U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
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Kinetics of Microbial Inactivation for Alternative Food Processing Technologies
High Pressure Processing

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Scope of Deliverables

This section covers high pressure processing as an alternative technology for preservation of foods. It includes critical process 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 described and tentative flow charts for the application of HACCP to high pressure processing are also included.

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 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.

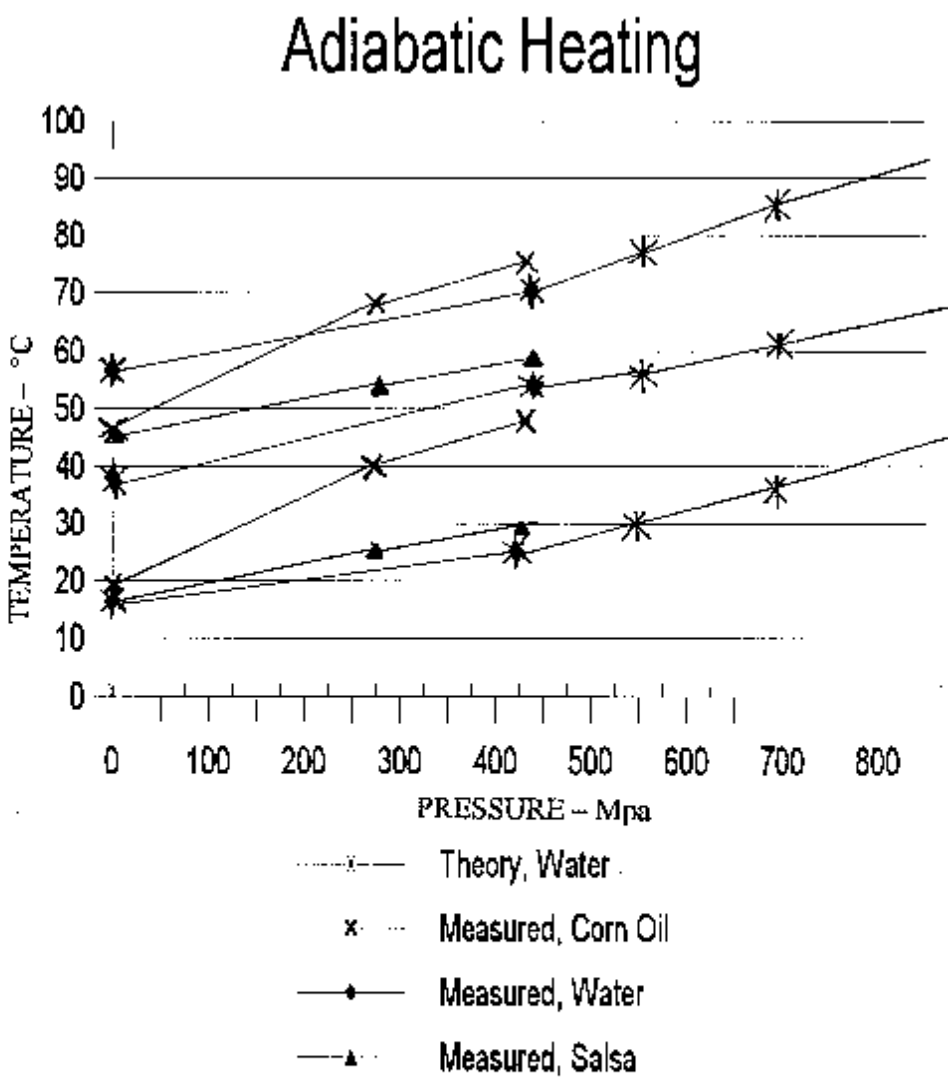

Figure 1 Increase in Temperature of Water, Corn Oil, and Salsa as a Result of Adiabatic Compression (Ting 1999). Note that the Increase in Temperature Upon Compression is also a Function of Initial Temperature (IT).

