as the causative agent in a foodborne illness outbreak of apple cider in New York State.

2.2. Shape of Inactivation Curve

The shape of the curve for microbial inactivation by UV light is sigmoidal. The initial plateau is due to an injury phase of the microorganism in response to UV exposure. After the initial plateau, the maximum amount of injury has been surpassed; thus, minimal additional UV exposure would be lethal for microorganisms and survivor numbers rapidly decline. The end of the curve has a tailing phase due to UV resistance of the microorganisms and to experimental components, such as suspended solids that may block the UV irradiation.

Much of the prior literature has focused on UV disinfection of water supplies. The literature is insufficient to develop comprehensive microbial inactivation reaction kinetics data or models. There are, however, studies relative to the UV radiant exposure required to obtain a 4-log reduction of various microorganisms, using 254 nm UV light (Table 1). These data suggest that the log reduction is related to the UV radiant exposure (J/m^2). The curve has a linear section with a shoulder and tailing effects (Hoyer 1998). All tested organisms were reduced by 4-log cycles with UV radiant exposure less than 400 J/m^2 (Hoyer 1998). With some bacterial cells, photoreactivation, a repair mechanism that is enhanced by visible light in the blue spectral range, may occur. Photoreactivated cells show greater resistance to UV radiation than non-reactivated cells (Table 1).

3. Mechanisms of Microbial Inactivation

3.1. Pathogen Culture Maintenance and Growth

The *E. coli* strains were kept in 30% glycerol at −80 °C and, when needed, were inoculated onto Tryptic Soy Agar, grown at 37 °C for 18h with 250 rpm shaking agitation that provided the necessary aeration for good growth. The innoculation procedure was repeated 3 times before use (Worobo 1998).

3.2. Microbial Enumeration Conditions and Methods

Apple cider was inoculated with *E. coli* O157:H7 to achieve an initial level of approximately 7- to 8-logs cfu/ml. High inoculum levels are required to ascertain a 5-log or greater reduction in the target pathogen. The initial levels of *E. coli* O157:H7 were deter-

Table 1—UV 254 nm radiant exposure (J/m^2) for 4-log reduction (from Hoyer 1998)

Microorganism	Exposure required without photoreactivation (J/m^2)	Exposure required with photoreactivation (J/m^2)
Escherichia coli ATCC 11229	100	280
E. coli ATCC 23958	50	200
E. coli NCTC 5934	90	215
E. coli NCIB 9481	100	180
E. coli wild isolate	110	270
Enterobacter cloacae	100	330
Klebsiella pneumoniae	110	310
Citrobacter freundii	80	250
Yersinia enterocolitica	100	320
Salmonella Typhi	140	190
Salmonella Typhimurium	130	250
Serratia marcescens	130	300
Enterocolitica faecium	170	200
Vibrio cholerae wild isolate	50	210
Pseudomonas aeruginosa	110	190
Mycobacterium smegmatis	200	270
Polio virus (Mahoney)	290	
Rotavirus SA 11	350	
Staphylococcus aureus phage A994	380	

mined and then cider was processed through the CiderSure machine. Enumeration of aseptically drawn samples was done with Tryptic Soy Agar and incubation at 37 °C for 24 to 48 h (Worobo 2000). The primary reference used in identifying these standard enumeration methods is: Compendium of Methods for the Microbiological Examination of Foods, Third Edition. 1992. Edited by: Carl Vanderzant, Ph.D. and Don F. Splittstoesser, Ph.D. compiled by the American Public Health Association (APHA) Technical committee on Microbiological Methods for Foods

3.3. Inactivation Mechanism(s)

The germicidal properties of ultraviolet irradiation are due to the DNA absorption of the UV light, causing crosslinking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Miller and others 1999). Due to the mutated base, formation of the hydrogen bonds to the purine bases on the opposite strand is impaired. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death. The amount of crosslinking is proportional to the amount of UV exposure. The level of mutations that can be reversed depends on the UV repair system present in the target microorganism. Once the threshold of crosslinking has been exceeded, the number of crosslinks is beyond repair, and cell death occurs (Miller and others 1999). This phenomenon is reflected in the shape of the inactivation curve described before. The crosslinking threshold corresponds to the point of rapid decline after the initial plateau phase on the sigmoidal survival plot of UV exposure.

3.4. Methods to Measure, Quantify or Mathematically Model Pathogen Inactivation

Ultraviolet light from germicidal lamps was exposed to inoculated apple cider by passing it through the processing tube at a constant flow rate. The UV exposure was monitored using a sensor placed on the outermost exterior wall of the inner surface of the flow chamber. The D-value of *E. coli* O157:H7 due to ultraviolet exposure was calculated by increasing the exposures in the same apple cider. *Escherichia coli* O157:H7 survival was determined for each successive level of UV exposure and D-value was extrapolated from this multiple point data (Worobo, 2000).

4. Validation/Critical Process Factors

4.1. Identification and Description of Critical Process Factors

Little data is available on critical process factors affecting microbial inactivation. Pressure, temperature, and pH of the medium appear to have little effect on the absorption properties. Product composition, solids content, color, starches, and the overall chemistry of the food have a major effect. The effects of these individual factors are not available.

For disinfection of water, it is essential that all parts (each volume element) of the product receive a UV radiant exposure of at least 400 J/m^2 (at 254 nm) to reduce human pathogens and virus by at least 4-log cycles (Bernhardt 1994). Thus, the homogeneity of the flow pattern and the radiation field may have critical effects on disinfection. An additional critical factor is the transmissivity of the material being disinfected. If the material is highly transparent to UV light, disinfection may be more effective; however, turbid materials would attenuate and scatter UV radiation, resulting in less microbial inactivation. The thickness of the radiation path through the materials is also of importance, since attenuation increases with the length of passage; thus, the geometric configuration of process systems is critical. Another critical factor to consider is the UV wavelength used since this will affect microbial inactivation.

It is important to note some of the key terms used in the UV literature. The common expressions of irradiance, fluence rate, and fluence, and their distinction (Bolton 1999), are defined in the Glossary. It is important to point out that fluence rate refers to power passing through a sphere, while irradiance refers to power passing through a surface. For a parallel and perpendicularly incident beam, not scattered or reflected, irradiance and fluence rate become identical. For any UV source within a 3-dimensional volume, the integration of UV irradiance over the interior surface of the volume yields the UV power of the lamp. This is not true for UV fluence rate. The appropriate term for UV disinfection is "UV fluence rate" because a microorganism can receive UV power from any direction, especially when multiple lamps are used. The light dose or fluence is the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere. It is given by the average fluence rate times the exposure time in s. The term "UV dose" is often used in UV disinfection literature and represents UV exposure of a given organism in the germicidal range.

It may be noted that although Bolton (1999) prefers the use of UV fluence rate, the term "irradiance" is commonly used in the water disinfection literature. Indeed, it appears that in some of the German standards the word irradiance was used (Hoyer 1998). Nevertheless, the use of the more technically accurate concept of UV fluence rate is preferred.

