Chapter 5
Effect of Preservation Technologies on Microbial Inactivation in Foods

1. Introduction

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

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

2. Validation of processing parameters

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

should be used for establishing and validating scheduled new thermal processes based on thermal effect on microorganisms, such as microwave heating.

For other preservation processes not based on heat inactivation, key pathogens of concern and nonpathogenic surrogates need to be identified and their significance evaluated. Surrogate microorganisms should be selected from well-known nonpathogenic populations, should mimic the target pathogenic microorganism in growth habits, should not be susceptible to injury, and should not exhibit non-reversible inhibition (thermal or otherwise). Surrogate microorganisms should be genetically stable and exhibit uniform thermal and growth characteristics from batch to batch over several generations. The durability to food and processing parameters should be similar to that of the target organism. Population of surrogates should be constant and maintain stable thermal and growth characteristics from batch to batch. Enumeration of surrogates should be rapid and should utilize inexpensive detection systems that easily differentiate them from natural flora. Genetic stability of surrogates is desirable to obtain reproducible results. It also is recommended that surrogates do not establish themselves as "spoilage" organisms on equipment or in the production area. The validation process should be designed so that the surrogate exhibits a predictable time-temperature process character profile that correlates to that of the target pathogen. Introduction of system modifications or variables, leading to inaccurate results should be avoided (for example, thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity, and so on).

3. Processing technologies

3.1. Water activity and pH

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

3.2. Technologies based on thermal effects

In addition to microbial inactivation by conventional methods of heating, microwave and ohmic and inductive heating are also considered to be heat-based processes that can inactivate microorganisms by thermal effects. Microwave and radio frequency heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material through two mechanisms—dielectric and ionic. Ohmic heating is defined as the process of passing electric currents through foods or other materials to heat them. Ohmic heating is distinguished from other electrical heating methods by the presence of electrodes contacting the food, frequency, and waveform. Inductive heating is a process wherein electric currents are induced within the food due to oscillating electromagnetic fields generated by electric coils. No data about microbial death kinetics under inductive heating have been published.

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

3.3. High pressure processing

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

HPP acts instantaneously and uniformly throughout a mass of food independent of size, shape, and food composition. Compression will uniformly increase the temperature of foods approximately 5 °F (3 °C)

per 100 MPa. Compression of foods may shift the pH of the food as a function of imposed pressure and must be determined for each food treatment process. Water activity and pH are among the critical process factors in the inactivation of microbes by HPP. An increase in food temperature above room temperature, and to a lesser extent, a decrease below room temperature increases the inactivation rate of microorganisms during HPP treatment. Temperatures in the range of 113 – 122 °F (45 – 50 °C) appear to increase the rate of inactivation of food pathogens and spoilage microbes. Temperatures ranging from 194 – 230 °F (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), initial product temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity, and concurrent processing aids. Interestingly, because HPP acts instantaneously and uniformly through a mass of food, package size, shape, and composition are not factors in process determination. High hydrostatic pressures can cause undesirable structural changes in structurally fragile foods such as strawberries or lettuce (for example, cell deformation and cell membrane damage). Food products that have been brought to market include raw oysters, fruit jellies and jams, fruit juices, pourable salad dressings, raw squid, rice cakes, foie gras, ham, and guacamole.

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

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

sporeformers of concern, *Bacillus cereus* has been the most studied because of its facultative anaerobic nature and very low rate of lethality.

Normally, gram-positive vegetative bacteria are more resistant to environmental stresses, including pressure, than vegetative cells of gram-negative bacteria. Among the pathogenic 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 *Escherichia 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. For vegetative bacteria, nonpathogenic *Listeria innocua* is a useful surrogate for the foodborne pathogen, *L. monocytogenes*. A nonpathogenic strain of *Bacillus* may be useful as a surrogate for HPP-resistant *E. coli* O157:H7 isolates.

3.3.1. Commercial implications

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

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

Acid foods with pH values between 4.0 and 4.5 can be made commercially sterile using a pressure of 580 MPa and a hold time of 15 min. Products would have an initial temperature of about 71.6 °F (22 °C). Shorter hold times are possible if the product is to be refrigerated. Actual hold-time values must be determined from challenge packs and storage studies perhaps twice the length of the intended shelf life of the product.

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

3.4. Pulsed electric fields

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

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

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

Many researchers have studied the effects of pulsed electric fields in microbial inactivation; however, due to the numerous critical process factors and broad experimental conditions used, definite conclusions about specific pathogen reductions cannot be made. Research that provides conclusive data on the PEF inactivation of pathogens of concern is clearly needed. Castro and others (1993) reported a 5-log reduction in bacteria, yeast, and mold counts suspended in orange juice treated with PEF. Zhang and others (1995) achieved a 9-log reduction in *E. coli* suspended in simulated milk ultrafiltrate treated with

PEF by applying a converged electric field strength of 70 KV/cm for a short treatment time of 160 μs. This processing condition may be adequate for commercial food pasteurization that requires 6- to 7-log reduction cycles (Zhang and others 1995). However, numerous critical process factors exist and carefully designed studies need to be performed to better understand how these factors affect populations of pathogens of concern. Currently, there is little information on the use of surrogate microorganisms as indicators of pathogenic bacteria when PEF is used as a processing method. Selection of surrogates will require the prior identification of the microorganism of concern in a specific food and PEF system. The selection of the appropriate surrogate(s) will depend on the type of food, microflora, and process conditions (that is, electric field intensity, number of pulses, treatment time, pulse wave), and should also follow the general guidelines listed in the validation section.

3.5. Irradiation

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

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

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

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

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

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

be considered. More important, the effectiveness of the technology will need to be validated for the specific application.

3.6. Other technologies

Some of the technologies present greater limitations or are at a development stage that requires extensive further scientific research before they can be commercially used. For instance, high voltage arc discharge (application of discharge voltages through an electrode gap below an aqueous medium) causes electrolysis and highly reactive chemicals. Although microorganisms are inactivated, improved designs need to be developed before consideration for use in food preservation. Likewise, oscillating magnetic fields have been explored for their potential to inactivate microorganisms; however, the results are inconsistent. Data on inactivation of food microorganisms by ultrasound (energy generated by sound waves of 20,000 or more vibrations per second) are scarce, and limitations include the inclusion of particulates and other interfering substances. Ultraviolet (UV) light is a promising technique, especially in treating water and fruit juices. A 4-log bacterial reduction was obtained for a variety of microorganisms when 400 J/m^2 was applied. Apple cider inoculated with *E. coli* O157:H7 treated in that manner achieved a 5-log reduction (Worobo 2000). Critical factors include the transmissivity, the geometric configuration of the reactor, the power, wavelength, and physical arrangement of the UV source, the product flow profile, and radiation path length.

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