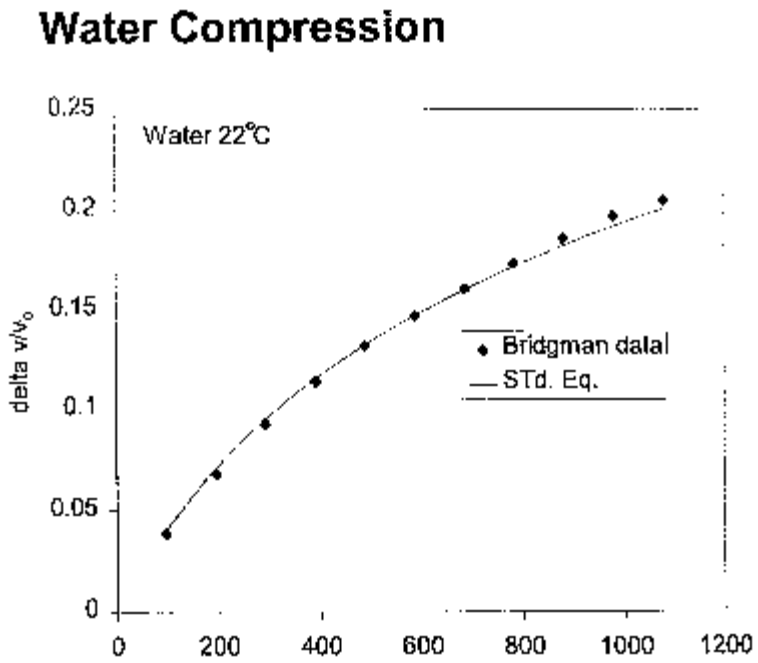

Figure 2. Fractional Decrease in Volume of Water as a Function of Imposed Pressure (Bridgeman 1912, Ting 1999)

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. Their monitoring and control must be included in HACCP plans for the HPP treatment of foods. 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.

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 sporeforming 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 (Hein zand 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.

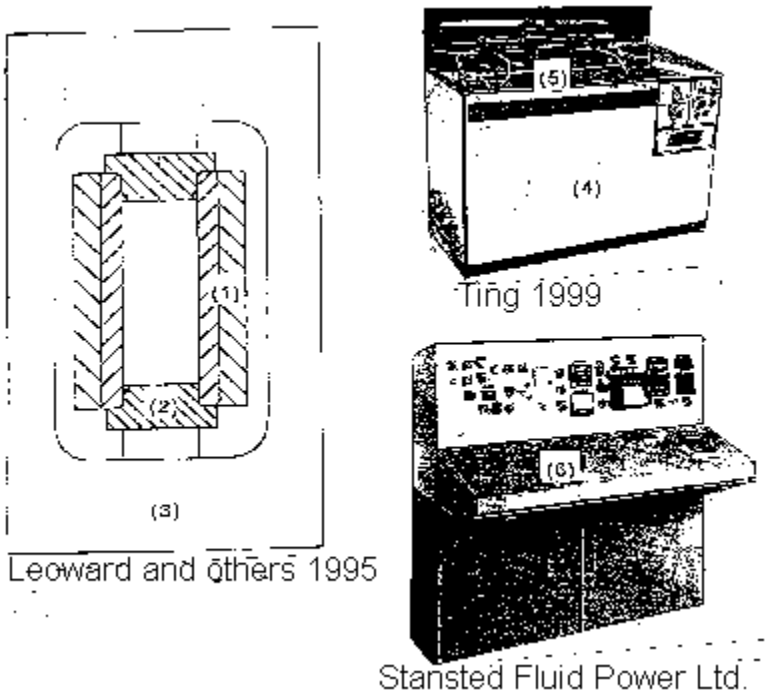

Figure 3. Equipment for Batch High Pressure Treatment of Foods

1. Pressure Vessel--shown in cross section with two concentric cylinders
2. End closures--high pressure water from the intensifier can be delivered to the pressure vessel through the bottom closure.
3. Yoke shown supporting end closures.
4. Low pressure pump--the pump and mother are contained in the pump/intensifier cabinet.
5. Intensifier--this unit sits on top of the low pressure pump in the cabinet.
6. System control cabinet--controls and recorders for pressure and temperature are shown.

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 $\geq$ 450 MPa/ 25 °C for 5 min and for *P. fluorescens* when treated at $\geq$ 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 *Listeria* 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 x10^8 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 L*isteria* 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 food processing plants, whether a dairy facility or not.

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 800MPa 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 ~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 x 10^2 *C. sporogenes* spores/mL in truffle cream. Total inactivation of *B. cereus* (starting concentration $4x10^5$ spores/mL), *Bacillus licheniformis* ($6x10^6$ spores/mL) and *B. stearothermophilus* ($4x10^5$ 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 variable 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* species 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 λ 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 and 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 conjunction 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 were 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. *C. 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 α-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. A HACCP plan is essential to insure that ingredients entering the process have low counts of pathogens and spoilage microbes. The Appendix shows typical HACCP programs for HPP-treated acidified foods. 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). Additional factors for 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). Other 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.

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 observation of strains of *S. cerevisiae* by comparing tolerance under different applications of heat shock and recovery, and different growth phases that also involved incorporation of HPP-resistant mutant strains. 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, integrity, 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 can be written to determine E', the activation energy, if the rates of the reaction, k_{T1} and k_{T0}, are known at two tempe ratures, T_1 and T_0. Pressure is constant.