4.2. Describe Methods to Measure/Monitor Critical Process Factors

In one system, sensors placed at a precise depth in the fluid stream measure the actual power through the material. The unit is programmed to deliver the same energy level to the material and, therefore, adjusts the exposure time to achieve the proper energy levels. In addition, if the material's absorption is very high, the unit does not operate.

UV irradiance may be monitored by radiometers, either thermal or photonic, UV sensors, or actinometer; however, the most reliable method is still biodosimetry, where the sample is inoculated with a surrogate microorganism and log reductions are counted at the outlet. The protocols for monitoring many of the other critical factors are not yet in place, and little or no information exists about them.

Detailed considerations monitoring UV radiant exposure have been described by Hoyer (1998) and are described below. Specific reference standards are in place in Germany for water disinfection.

To verify that UV radiant exposure exceeds 400 J/m^2 during operation requires a link that assures the conditions approved as sufficient by the biodosimetric test are met. This includes flow control, achieved with calibrated UV selective monitoring sensors mounted in a port at a defined position of the UV system. In a specified range of flow, the UV irradiance (W/m^2) at that port must be kept above the minimum level of the identical prototype during the biodosimetric test. It must allow for independent control with a reference sensor. This monitoring ensures that changes which may reduce the disinfection effect are detected and measures to handle insufficient disinfection are taken.

UV sensors must be standardized. For independent control, a single type of reference sensor should monitor the irradiance for every UV system. This includes a standardized size for a measuring port enclosed with a quartz glass panel of greater than 90 percent UV transparency. The manufacturer decides whether to use the standard port for system monitoring or an independent system sensor in the equivalent position, provided the signal is the same compared with a reference sensor. Prerequisites for a reproducible measurement of UV irradiance are a defined opening angle, spectral selectivity, and a defined physical size of UV sensors. These are detailed within German standards (DVGW 1997).

Because the mercury arc also emits radiation of higher wavelength, UV sensors must have a spectral selectivity radiation of 240-290 nm. Sensors sensitive to longer wavelengths would not detect the decay of microbiocidic radiation with sufficient liability because spectral absorption of FeO(OH), $MnO(OH)_2$, and humic acids decreases with increasing wavelength and because aging of UV lamps predominantly occurs in the low wavelength range, leaving the long-range radiation intensity almost unchanged. Therefore, radiation above 290 nm on stock contributes to more than 10 percent of the sensors' signal. Calibration of sensors for irradiance in W/m^2 is standardized at 253.7 nm, the resonant band of mercury. This standardization is done by actinometry with uridine, via a transfer standard calibrated by a national standardization body. The advantage of uridine is that its spectral absorption is nearly identical with the DNA. Radiant exposure is simply determined using a common laboratory spectrophotometer.

The sensor port position must also allow for the detection of water transmission changes to nearly the same degree as the decrease of irradiation from aging or deposits. The position depends on individual construction of UV units and is tested with changing UV lamps and changing the UV transmission properties of the water by adding a UV dye. Along with this procedure the sensitivity and spectral selectivity of the sensor is verified by comparison with the reference system.

Because one sensor can only monitor 1 or 2 lamps, in systems with several lamps all UV lamps need to be monitored through electrical parameters. Additionally, all UV lamps in a system unit must be of the same age and quality and the quartz of the glass panel must be guaranteed by the manufacturer and documented through identification marks.

4.3. Description of Microbial or Chemical Surrogates/ Indicators

Escherichia coli ATCC 25922 shows similar ultraviolet irradiation sensitivity to *E. coli* O157:H7 and therefore would seem like an appropriate surrogate microorganism. For the validation work, apple cider would be inoculated with the surrogate microorganism at high levels to determine the inactivation effect of UV on the surrogate microorganism. The validations were repeated in triplicate and the microbiological plating performed in duplicate (Worobo, 2000).

Hoyer (1998) describes the following set of recommendations for testing drinking water systems. According to Hoyer (1998) the minimum required irradiance (W/m^2) should be tested by biodosimetry measured by the system sensor (and in parallel with the reference sensor) at the minimum and maximum throughput as declared by the manufacturer. The biodosimetric test with minimum flow is necessary due to the incomplete mixing expected under laminar flow. The most important features of the biodosimetry test are the mixing devices before and after the UV unit, proper mixing to better than 99% of added germs, and representative sampling.

The disinfection performance at the minimum required irradiance at the sensor port of the UV system is tested in 2 ways (Hoyer 1998):

1. At the lowest 254 nm spectral absorption coefficient of water (less than or equal to 1 1/m), reducing the UV output of the lamps to the minimum required irradiance.

2. At full-lamp power and higher UV absorption of the water by adding a UV dye until the minimum required irradiance is read from the sensor.

To test both sets of conditions (that is, effects of varying lamp power or increasing UV absorption in the medium), a 4-log reduction with proper hydraulics and a 400 J/m^2 UV radiant exposure should be achieved. An ideal surrogate microorganism should have a 5-log reduction at 400 J/m^2. At present, such a germ is not available. Therefore a split test with 2 different bacteria is performed. Germs of *E. coli* ATCC 11229 being too sensitive to test for 400 J/m^2 but suitable to test for hydraulics, and spores of *Bacillus subtilis* ATCC 6633 (2.5-log reduction at 400 J/m^2) being too insensitive to test for hydraulics but suitable to test for 400 J/m^2 are used.

5. Process Deviations

PROCESS DEVIATIONS IN THIS TECHNOLOGY ARE EQUIPMENT-specific. Since no standard equipment design currently exists, the character of the deviation and the corrective action cannot be characterized in a simple manner within the scope of this document; however, the procedures described by Hoyer (1998) would appear to provide some hints.

To provide the required dosage, adjustment of the exposure time is done by computers linked to sensors. The scan rate is about 20 ms. This information is used for varying lamp power and/or changes in the absorption. UV irradiance measurement should be done at various points in the system, but protocols are not available at this time.

When the UV radiant exposure drops below a particular value, as described by the above descriptions, the product would have to be diverted and reprocessed or the system shut down.

6. Research Needs

Research that needs to be addressed includes:

- Effects of individual parameters, such as suspended and dissolved solids concentration.
- Identification of the pathogens most resistant to UV light
- Identification of surrogate microorganisms for pathogens.
- Development of validation methods to ensure microbiological effectiveness.
- Development and evaluation of kinetic models.
- Studies to optimize critical process factors.

Glossary

Fluence rate, Eo. The radiant power of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area dA, divided by dA (W/m^2).

Irradiance, E. The total radiant power of wavelengths incident on an infinitesimal element of surface area dS containing the point under consideration divided by dS. Note that for the receipt of light, "irradiance" is the counterpart to "emittance" for the emission of light (W/m^2).

Light dose, fluence, or UV radiant exposure, H. The total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area dA divided by dA. It is given by the average fluence rate times the exposure time in s. The term UV dose is often used in UV disinfection literature. It represents UV exposure of a given organism in the germicidal range (J/m^2).