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

R is Å 2 (calories/ °C-mole), 2.3 converts from natural to common logarithms, and temperature is in degrees Kelvin. A similar equation 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.

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

If the pressure is in atmospheres, R = 82 (cm^3 /mole). 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 ÆV* 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 much 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 requirements are discussed in the next section and are the basis for a HACCP program for food preservation by HPP.

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.

4.1.4.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

As part of any HACCP system, critical control points are required. 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 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 can be expected in any repetitive manufacturing process. A HACCP program requires that critical control points (CCP) are determined and that values for the CCP are established and monitored. Additionally, limits must be established for the values of each CCP and a plan prepared to determine corrective action. The corrective action would depend on the magnitude the CCP deviates from the established limit or limits (see Appendix for examples of HACCP programs).

This section identifies CCP used in high pressure food preservation. The CCP 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 control points 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 control points and parameters to be controlled for batch, semi-continuous, and pulsed HPP, including processes where temperature is specified, are as follows.

Batch Systems

Critical Control Point/ 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 CCP and 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 and factors to be controlled are those shown in 5.1.1. for batch systems and 5.1.2. for semi-continuous systems.

The development of a valid HPP process must include information on the limits of critical control factors deviations. These limits must include appropriate corrective actions as a function of severity of each deviation. A cumulative estimate of deviation severity should show several CCP 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

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. Accurate predictions could be used to develop HACCP plans.
- 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/cm^2**	**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/cm^2	**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 parahaemolyticu*s (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).

REFERENCES

Adegoke, G. O., Iwahashi, H. and Komatsu, Y. 1997. Inhibition of Saccharomyces cerevisiae by combination of hydrostatic pressure and monoterpenes. J Food Sci. 62(2): 404-405.

Alderton, G. and Snell, N. 1969. Bacterial spores: Chemical sensitization to heat. Science. 163:1212

Alderton, G., Ito, K. A. and Chen, J. K. 1976. Chemical manipulation of the heat resistance of Clostridium botulinum spores. Appl Environ Microbiol. 31:492-498

Aleman, G. D., Farkas, D. F., McIntyre, S., Torres, J. A. and Wilhelmsen, E. 1994. Ultra-high pressure pasteurization of fresh cut pineapple. J Food Protect. 57(10):931-934

Aleman, G. D., Ting, E. Y., Mordre, S. C., Hawes, A. C. O., Walker, M., Farkas, D. F. and Torres, J. A. 1996. Pulsed ultra high pressure treatments for pasteurization of pineapple juice. J Food Sci. 61(2):388-390

Alpas, H., Kalchayandand, N., Sikes, T., Dunne, C. P. and Ray, B. 1994. Hydrostatic pressure among strains of foodborne pathogens. Applied and Environmental Microbiology. 65(9):4248-4251

Ananth, V., Dickson, J. S., Olson, D. G. and Murano, E. A. 1998. Shelf-life extension, safety and quality of fresh pork loin treated with high hydrostatic pressure. J Food Protect. 61(12):1649-1656

Arroyo, G., Sanz, P. D. and Prestamo, G. 1997. Effect of high pressure on the reduction of microbial populations in vegetables. J Appl Microbiol. 82:735-742

Ballestra, P., Da Silva, A. A. and Cuq, J. L. 1996. Inactivation of Escherichia coli by carbon dioxide under pressure. J Food Sci. 61(4):829-836

Benito, A., Ventoura, G., Casadei, M., Robinson, T. and Mackey, B. 1999. Variation in resistance of natural isolates of Escherichia coli O157 to high hydrostatic pressure, mild heat, and other stresses. Appl Environ Microbiol. 65(4):1564-1569

Berlin, D. L., Herson, D. S., Hicks, D. T. and Hoover, D. G. 1999. Response of pathogenic vibrio species to high hydrostatic pressure. Appl Environ Microbiol. 65(6):2776-2780

Brauch, G., Haensler, U. and Ludwig, H. 1990. The effect of pressure on bacteriophages. High Pressure Res. 5:767-769

Bridgman, P. W. 1912. Watern in the liquid and five sloid forms under pressure. Proc Amer Acad Art Sci. 47:441-558

Butz, P., Trangott, V., Ludwig, H., Ries, J. and Weber, H. 1990. The high pressure inactivation of bacteria and bacterial spores. Die Pharm Ind. 52:487-491

Butz, P., Habison, G. and Ludwig, H. 1992. Influence of high pressure on a lipid-coated virus. R. Hayashi, K. Heremans and P. Masson(eds.). High Pressure and Biotechnology. London. John Libby & Co., Ltd. 61-64.