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Contacts: Randy Worobo, Ph.D., Assistant Professor, Cornell University, New York State Agricultural Experiment Station W. North Street, Geneva, NY 14456. Phil Hartman, FPE Inc., 2453 Canandaigua Road, Macedon, NY 14502

Ultrasound

DALLAS G. HOOVER

Scope of Deliverables: This section describes the uses of ultrasound in the food industry. A general theory about the mechanism of microbial inactivation is presented. Data from inactivation of food microorganisms are scarce, and most applications of ultrasound involve its use in combination with other preservation methods. This review points to the need for more research on microbial inactivation in food systems when ultrasonication is used with other methods.

1. Introduction

1.1. Definition, Description and Applications

The textbook definition of ultrasound is energy generated by sound waves of 20,000 or more vibrations per second. Presently, most developments of ultrasonics (sonication) for food applications are nonmicrobial in nature (Hoover 1997). High frequencies in the range of 0.1 to 20 MHz, pulsed operation and low power levels (100 mW) are used for nondestructive testing (Gunasekaran and Chiyung1994). Ultrasonic excitation is being examined for nondestructive evaluation of the internal quality and latent defects of whole fruits and vegetables in a manner similar to the use of ultrasound for viewing the developing fetus in a mother's womb (Mizrach and others 1994). Floros and Liang (1994) noted the use of low intensity high-frequency ultrasound for improvement of food product/process monitoring due to the acceleration of diffusion. These industrial applications include texture, viscosity and concentration measurements of many solid or fluid foods; composition determination of eggs, meats, fruits and vegetables, dairy and other products; thickness, flow level and temperature measurements for monitoring and control of several processes; and nondestructive inspection of egg shells and food packages. Floros and Liang (1994) also listed direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction processes and enhancement of any process dependent upon diffusion. It is evident that ultrasound technology has a wide range of current and future applications in the food industry.

1.2. Mechanisms of Microbial Inactivation

The bactericidal effect of ultrasound is generally attributed to intracellular cavitation (Hughes and Nyborg 1962). It is proposed that micro-mechanical shocks are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrasonication process. These shocks disrupt cellular structural and functional components up to the point of cell lysis.

1.3. Summary of Microbial Inactivation Kinetics

The use of ultrasound alone to lyse microbial cells is a well-established laboratory method to extract intracellular components (Skauen 1976). Stumpf and others (1946) published an improved ultrasonic method to disintegrate a broader range of bacteria to obtain cell-free bacterial enzyme extracts efficiently and aseptically, acknowledging some difficulties with bacteria from genera *Sarcina, Micrococcus, Acetobacter* and the yeast, *Saccharomyces cerevisiae.*

In the laboratory, the efficiencies of lysis approach 100%; however, such cellular disruption is conducted as a small-scale batch operation, with temperature controlled by placement of the sample and its container in an ice bucket, and with immersion of the sonicator probe into a small volume of cells suspended in buffer. In an industrial food processing setting, less control of critical factors would prevent lysis efficiencies from reaching such high levels. In addition, the protective nature of foods to the ultrasonic inactivation of bacterial cells or spores is quite evident when comparing results from microorganisms suspended in buffer to those present in a food system (see Section 2). The heterogeneous nature of food with the inclusion of particulates and other interfering substances severely curtails the singular use of ultrasound as a preservation method. Although these limitations make the current probability of commercial development low, combination of ultrasound with other preservation processes (for example, heat and mild pressure) appears to have the greatest potential for industrial applications. Review of the literature indicates that no published information on microbial inactivation rates in foods is available. Such information is necessary to generate kinetic data.

1.4. Summary of Critical Process Factors

Critical processing factors are assumed to be the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food and the temperature of treatment. When ultrasound is used in combination with other processes, then the critical process factors of these methods must be taken into account.

2. Pathogens of Public Health Concern Most Resistant to the Technology

THE LIMITED CURRENT LITERATURE ON PROCESS EFFECTIVENESS indicates that all pathogens should be considered resistant to ultrasound, especially when ultrasound is the lone preservation treatment. Particular attention should be given when combining ultrasound with other methods with greater antimicrobial potency, because assumed optimal conditions can result in higher or lower inactivation rates of target microorganisms than expected.

2.1. Critical Process Factors When Used in Combination with Other Treatments

Survey of the literature shows that enteric gram-negative pathogens have been targeted with ultrasound in perishable animal-derived foods, such as poultry and milk (Lilliard 1994). In these instances, the application has most often been in a liquid environment. For example, Lee and others (1989) reduced populations of salmonellae by approximately 4-log cycles in peptone water using a 10-min treatment; however, in chocolate milk, a 30-min ultrasonic treatment only lowered the number of salmonel-

lae by 0.8-log cycle, suggesting that the chocolate milk offered significant protection against microbial inactivation.

Attempts have been made to reduce salmonellae attached to poultry skin. Lilliard (1993) studied the effects of sonicating poultry in chilled baths containing chlorinated water. Reductions of *Salmonella* in the range of 2.5 to 4-log cycles were obtained with a combination of immersion in chlorinated water and sonication compared to reductions of less than 1-log when using immersion in chlorinated water alone. Sonication alone reduced the counts by only 1-1.5-log. Results were similar in the examination of pre- and post-chill broiler drumsticks treated with ultrasonic energy in 1% lactic acid at pH 2.0 and 4.0 for 0.5, 2, and 3.5 min. After 0, 7, and 15 d, aerobic plate counts showed no significant effect of ultrasonification (Sams and Feria 1991).

Ordoñez and others (1984) combined ultrasound of 20 kHz/160 W using a cell disrupter with heating over a range of 5 to 62 °C for the inactivation of *Streptoccocus faecium* and *Streptoccocus durans*. They found that the combination of ultrasound and heat applied together was significantly more effective in inactivating these bacteria than the 2 methods used alone. Compared to a singular heat treatment, the simultaneous use of ultrasound and heat reduced the populations of thermoduric enterococci approximately 1 additional log cycle. Data presented in this paper evaluated the application using cell suspensions in 0.1 M dimethylglutaric acid buffer (pH 6.6). Although no food system was tested, the authors suggested that a milk pasteurization process incorporating ultrasonic treatment would allow for reduction of processing temperature and time.

A subsequent work by Ordoñez and others (1987) examined a similar application (referred to as thermoultrasonication) against survival of a strain of *Staphylococcus aureus* suspended in 0.05 M phosphate buffer (pH 6.8) and UHT milk. In this work, the combined process reduced D-values by 63% in the buffer as compared to the D-values of the heat treatment alone, and by 43% when tested in UHT milk. (These percentages represent less than a 10-fold reduction in the time in min to reduce *S. aureus* 1-log cfu/ml since a 1-log reduction represents a 90% reduction.)

Spore suspensions of *Bacillus subtilis* were targeted by Garcia and others (1989) employing thermoultrasonication in the temperature range of 70 to 95 °C. Distilled water, glycerol and milk were used as the treatment media. Ultrasound alone had no effect, but thermoultrasonication reduced the spore population by 63 to 73% (<1-log cycle cfu/ml) in glycerol and by 40 to 79% in milk. In distilled water the reductions ranged from 70 to 99.9% (3-log cycle cfu/ml). The effect of thermoultrasonication was dramatically diminished as the temperature of the treatment approached 100 °C. The optimum temperature for maximum inactivation of spores of *B. subtilis* under the experimental conditions was 70 °C. The mechanism for this phenomenon was unclear.

Raso and coworkers examined the response of *Yersinia enterocolitica* and spores of *B. subtilis* to a combination of heat, pressure and ultrasound. For the *Y. enterocolitica* study, cells were suspended in citrate-phosphate buffer (pH 7.0) (Raso and others 1998a). They used combined ultrasound and static pressures [manosonication (MS)] as well as heat/ultrasound and pressure [manothermosonication (MTS)] against *Y. enterocolitica*. At ambient temperature and pressure, the effect of ultrasound on *Y. enterocolitica* was insignificant. Moderate pressures of 600 kPa did not affect the survival of *Y. enterocolitica* to heat. Heat and ultrasound under pressure functioned independently. It appeared that the individual contributions of heat and ultrasound under pressure to the total effect of MTS depended primarily upon the temperature. Above 58 °C, any added inactivation caused by pressure disappeared. These results suggest that inactivation is not a simple additive reaction of the 3 treatment types. Optimal inactivation using these 3 methods requires a trial-and-error approach until the mechanisms of inactivation are resolved. It was noted that MS treatment resulted in cellular disruption. D-values recorded for *Y. enterocolitica* ATCC 9610 were 1.39 min at 59 °C, 1.5 min for the highest ultrasound setting (an amplitude of 150 μm at 20 kHz), and 0.28 min for an MS treatment of 300 kPa and 150 μm at 30 °C. The latter treatment was similar to an MTS treatment at 63 °C. The same authors (Raso and others 1998b) found that a 12-min treatment of 500 kPa and 117 μm at 20 kHz killed approximately 99% (2-log reduction) of a spore suspension of *B. subtilis* ATCC 9372 in McIlvaine citrate-phosphate buffer (pH 7.0). The sporicidal effect of MS treatments depended upon the static pressure, amplitude of ultrasonic waves and the treatment temperature. Above 500 kPa, additional increments of pressure magnitude did not increase the amount of spore inactivation. In the range of 70 to 90 °C, combination with 20 kHz, 300 kPa, and 117° m for 6 min had a synergistic effect on spore inactivation. Although the authors point out the possible application of MT and MTS as a preservation system for highly heat-sensitive liquids, no food system was investigated as a test medium in either of these studies.

A focused 1-MHz ultrasound transducer, capable of generating a spatial peak pulse average intensity of 500 W/cm^2 was used to treat culture broths of *Escherichia coli* containing microbubbles by Vollmer and others (1998). It was found that stress response was induced in *E. coli* and under some conditions, caused death. They also reported that stationary-phase cells were more resistant to sonication than those in exponential-phase growth stage. The intent of the work was not the development of ultrasound technology as a food preservation method, but rather to study stress in bacteria and perhaps to develop the technology to treat drinking water.

The articles overviewed here demonstrate that ultrasound lacks the power and versatility to inactivate microorganisms reliably for purposes of food preservation; however, ultrasound may be used in combination with other preservation processes primarily to enhance microbial inactivation in foods. Such applications will require further exploration (for example, validation studies) of important synergistic effects that are relevant for industrial use. In conclusion, ultrasound technology has the potential for future use as a preservation process; however, food systems present a very challenging environment for ultrasound to achieve the degree of microbial inactivation necessary for practical use. At the present and probably for the next several years, its applications in this area are not commercially feasible. Also, an important component of research that has received little notice with this technology is the possible effects of ultrasound (developed for food preservation purposes) on the sensory quality of the food.

3. Mechanisms of Inactivation

In general, a relatively low number of studies employ ultrasound for microbial inactivation. As stated above in Section 1.3, the mechanism of inactivation of vegetative bacteria appears to be intracellular cavitation. Maximum effectiveness results in cellular lysis. For spores, the mechanism is not clear. Cavitation must play a role, but it is an auxiliary one since ultrasound alone has no effect on spores. The other co-treatments have a main effect in any spore inactivation. Inactivation mechanisms of ultrasound used in combination with other treatments are not understood. Also unknown are ways to determine the occurrence of ultrasound-induced injury and repair, and to predict the effects of process variables and post-treatment storage of food products treated with ultrasound in combination with other inactivation methods. In the literature, conventional plating methods specific for the organism under examination have been used to enumerate microorganisms in studies involving ultrasound.

At the current state of commercial development for purposes of food preservation, ultrasound has potential to enhance the effectiveness of other processing methods. No mathematical model has been formulated for the inactivation of microorganisms by ultrasonic methods.

4. Validation/Critical Process Factors

SUCH IS THE CURRENT STATE OF LITERATURE REGARDING THE application of ultrasound as a preservation process that, qualifying, prioritizing, and quantifying its critical process factors is by assumption or implication. Factors that appear to substantially affect the destruction of microorganisms by ultrasound are the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food and the temperature of treatment. When ultrasound is used in combination with other processes, the critical process factors of these methods must be taken into account. For example, the presence of disinfectants or preservative compounds and levels of static pressure, irradiation, or electrical energy are critical process factors when hurdle treatment is the processing approach. Further maturity of ultrasonic processing in the food industry will define its critical process factors.

5. Process Deviations

AS SHOWN IN THE REVIEW OF THE LITERATURE AND DUE TO THE limited extent of research in the area, critical process factors and, therefore, possible process deviations are not well understood.

6. Research Needs

IT IS EVIDENT FROM THIS REVIEW THAT FURTHER RESEARCH IS needed to determine the feasibility and usefulness of ultrasound as a food preservation method or supplementary treatment. The main areas to be addressed are:

- Determination of the effect of ultrasound on microbial inactivation efficiency when used with other processing technologies (high pressure, heat, or others).
- Identification of mechanisms of microbial inactivation when used in combination with other technologies.
- Identification of critical process factors when ultrasound is used in hurdle technology.
- Evaluation of the influence of the food properties, such as viscosity and size of particulates, on microbial inactivation.

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Pulsed X-rays

GUSTAVO V. BARBOSA-CANOVAS, DONALD W. SCHAFFNER, MERLE D. PIERSON, AND Q. HOWARD ZHANG.

Scope of Deliverables: This section reviews current applications of X-ray technology for food processing. The advantages of X-rays over other types of radiation are described. A study on the inactivation of *Escherichia coli*, a pathogen of concern, with pulsed X-rays in ground meat is described. Little is known about the inactivation kinetics when X-rays or pulsed X-rays are used for microbial inactivation. Comprehensive research needs to be done to validate the potential use of X-rays in food preservation.

1. Definition, Description and Applications

1.1. X-rays

Several studies have compared the effects of electron beam, gamma rays and X-rays, but comparison between these technologies is inconclusive due to differences in the doses applied (Thorne 1991). It has been suggested that there may be differences in the free radicals formed by these different processes (Taub and others 1979). Electrons have a limited penetration depth of about 5 cm in food (Josephson and Peterson 1982), while X-rays have significantly higher penetration depths (60 – 400 cm) depending upon the energy used (Curry and others 1999).