Butz, P., Funtenberger, S., Haberditzl, T. and Tauscher, B. 1996. High pressure inactivation of Byssochlamys nivea ascospores and other heat-resistant moulds. Lebensm Wiss Technol. 29:404-410

Carlez, A., Cheftel, J. C., Rosec, J. P., Richard, N., Saldana, J. L. and Balny, C. 1992. Effects of high pressure and bacteriostatic agents on the destruction of citrobacter

freundii in minced beef muscle. C. Balny, R. Hayashi, K. Heremans and P. Masson(eds.). High Pressure and Biotechnology. London, U.K. John Libby & Co., Ltd. 365-368.

Carlez, A., Rosec, J. P., Richard, N. and Cheftel, J. C. 1994. Bacterial growth during chilled storage of pressure-treated minced meat. Lebens Wiss Technol. 27:48-54

Carpi, G., Buzzoni, M., Gola, S., Maggi, A. and Rovere, P. 1995. Microbial and chemical shelf-life of high-pressure treated salmon cream at refrigeration temperatures. Industria Conserve. 70:386 - 397

Cheftel, J. C. 1992. Effects of high hydrostatic pressure on food constituents: An overview. C. Balny, Hayashi, R., Heremans, K., Masson, P. High Pressure Biotechnol. 224. 195-209.

Cheftel, J. C. 1995. Review: high pressure, microbial inactivation and food preservation. Food Sci Technol Int. 1(2/3):75-90

Chen, C. and Tseng, C. W. 1997. Effect of high hydrostatic pressure on the temperature dependence of Saccharomyces cerevisiae and Zygosaccharomyces rouxii. Proc Biochem. 32(4):337-343

Clouston, J. G. and Wills, P. A. 1969. Initiation of germination and inactivation of Bacilluspumilus spores by hydrostatic pressure. J Bacteriol. 97:684-690

Crawford, Y. J., Murano, E. A., Olson, D. G. and Shenoy, K. 1996. Use of high hydrostatic pressure and irradiation to eliminate Clostridium sporogenes in chicken breast. J Food Protect. 59:711-715

Dring, G. J. 1976. Some aspects of the effects of hydrostatic pressure on microorganisms. S. A. Skinner and V. Hugo(eds.). Inhibition and inactivation of vegetative microbes. New York. Academic Press Inc.

El Moueffak, A. C., Antoine, M., Cruz, C., Demazeau, G., Largeteau, A., Montury, M., Roy, B. and Zuber, F. 1995. High pressure and pasteurization effect on duck foie gras. Int J Food Sci Technol. 30(6):737-743

Enomoto, A., Nakamura, K., Hakoda, M. and Amaya, N. 1997. Lethal effect of high-pressure carbon dioxide on a bacterial spore. J Ferment Bioeng. 83(3):305-307

Estiaghi, M. N., Stute, R. and Knorr, D. 1994. High pressure and freezing pretreatment effects on drying, rehydration, texture and color of greenbeans, carrots and potatoes. J Food Sci. 59:1168-1170

Fujii, T., Satomi, M., Nakatsuka, G., Yamaguchi, T. and Okuzumi, M. 1994. Changes in freshness indexes and bacterial flora during storage of pressurized mackerel. Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Japan). 35(2):195-200

Fujii, T., Satomi, M., Nakatsuka, G. and Yamaguchi, T. 1995. Effect of media on the detection rate of pressure-injured bacteria. Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Japan). 36(1):17-21

Garcia-Graells, V., Hauben, E. J. A. and Michiels, C. W. 1998. High-pressure inactivation and sublethal injury of pressure-resistant Escherichia coli mutants in fruit juices. Appl Environ Microbiol. 64:1566-1568

Gervilla, R., Capellas, M., Ferragut, V. and Guamis, B. 1997a. Effect of high hydrostatic pressure on Listeria innocua 910 CECT inoculated into ewes' milk. J Food Protect. 60(1):33-37

Gervilla, R., Felipe, X., Ferragut, V. and Guamis, B. 1997b. Effect of high hydrostatic pressure on Escherichia coli and Pseudomonas fluorescens strains in bovine milk. J Dairy Sci. 80:2297-2303

Giddings, N. J., Allard, H. A. and Hite, B. H. 1929. Inactivation of the tobacco mosaic virus by high pressure. Phytopatology. 19:749-750

Gola, S., Foman, C., Carpi, G., Maggi, A., Cassara, A. and Rovere, P. 1996. Inactivation of bacterial spores in phosphate buffer and in vegetable cream treated at high pressures. R. Hayashi and C. Balny(eds.). High Pressure Bioscience and Biotechnology. Amsterdam. 253-259. Elsevier Science B. V.