1.2. Pulsed X-rays

Pulsed X-ray is a new technology that utilizes a solid state-opening switch to generate electron beam X-ray pulses of high intensity. The Institute of Electrophysics in Russia developed a novel nanosecond opening switch in the late 1980's that could deliver power pulses in the gigawatt range, open at voltages of 100s of kV, and operate repetitively (Curry and others 1999). Opening times range from 30 ns down to a few nanoseconds. Repetition rates have been demonstrated up to 1000 pulses/s in burst mode operation. The specific effect of pulsed in contrast to non-pulsed X-rays has yet to be investigated.

Electrically driven radiation sources that switch off when the radiation is no longer needed are easier to incorporate into existing food processing plants (Martens and Knorr 1992). In contrast, radionuclide sources require permanent massive concrete shielding to protect workers and the environment from their permanent radiation. The practical application of food irradiation in conjunction with existing food processing equipment is further facilitated by: (1) the possibility of controlling the direction of the electrically produced radiation; (2) the possibility of shaping the geometry of the radiation field to accommodate different package sizes; and (3) its high reproducibility and versatility.

Potentially, the negative effects of irradiation on the food quality can be reduced. The radionuclides Co-60 and Cs-137 are produced by neutron bombardment of Co-59 and Cs-136 as a fission fragment of a nuclear power reactor operation. They emit g-radiation of discrete energy. By contrast, the linear induction electron acceleration (LIEA) generates broad spectrum ionizing radiation by targeting the accelerated electron beam to collide with a heavy metal converter plate. This plate converts the electron beam in X-rays with a broad-band photon-energy spectrum. Then, by filtering the energy spectrum of the radiation, high-energy, highly penetrating radiation is produced, resulting in smaller variations in dose uniformity of food packages (Mertens and Knorr 1992) and higher quality. LIEA can deliver dose rates many orders of magnitude higher than possible with Co-60 sources. Consequently, ultra-short, high-intensity radiation treatments can be applied, resulting in higher local radical concentrations and favoring radical-radical recombination reactions. This reduces the diffusion of radical species, which are thought to be responsible for undesirable effects of irradiation on food quality.

Curry and others (1999) used pulsed X-rays to produce up to a 3-log reduction of *E. coli* O157:H7 in ground beef. The system consists of an X-ray accelerator with a thyristor-charging unit, a magnetic pulse compressor, a solid state opening switch, an electron beam diode load and an X-ray convertor (Fig. 1). The thyristor charging unit converts 3 phase (ϕ), 240 V – 440 V power to direct current. A thyristor capacitor charging circuit is used to charge the magnetic pulse compressor (Fig. 2). A 2-stage circuit compresses and sequentially steps-up the voltage pulse before it is used to charge an inductive load or inductance L- as illustrated in Fig. 2. Energy from capacitor C3 is transferred from the inductive load in approximately 100 ns. Upon current reversal through L-, the reverse current through the solid state-opening

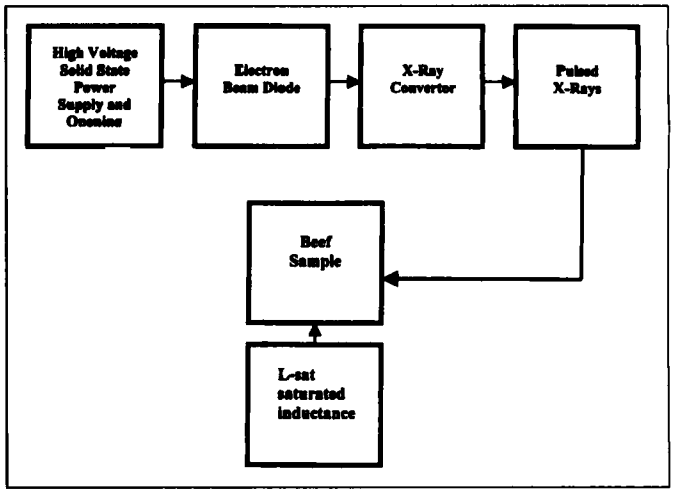

Fig. 1—Schematic flow diagram for pulsed X-rays

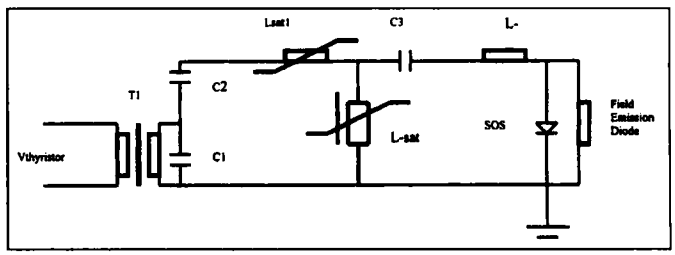

Fig. 2—Electrical circuit for solid state opening switch accelerator system. L-sat, saturated inductance; L, inductance C1,2,3, capacitors; T1, transformer; SOS, switch

switch (SOS) rises to tens of kiloamperes. At current reversal, the SOS opens 5-10 ns, generating a 220 keV pulse. The 220 keV, 500 A, 40 ns pulse is delivered to a field emission diode. A prototype accelerator was constructed to operate only on X-ray mode, even though it can be operated either in the pulsed X-ray mode or electron beam mode. A convertor was installed on the accelerator, and the electron beam was converted to pulsed X-rays to allow thick samples to be processed.

2. Pathogens of Public Health Concern

Bremssthrahlung X-rays have been used to eliminate 99.9% of *E. coli* O157:H7 added to ground beef prior to treatment (Curry and others 1999). A high peak rate of $10^7 - 10^8$ rads/sec X-ray pulses were used. The study compared X-rays with gamma rays from a cobalt-60 source. Doses in the range of 0.2 to 0.4 and 0.7 to 0.9 KGy were used. The results showed a slighly higher inactivating rate (6%) per log of reduction for X-rays in contrast to gamma radiation.

X-ray irradiation has been used to eliminate *Salmonella* Senftenberg 775W from turkey carcass. The food was irradiated using a General Electric Maxitron 300 X-ray machine, operated at 300 kV peak, 20 MA with a half layer 2.8 mm copper, delivering 268 rads/min at an effective energy of 49 kV (Teotia and Miller 1975). Each carcass or drumstick was placed in the center of the X-ray beam to obtain as complete irradiation of the carcass or drumstick as possible. Each carcass or drumstick was irradiated continuously for 2 h to give a total exposure of 30,000 to 80,000 rads. The temperature in the X-ray room varied between 20 and 24 °C. Results showed that 80,000 rads of X-ray failed to eliminate the test organism on whole turkey carcasses but reduced the test organism to an undetectable level on turkey drumsticks. Each irradiated carcass or drumstick was aseptically transferred from its packaging to a sterile stainless steel container. As the drumsticks or carcasses were removed from its packaging, they were examined, and no change in appearance of the skin or meat was observed (Teotia and Miller 1975).

3. Mechanisms of Microbial Inactivation

TO INACTIVATE SURFACE AND SUBSURFACE BACTERIA, FULLY packaged foods are sterilized by X-ray treatment (Brynjolfsson 1979). X-ray treatment reduces or eliminates *Salmonella* serovars in poultry, mold growth on strawberries, and sprout development in potatoes. *Salmonella* serovars have been found to be the most radiation sensitive of all pathogenic organisms on foods (Teotia and Miller 1975). As a method of food preservation, X-ray treatment has low energy requirements.

Radionuclides emit gamma radiation with discrete energy levels (1.17 MeV and 1.3 MeV for Co-60 and 0.67 MeV for Cs-137), while X-ray spectra (from a 5 MeV electron accelerator) have broad energy distributions spanning the same general energy levels (Martens and Knorr 1992). Given these overlapping energy distributions, it is reasonable to assume that mechanisms for microbial inactivation are also similar.

Microbial inactivation by all types of ionizing radiation is thought to happen through 2 main mechanisms: direct interaction of the radiation with cell components and indirect action from radiolytic products, such as the water radicals $\cdot H^+$, $\cdot OH^-$ and e_{aq}^- (Farkas 1997). The primary target of ionizing radiation appears to be chromosomal DNA, although effects on the cytoplasmic membrane may also play a role (Grecz and others 1983). Changes to chromosomal DNA and/or cytoplasmic membrane can cause microbial inactivation or growth inhibition. Many studies have shown that ions, excited atoms and molecules generated during irradiation have no toxic effect on humans.

4. Research Needs

Research needs include:

- Identifying pathogens of concern resistant to the technology and validating surrogates.
- Understanding mechanisms of inactivation.
- Understanding critical process factors and how they affect microbial inactivation.
- Identifying process deviations and how to correct them.
- Investigating the specific effects of pulsed X-rays in contrast to X-rays.

Glossary

A COMPLETE LIST OF DEFINITIONS REGARDING ALL THE TECHnologies is located at the end of this document.

Bremsstrahlung. One of the 3 possible ways to generate X-rays, and the one commonly used to create X-rays for food irradiation. Literally translated from the German it means "breaking" (brems) "radiation" (strahlung). Bremsstrahlung X-rays are generated when electrons accelerate on coulomb collision with other particles or when a beam of particles decelerates on encountering an obstacle. Synchrotron radiation or Compton scattering can also generate X-rays.

Converter Plate. A heavy metal (usually Pb) plate that converts an electron beam into Bremsstrahlung X-rays with a broad band photon energy spectrum.

LIEA. Linear Induction Electron Accelerator.

SOS. For X-rays technology, solid state opening switch. It can deliver pulses in the gigawatt range.

Thyristor. Charging unit used to convert 3-phase power to direct current.

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Research Needs[1]

DENNIS R. HELDMAN AND FRANK F. BUSTA

All Technologies

- Evaluate the adequacy of the linear first-order survivor curve model. Although there is evidence of various types of deviations from this historical model, a universally accepted alternative has not evolved. Future research on an appropriate model(s) would be beneficial to all preservation technologies.
- Establish experimental protocol for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to various alternative technologies, especially pulsed electric fields, pulsed light, oscillating magnetic fields and X-rays. For example, PEF studies should incorporate multiple levels of electric field intensity, as well as test the potential for synergy with temperature.
- Identify differences of inactivation action/mechanism(s) among alternative technologies. For example, pulsed light and ultraviolet light, ohmic and microwave, PEF and thermal, and so on.
- Determine the synergism or antagonism of one alternative process used with another and their combined effect on microbial inactivation efficiency.
- Determine potential formation of unpalatable and toxic by-products of processing with alternative technologies.
- Develop methods for measuring and monitoring temperatures or other treatment actions within individual, large, solid particulates.
- Identify new or changing critical process factors and their effect on microbial inactivation.
- Investigate the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) and/or activation volumes (V) are quantified. Synergistic effects among pressure, temperature, and other measurable variables also should be evaluated.

Pulsed Electric Fields

- Develop reliable kinetic parameters for the microbial inactivation models using PEF for the microbial population of interest in food safety. Develop and evaluate the subsequent kinetic models.
- Determine mechanisms of microbial and enzyme inactivation by PEF.
- Identify the pathogens of concern most resistant to PEF.
- Identify surrogate microorganisms for these pathogens.
- Develop validation methods to ensure microbiological effectiveness of PEF.
- Conduct studies to optimize critical process factors identified with PEF.
- Design PEF treatment chambers for uniformity and processing capacity.
- Develop PEF electrode materials for longer operation time and lower metal migration.
- Design, evaluate and reduce costs of process systems.

Ohmic and Inductive Heating

- Investigate more fully the combined influence of temperature and electric fields on the inactivation kinetics of key pathogenic microorganisms.
- Develop the knowledge base to assess the impact of deviations for specific designs of ohmic heaters, including improved models for ohmic processes.

Microwave

- Determine the effects of food formulation on heating patterns and assess their impact on overall process effectiveness.
- Determine the effects of equipment design factors, including frequency (for example, 915 MHz is sometimes proposed instead of the commonly used 2450 MHz for better uniformity of heating).
- Develop variable frequency ovens and assess their usefulness in food applications for improved uniformity of heating.
- Understand the factors affecting heating patterns, including qualitative changes occurring with frequency changes.
- Develop ways to monitor and real-time adjust for process deviations in microwave and radio frequency processing.

Pulsed Light

- Determine the suitability of the technology for solid foods and non-clear liquids where penetration depth is critical.
- Quantitatively determine the resistance of common pathogens or surrogate organisms to pulsed light treatments.
- Understand the differences between this technology and that of the more conventional UV (254 nm) light treatment.
- Determine the mechanisms of microbial inactivation to determine whether they are significantly different from those proposed for UV light.
- Understand the mechanism and quantification of the benefit attributed to the pulse effect.

Ultrasound

- Determine the effect of ultrasound on microbial inactivation efficiency when used with other processing technologies (high pressure, heat or others).
- Identify the mechanisms of microbial inactivation when used in combination with other technologies.
- Identify the critical process factors when ultrasound is used in hurdle technology.
- Evaluate the influence of the food properties, such as viscosity and size of particulates, on microbial inactivation.

High Voltage Arc Discharge

- Determine how delivery of highly reactive ozone and UV radiation by electric arc discharge inactivates microorganisms.
- Quantify the inactivation kinetics and mechanisms.
- Identify process by-products generated during the submerged arc discharge process due to the highly reactive nature of ozone and UV irradiation.
- Define maximum allowable dose similar to food irradiation.

Oscillating Magnetic Fields

- Establish the effects of magnetic fields on microbial inactivation.

- Elucidate the destruction kinetics of magnetic fields.
- Determine the mechanism of action of magnetic fields.
- Determine critical process factors and effects on microbial inactivation.
- Validate the process and evaluate the need for indicator organisms.
- Identify process deviations and determine ways to address them.

Ultraviolet Light

- Quantitatively determine the effects of individual parameters, such as suspended and dissolved solid concentration, on the effectiveness of the treatment.