Gould, G. W. and Sale, A. J. H. 1970. Initiation of germination of bacterial spores by hydrostatic pressure. J Gen Microbiol. 60:335-346

Haas, G. J., Prescott, H. E., Dudley, E., Dik, R., Hintlan, C. and Keane, L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. J Food Safety. 9:253-265

Hara, A., Nagahama, G., Ohbayashi, A. and Hayashi, R. 1990. Effects of high pressure on inactivation of enzymes and microorganisms in nonpasteurized rice wine (Namazake). Nippon Nogeikagaku Kaishi. 64(5):1025-1030

Hayakawa, I., Kanno, T., Tomita, M. and Fujio, Y. 1994a. Application of high pressure for spore inactivation and protein denaturation. J Food Sci. 59(1):159-163

Hayakawa, I., Kanno, T., Yoshiyama, K. and Fujio, Y. 1994b. Oscillatory compared with continuous high pressure sterilization on Bacillus stearothermophilus spores. J Food Sci. 59(1):164-167

Hayashi and Balny, C. High Pressure Science and Biotechnology. Hayashi and C. Balny. Amsterdam. Elsevier Science, B.V. 171-174, 253-259.

Hayashi, R. 1991. Pressure-processed food: Research and development. San-Ei Publishing. Kyoto, Japan.

Heinz, V. and Knorr, D. 1998. High pressure germination and inactivation kinetics of bacterial spores. N. S. Isaacs(ed.). High Pressure Food Science, Bioscience and Chemistry. Cambridge, UK. The Royal Society of Chemistry. 436-441

Heinz, V. and Knorr, D. 1999. The effects of high pressure on microbial spores. European Conference on Emerging Food Science and Technology. Tempere, Finland. November 22-24 1999.

Heredia, N. L., Garcia, G. A., Luevanos, R., Labbe, R. G. and Garcia-Alvarado, J. S. 1997. Elevation of the heat resistance of vegetative cells and spores of Clostridium perfringens type A by sublethal heat shock. J Food Prot. 60(8):998

Heremans, K. 1995. High pressure effects on biomolecules. D. A. Ledward, D. E. Johnston, R. G. Earnshaw and A. P. M. Hasting. High Pressure Processing of Foods. Leicestershire, UK. Nottingham University Press.

Hite, B. H. 1899. The effects of pressure in the preservation of milk. Morgantown. Bull WV Univ Agric Exp Sta Morgantown. 58. 15-35.

Hite, B. H., Giddings, N. J. and Weakly, C. E. 1914. The effects of pressure on certain microorganisms encountered in the preservation of fruits and vegetables. Morgantown. Bull WV Univ Agric Exp Sta Morgantown. 146. 1-67.

Hong, S. I., Park, W. S. and Pyun, Y. R. 1997. Inactivation of Lactobacillus sp. from kimchi by high pressure carbon dioxide. Lebensm Wiss Technol. 30:681-685

Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F. and Knorr, D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. Food Technol. 43(3):99-107

Hoover, D. G. 1993. Pressure effects on biological systems. Food Technol. 47(6):150-155

Horie, Y., Kimura, K., Ida, M., Yosida, Y. and Ohki, K. 1991. Jam preparation by pressurization. Nippon Nogeikagaku Kaishi. 65(6):975-980

Ishiguro, Y., Sato, T., Okamoto, T., Okamoto, K., Sakamoto, H., Inakuma, T. and Sonoda, Y. 1993. Effects of hydrostatic pressure and antimicrobial substances on the sterilization of tomato juice. Nippon Nogeikagaku Kaishi. 67(12):1707-1711

Iwahashi, H., Fujii, S., Obuchi, K., Kaul, S. C., Sato, A. and Komatsu, Y. 1993. Hydrostatic pressure is like high temperature and oxidative stress in the damage it causes to yeast. FEMS Microbiology Letters. 108:53-58

Jaenicke, R. 1981. Enzymes under extremes of physical conditions. Ann Rev Biophys Bioeng. 10:1-67

Jay, J. J. 1996. Modern food microbiology. Chapman Hall. New York.

Johnson, F. H. and Campbell, D. H. 1945. The retardation of protein denaturation by hydrostatic pressure. Journal of Cell Comp Physiol. 26:43-46

Johnson ,F. H., Eyring, H., and Jones-Stover, B. 1974. The theory of rate processes in biology and medicine. p. 82. Wiley, New York.

Kakugawa, K., Okazaki, T., Yamauchi, S., Morimoto, K., Yoneda, T. and Suzuki, K. 1996. Thermal inactivating behavior of Bacillus stearothermophilus under high pressure. R. Hayashi and C. Balny. High Pressure Bioscience and Biotechnology. Amsterdam. Elsevier Science B. V. 171-174.