High Pressure

- Conduct additional modeling research, using data generated by multiple-cell pressure units that allow for similar come-up times. Although HPP-derived semi-logarithmic survival curves appear nonlinear (for example, sigmoidal or biphasic), in HPP predictive microbiology, a logarithmic order of reduction is normally assumed. This assumption carries the danger of underestimating the subpopulation of pressure-resistant organisms.
- Investigate the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that kinetic parameters are quantified. In this way, critical process factors can be evaluated for survival of pathogens or surrogates in a statistical manner. Accurate predictions could be used to develop HACCP plans.
- Evaluate synergistic effects among pressure, temperature and other variables.

[1]authors collected and summarized the research needs from the report.

Glossary

Activation energy constant, E. Temperature coefficient determined from the slope of a ln (k) versus $1/T_A$ plot.

Activation volume constant, V. Pressure coefficient obtained from the slope of the ln (k) versus a pressure difference ($P-P_R$) plot.

Adiabatic compression. Compression or decompression processes occurring without heat transfer.

Bacteriophage. A bacterial virus; a virus capable of infecting bacteria.

Batch treatment. Treatment of a static mass of food in bulk or packaged.

Biological indicator system. A system (bacteria or enzyme) used to determine whether a process cycle has met the specified requirements.

Bremasstrahlung. One of the three possible ways to generate X-rays, and the one commonly used to create X-rays for food irradiation. Literally translated from the German it means "breaking" (brems) "radiation" (strahlung). Bremsstrahlung X-rays are generated when electrons accelerate on coulomb collision with other particles or when a beam of particles decelerates on encountering an obstacle. Synchrotron radiation or Compton scattering can also generate X-rays.

Broad spectrum light. For pulse light technology, it refers to ultraviolet, visible, and infrared spectrum of light.

Capacitor bank. Network of two or more capacitors used to store the energy supply from a DC power source.

Cavity. The metallic enclosure in the microwave system where the microwaves coming from the waveguide do the heating.

Cell lysis. The rupturing of a bacterial cell.

Chemical indicator system. A system using calibrated chemical agents (one type of which changes color as a function of time and temperature of exposure to heat) to determine whether a process cycle has met the specified requirements.

Co field flow. One possible configuration for a PEF continuous chamber

Compression time. Recorded time to bring a mass of food from 0.1 MPa to process pressure (s).

Conductivity (Electrical), σ. Physical property of a food material that determines its ability to conduct electricity and is expressed in Siemens per cm (S/cm). In ohmic heating, it enables heating to occur.

Conductivity (Thermal). Physical property of a food material which determines its ability to conduct heat. Expressed in Watts/meter °C.

Continuous chamber. Opposite of static chamber, it processes liquid foods that are pumped between pulsing electrodes.

Continuous HPP process treatment. Treatment of liquiform products using a hold chamber designed to insure every food element receives a specified residence time at process pressure (and temperature) with subsequent means for the product to do work during decompression followed by aseptic or clean filling of packages.

Conventional heating. Heating of a substance by transfer of thermal energy from a heating medium to a low temperature product.

Converter Plate. A heavy metal (usually Pb) plate that converts an electron beam into Bremsstrahlung X-rays with a broad-band photon energy spectrum.

Critical process factor. Any specified process condition and specified limit (see process deviation) required to achieve a desired/specified residual level of activity of a specified pathogen. For instance in HPP critical process factors can include, but not be limited to, process pressure, product IT, process temperature, pH, A_w, product composition, compression time, and process pressure hold time.

Cross-field. An ohmic heating system where the electric field is aligned across the product flow path.

Cyclotron. An accelerator in which particles move in spiral paths in a constant.

Cyclotron resonance. Phenomenon that occurs when the frequency of revolving ions induced by a specific magnetic field intensity is similar to the frequency of that magnetic field and parallel to it. In these instances, energy may be transferred to the ions, affecting cell metabolic activities.

D.C. power supply. Electrical device to deliver direct current to the capacitor bank

Decompression time. Recorded time to bring a mass of food from process pressure to 37% of process pressure. If decompression time is 0.5% or less of process pressure hold time it may be neglected in process determination calculations (seconds).

Density. Mass per unit volume of a material.

Dielectric constant. Property of a material representing the ability to store electromagnetic energy.

Dielectric loss. Property of a material representing the ability to dissipate electromagnetic energy as heat.

Dipole. For oscillating magnetic fields, a magnetic particle that contains a "north" and "south" magnetic pole.

D-value, decimal reduction time. Time required for a one-log cycle reduction in the microbial population, at a specific temperature, pressure, or electric field intensity. For the D-value to be meaningful, the semi logarithmic survivor curve must be a straight line.

Electric field intensity, E. A force on a stationary positive charge per unit charge in an electrical field. For ohmic heating and PEF, this can be calculated in an average sense as the voltage divided by the distance between the electrodes.

Electric field strength. See electric field intensity

Electrical breakdown. An abrupt rise in electric current in the presence of a small increase in voltage. As a consequence, rupture of bacterial cell membranes may occur with the application of an electric field. This effect is more pronounced in pulsed electric field treatment. In microwaves, this can happen if operating at very low pressures, as in freeze-drying.

Electrode gap. Distance (cm) between the inner and outer electrode.

Electroheating. See ohmic heating

Electrohydraulic treatment. A rapid discharge of high voltage electricity across an electrode gap below the surface of aqueous suspensions.

Electroporation. Phenomenon in which a microbial cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes.

Energy density or fluence. Energy delivered from a light

source per unit area (Joules/ cm^2).

Focussing. Concentration of electromagnetic waves inside a food due to its curved surface, much like a lens focussing light waves. It leads to enhanced heating at the interior.

Gyrofrequency. Frequency at which the ions revolve in a magnetic field.

Heterogeneous magnetic fields. Magnetic field that exhibits a gradient depending on the nature of the magnet

High pressure processing (HPP). Adiabatic compression, hold, and decompression of foods at pressures in the range of 100 to 800 MPa for hold times of 0.001 to 1200 seconds or longer.

High voltage electrical impulse. Application of high voltage discharges to a liquid medium in a very short time.

High voltage switch. Device used to trigger the delivery of high intensity light pulses to foods or packaging materials.

Homogeneous magnetic fields. Magnetic field with a constant strength over space.

Homogeneous material. Material which does not exhibit spatial variation in composition

Inclusion particle. A food particle of significantly different electrical conductivity than its surroundings.

Inhmogeneous material. Material which exhibits spatial variation in composition

In-line field. An ohmic heating system where the electric field is aligned along the product flow path.

Input voltage. Voltage (kV) supplied from a DC power source.

Intensifier. Device for delivering high pressure process liquid generally by using a large diameter low pressure piston to drive a small diameter high pressure piston. The ratio of intensification is directly proportional to the ratio of the area of the large diameter piston divided by the area of the small diameter piston. A 20:1 intensification ratio is common. The pressure in the low pressure cylinder may be used to estimate the pressure of the high pressure process liquid. Intensifiers may be operated as single or multiple stroke devices. Single stroke intensifiers may be used to control the decompression rate of an HPP system.