Kalchayanand, N., Sikes, T., Dunne, C. P. and Ray, B. 1994. Hydrostatic pressure and electroporation have increase bactericidal efficiency in combination with bacteriocins. Applied and Environmental Microbiology. 60:4174-4177

Kalchayanand, N., Sikes, A., Dunne, C. P. and Ray, B. 1998. Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. J Food Protect. 61(4):425-431

Kinugasa, H., Takeo, T., Fukumoto, K. and Ishihara, M. 1992. Changes in tea components during processing and preservation of tea extract by hydrostatic pressure sterilization. Nippon Nogeikagaku Kaishi. 66(4):707-712

Laidler, K. J. 1951. The Influence of pressure on rates of biological reaction. Arch Biochem. 30:226-240

Larkin, J. and Reddy, N. R. 1999. Personal communication.

Larsen, W. P., Hartzell, T. B. and Diehl, H. S. 1918. The effects of high pressure on bacteria. J Inf Diseases. 22:271-279

Ledward, D. A., Johnston, D. E., Earnshaw, R. G. and Hasting, A. P. M. 1995. High Pressure Processing of Foods. Nottingham University Press. Leiscestershire, UK.

Leistner, L. and Gorris, L. G. M. 1995. Food preservation by hurdle technology. Trends in Food Science & Technology. 6(2):41-46

Linton, M., McClements, J. M. J. and Patterson, M. F. 1999. Inactivation of Escherichia coli O157:H7 in orange juice using a combination of high pressure and mild heat. J Food Protect. 62(3):277-279

Lüdemann, H.-D. 1992. Water and aqueous solutions under high pressure. C. Balny, R. Hayashi, K. Heremans and P. Masson. High Pressure and Biotechnology. London. Colloque INSERM/John Libby Eurotext Ltd. 224. 25-32.

Ludwig, H., Bieler, D., Hallbauer, K. and Scigalla, W. 1992. Inactivation of microorganisms by hydrostatic pressure. C. Balny, R. Hayashi, K. Heremans and P. Masson. High Pressure and Biotechnology. London. Colloque INSERM/John Libby Eurotext Ltd. 224. 25-32.

Ludwig, H., Van Almsick, G. and Sojka, B. 1996. High pressure inactivation of microorganisms. R. Hayashi and C. Balny. High pressure Bioscience and Biotechnology. Amsterdam. Elsevier Science B. V. 237.

Mackey, B. M., Forestiere, K. and Isaacs, N. 1995. Factors affecting the resistance of Listeria monocytogenes to high hydrostatic pressure. Food Biotechnol. 9:1-11

Marquis, R. E. and Bender, G. R. 1987. Barophysiology of prokaryotes and proton-translocating ATPases. H. W. Jannisch, R. E. Marquis and A. M. Zimmermann. Current Perspectives in High Pressure Biology. London. Academic Press, Ltd. 65-73.

Masuda, M., Saito, Y., Iwanami, T. and Hirai, Y. 1992. Effects of hydrostatic pressure on packaging materials for food. High Pressure and Biotechnology. London. Colloque INSERM/John Libby Eurotext Ltd. 224. 545-547.

Metrick, C., Hoover, D. G. and Farkas, D. F. 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of Salmonella. J Food Sci. 54:1547-1564

Meyer, P. 2000. Ultra high pressure, high temperature, food preservation process. US 6,017,572.

Miyakawa, H., Anjitsu, K., Ishibashi, N. and Shimanura, S. 1994. Effects of pressure on enzyme activities of Lactobacillus helveticus LHE-511. Biosci Biotechnol Biochem. 58(3):606-607

Miyao, S., Shindoh, T., Miyamori, K. and Arita, T. 1993. Effects of high pressurization on the growth of bacteria derived from surimi (fish paste). Nippon Shokuhin Kogyo Gakkaishi (Journal of the Japanese Society for Food Science and Technology). 40(7):478-484

Moorman, J. E., Toledo, R. T. and Schmidt, K. 1996. High-pressure throttling (HPT) reduces microbial population, improves yogurt consistency and modifies rheological properties of ultrafiltered milk. IFT Annual Meeting: Book of Abstracts. 49.

Mussa, D. M., Ramaswamy, H. S. and Smith, J. P. 1999. High-pressure destruction kinetics of listeria monocytogenes on pork. J Food Protect. 62(1):40-45

Nakayama, A., Yano, Y., Kobayashi, S., Ishikawa, M. and Sakai, K. 1996. Comparison of pressure resistances of spores of six Bacillus strains with their heat resistances. Appl Environ Microbiol. 62(10):3897-3900

Ng, H., Bayne, G. G. and Garibaldi, J. A. 1969. Heat resistance of Salmonella, the uniqueness of Salmonella senftenberg 775W. Appl Microbiol. 17:78-82

Nishi, K., Kato, R. and Tomita, M. 1994. Activation of Bacillus spp. spores by hydrostatic pressure. Nippon Shokuhin Kogyo Gakkaishi (Journal of the Japanese Society for Food Science and Technology). 41(8):542-548

Ohnishi. 1993. Pressurization of Trichinella spiralis. Japanese-only book of Japanese pressure consortium proceedings. Chapter 17.

Okazaki, T., Yoneda, T. and Suzuki, K. 1994. Combined effects of temperature and pressure on sterilization of Bacillus subtilis spores. Nippon Shokuhin Kogyo Gakkaishi (Journal of the Japanese Society for Food Science and Technology). 41(8):536

Okazaki, T., Kakugawa, K., Yamauchi, S., Yoneda, T. and Suzuki, K. 1996. Combined effects of temperature and pressure on inactivation of heat-resistant bacteria. R. Hayashi and C. Balny. High Pressure Bioscience and Biotechnology. Amsterdam. Elsevier Science B. V. 415-418.

Otake, T., Mori, H., Kawahata, T., Izumoto, Y., Nishimura, H., Oishi, I., Shigehisa, T. and Ohno, H. 1997. Effects of high hydrostatic pressure treatment of HIV infectivity. K. Heremans. High Pressure Research in the Biosciences and Biotechnology. Leuven. Leuven University Press. 223-236.

Oxen, P. and Knorr, D. 1993. Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*. Lebensmittel-Wissenschaft und Technologie. 26:220-223

Pagan, R., Jordan, S., Benito, A. and Mackey, B. 1999. Enhanced acid sensitivity of pressure-damaged escherichia coli. European Conference on Emerging Food Science and Technology, European Federation of Food Science and technology. Tempere, Finland. November 22-24, 1999.

Pagan, R. and Mackey, B. 1999. Membrane damage in pressure-treated cells of escherichia coli. European Conference on Emerging Food Science and Technology, European Federation of Food Science and Technology. Tempere, Finland. November 22-24, 1999.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., Welti-Chanes, J. and Swanson, B. G. 1998. Oscillatory high hydrostatic pressure inactivation of Zygosaccharomyces bailii. J Food Protect. 61(9):1213-1215

Papineau, A. M., Hoover, D. G., Knorr, D. and Farkas, D. F. 1991. Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure. Food Biotechnol. 5:45-47

Parish, M. E. 1998. High pressure inactivation of Saccharomyces cerevisiae, endogenous microflora and pectinmethylesterase in orange juice. J Food Protect. 18(1):57-65

Patterson, M. F., Quinn, M., Simpson, R. and Gilmour, A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. J Food Protect. 58(5):524-529

Patterson, M. F. and Kilpatrick, D. J. 1998. The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. J Food Protect. 61(4):432-436

Paul, P. L. and Morita, R. Y. 1971. Effects of hydrostatic pressure and temperature on the uptake and respiration of amino acids by a faculttively psychrophilic marine bacterium. J Bacteriol. 108:835-843

Paul, P., Chawala, S. P., Thomas, P. and Kesavan, P. C. 1997. Effect of high hydrostatic pressure, gamma-irradiation and combined treatments on the microbiological quality of lamb meat during chilled storage. J Food Safety. 16(4):263-271

Perrier-Cornet, J. M., Marechal, P. A. and Gervais, P. 1995. A new design intended to relate high pressure treatment to yeast cell mass transfer. J Biotechnol. 41:49-58

Pierson, M. D. and Corlett, D. A. J. 1992. HACCP principles and applications. Van Nostrand Reinhold Publishing. New York.

Ponce, E., Pla, R., Mor-Mur, M., Gervilla, R. and Guamis, B. 1998. Inactivation of Listeria innocua inoculated in liquid whole egg by high hydrostatic pressure. J Food Protect. 61(1):119-122

Popper, L. and Knorr, D. 1990. Applications of high-pressure homogenization for food preservation. Food Technol. 44:84-89

Raso, J., Pagan, R., Condon, S. and Sala, F. J. 1998a. Influence of temperature and pressure on the lethality of ultrasound. Appl Environ Microbiol. 64(2):465-471

Raso, J., Palop, A., Pagan, R. and Condon, S. 1998b. Inactivation of Bacillus subtilis spores by combining ultrasonic waves under pressure and mild heat treatment. J Applied Microbiol. 85:849-854

Raso, J., Barbosa-Canovas, G. V. and Swanson, B. G. 1998c. Initiation of germination and inactivation by high hydrostatic pressure of Bacillus cereus sporulated at different temperatures. IFT Annual Meeting: Book of Abstracts. Atlanta, GA. 154.