Internal energy generation. Heat generation within a material and throughout its volume due to the presence of an energy source that is dissipated throughout the volume (see also volumetric heating).

Interstitial fluid motion. The motion of fluid in the spaces between solid particles.

Irreversible breakdown. Irreversible generation of pores in the bacterial cell membranes.

k, reaction rate constant (first-order). The slope of the logarithm of survivor ratio (log S) versus time of treatment for the microbial population.

LIEA. Linear Induction Electron Accelerator

Liquid crystals. Materials, which have properties that are useful for thermal sensing. Liquid crystals typically change color with temperature.

Magnetic flux density. Force that an electromagnetic source exerts on charged particles. Magnetic flux density is measured in Telsa (1 Telsa = 104 gauss).

Magnetron. The physical component of a microwave system that generates the microwaves.

Microwaves. Electromagnetic waves at frequencies 915, 2450, 5800, and 24225 MHz.

Non-thermal effects. Effects due to the exposure to a process that are not of thermal origin, i.e., cannot be explained by measured temperature changes.

Oscillating magnetic fields. Magnetic fields generated with electromagnets of alternating current. The intensity varies periodically according to the frequency and type of wave in the magnet.

Pasteurization. A process designed to reduce the population of pathogenic bacteria in a product, sufficient to ensure product safety but with modest impact on the nutritional properties and flavor of the product. Traditionally, this term has been applied to thermal processes but it can also refer to emergent alternative technologies with the purpose of pathogens inactivation.

Peak voltage. Maximum voltage (kV) delivered by PEF system.

Penetration depth. The distance the electromagnetic waves (of a certain frequency) travel in a material before it loses 63% of its energy.

Power cycling. The process of the microwave source turning on and off .

Process deviation. Any critical process factor which differs outside an specified value and limit or range limit during the treatment and subsequent handling of a treated food.

Process pressure (PP). Constant holding pressure for any HPP treatment (MPa) (psi). Process pressure should be controlled to ± 0.5% and recorded to the same level of accuracy. (± 500 psi at 100,000 psi) or (± 3.4 MPa at 680 MPa).

Process pressure hold time. Recorded time from end of compression to beginning of decompression (s).

Product composition. Specified percent by weight and range limit of stated product ingredients (%).

Product initial temperature (IT). Product IT can be specified as a critical process factor. For HPP processes, IT must be not less than 0.50 °C below value-value in all food locations from start of compression time to end of decompression time (°C).

Product pH. Value of pH measured at product IT at atmospheric pressure.

Product process temperature. Temperature at which the process is performed (°C). Initial temperature and process temperature must be monitored at all points of the process if it is an integral condition for microbial inactivation. With some processes, such as HPP, foods will increase in temperature as a function of the imposed treatment and their composition. Final product temperature at process pressure is independent of compression rate as long as heat transfer is negligible.

Pulse rate. Number of pulses per second or input frequency (1/s).

Pulse width. Duration of the pulse. In PEF and for exponential decaying pulse, pulse width can be calculated as the resistance of the food times the capacitor capacitance. This is also called time constant.

Pulsed treatment. Treatment of a food using more than one treatment cycle of specified conditions such that each cycle element is accurately and precisely reproduced until a specified number is achieved. Cycle parameters (i.e. pressure, electrical field) may display a square, ramp, sinusoidal, or other waveform when recorded.

Radio frequency. Electromagnetic waves at frequencies of 13.56, 27.12, and 40.68 MHz.

Rate of inactivation or survival ratio, S. Initial number of viable microorganisms (N_0) divided by the number survivor microorganisms after treatment (N).

Residence time distribution. The distribution of times spent by the various components of a food product through a process vessel.

Resonance. Electromagnetic wave patterns formed due to superposition of oncoming and reflected waves, leading to very high rates of heating. Resonance can occur inside a food for specific combinations of size, shape, and food property.

Reversible breakdown. Formation of reversible pores in the bacterial cell membranes.

Runaway heating. A cycle of increasing temperature in food causing increasing rate of enegy (microwave/ohmic) absorption that further increases the rate of temperature rise. It is more prominent in foods undergoing phase change from ice to water and in foods containing significant salt and other ions.

Semi-continuous HPP. Treatment of liquiform products using one or more chambers fitted with a free piston to allow compression, hold, and decompression with discharge under clean or sterile conditions.

Sinusoidal wave. A mode of propagation of the magnetic field.

SOS. In X-rays technology, solid state opening switch that can deliver pulses in the gigawatt range.

Specific heat. The ability of a material to store heat. Described technically as the amount of energy required to raise the temperature of unit mass of an object by a unit increment in temperature.

Static chamber. Chamber that processes a given volume of food at a time.

Static magnetic field. Magnetic fields that have constant intensity (B) over time and whose field direction is constant. The intensity varies periodically according to the frequency and type of wave in the magnet.

Sterilization. Any process, physical or chemical, which will destroy all forms of life; applied especially to microorganisms, including bacterial and mold spores and the inactivation of viruses.

Surrogate Microbe. A non-pathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain. The surrogate allows biological verification of the treatment without introducing pathogens into a food processing area. For example, PA 3679 is used as a surrogate microbe for *Clostridium botulinum* in thermal process validation. *Listeria innocua* is a possible surrogate for *L. monocytogenes.*

Tesla. Unit to express magnetic flux density (B). 1 Tesla (T) = 104 gauss.

Thermophysical properties. Properties that influence the heating rate of a material. Examples of thermophysical properties are thermal conductivity (the ability of the material to conduct heat), specific heat (the ability of the material to store heat), and density (the mass per unit volume of the material).

Thyristor. Charging unit used to convert three-phase power to direct current.

Treatment time. Time that a food product is subjected to a process. For instance in PEF, it is calculated as the product of the number of pulses times the duration of the pulses.

Ultrasonic. Term used to describe a vibrating wave of a frequency above that of the upper frequency limit of the human ear; it generally embraces all frequencies above 16 kilocycles/second.

Variable frequency. Sweeping over a range of frequencies during the microwave heating process to improve uniformity.

Volumetric heating. Heating by internal energy generation throughout the volume of a material (see also internal energy generation).

Water Activity, a_w. Qualitatively, a_w is a measure of unbound, free water in a system, available to support biological and chemical reactions. Water activity affects microorganisms survival and reproduction, enzymes, and chemical reactions. The water activity of a substance is quantitatively equal to the vapor pressure of the substance divided by the vapor pressure of pure water (both measured at the same temperature).

Waveform/Waveshape. Type of electric or pressure pulses generated by the high voltage pulser.

Waveguide. The physical component of a microwave system that guides the microwaves from magnetron to the cavity where the food is heated. When applied in the form of pulses, it reverses the charge for each pulse and pulse intensity gradually decreases.

z(T) [z(P) or z(E)]. Thermal (pressure or electric field) resistance constant (z) is the temperature (°C) (pressure or electric field) increase needed to accomplish a one-log cycle reduction in the D–value.

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