Raso, J., Gongora, M., Barbosa-Canovas, G. V. and Swanson, B. G. 1998d. Effect of pH and water activity on the initiation of germination and inactivation of Bacillus cereus by high hydrostatic pressure. IFT Annual Meeting: Book of Abstracts. Atlanta, GA. 153.

Roberts, C. M. and Hoover, D. G. 1996. Sensitivity of Bacillus coagulans spores to combinations of high hydrostatic pressure, heat acidity, and nisin. J Applied Bacteriol. 81:363-368

Rovere, P., Tosoratti, D. and Maggi, A. 1996a. Prove di sterilizzazione a 15.000 bar per ottenere la stabilita microbiologica ed enzimatica. Industrie Alimentari. XXXV:1062-1065

Rovere, P., Carpi, G., Dall'Aglio, G., Gola, S., Maggi, A., Miglioli, L. and Scaramuzza, N. 1996b. High-pressure heat treatments: Evaluation of the sterilizing effect and of thermal damage. Industria Conserve. 71:473-483

Russell, N. J., Evans, R. I., ter Steeg, P. F., Hellemons, J., Verheul, A. and Abee, T. 1995. Membranes as a target for stress adaptation. Int J Food Microbiol. 28:255-261

Sale, A. J. H., Gould, G. W. and Hamilton, W. A. 1970. Inactivation of bacterial spores by hydrostatic pressure. J Gen Microbiol. 60:323-334

Satomi, M., Yamaguchi, T., Okuzumi, M. and Fujii, T. 1995a. Effect of conditions on the barotolerance of Escherichia coli. Shokuhin Eiseigaku Zasshi (J of Food Hyg Soc Japan). 36(1):29-34

Satomi, M., Yamaguchi, T., Okuzumi, M. and Fujii, T. 1995b. Effect of several conditions on the recovery of pressure-injured bacteria. Shokuhin Eiseigaku Zasshi (J of Food Hyg Soc Japan). 36(3):344-351

Seyderhelm, I. and Knorr, D. 1992. Reduction of Bacillus stearothermophilus spores by combined high pressure and temperature treatments. ZFL Eur Food Sci. 43:17

Shigehisa, T., Ohmori, T., Saito, A., Taji, S. and Hayashi, R. 1991. Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. Int J Microbiol. 12:207-216

Shigehisa, T., Nakagami, H., Ohno, H., Okate, T., Mori, H., Kawahata, T., Morimoto, M. and Ueba, N. 1996. Inactivation of HIV in bloodplasma by high hydrostatic pressure. R. Hayashi and C. Balny. High pressure bioscience and biotechnology. Elsevier Science B. V. 273-278.

Simpson, R. K. and Gilmour, A. 1997a. The effect of high hydrostatic pressure on Listeria monocytogenes in phosphate-buffered saline and model food systems. J Appl Microbiol. 83:181-188

Simpson, R. K. and Gilmour, A. 1997b. The effect of high hydrostatic pressure on the activity of intracellular enzymes of Listeria monocytogenes. Let Appl Microbiol. 25:48-53

Smelt, J. P. P. M., Rijke, A. G. F. and Hayhurst, A. 1994. Possible mechanism of high pressure inactivation of microorganisms. High Press Res. 12:199-203

Smelt, J. P. P. 1998. Recent advances in the microbiology of high pressure processing. Trends Food Sci Technol. 9:152-158

Smelt, J. P. P. M. and Hellemons, J. C. 1998. High pressure treatment in relation to quantitative risk assessment. VTT Symposium. Espoo: Technical Research Centre of Finland. 27-38

Sonoike, K., Setoyama, K. and Kobayashi, S. 1992. Effect of pressure and temperature on the death rates of lactobacillus casei and escherichia coli. C. Balny, R. Hayashi, K. Heremans and P. Masson. High Pressure and Biotechnology. 224:297-301

Standsted. 2000. Personal communication. Standsted Fluid Power, Ltd. Standsted, Essex CM24 8HT, UK.

Stewart, C. M., Jewett Jr., F. F., Dunne, C. P. and Hoover, D. G. 1997. Effect of concurrent high hydrostatic pressure, acidity and heat on the injury and destruction of Listeria monocytogenes. J Food Safety. 17:23-36

Styles, M. F., Hoover, D. G. and Farkas, D. F. 1991. Response of Listeria monocytogenes and Vibrio parahaemolyticus to high hydrostatic pressure. J Food Sci. 56(5):1404-1407

Suzuki, C. and Suzuki, K. 1962. The protein denaturation by high pressure. J Biochem. 52:67-71

Suzuki, K. and Taniguchi, T. 1972. Effect of pressure on biopolymers and model systems. M. A. Sleigh and A. G. Macdonald. The Effect of Pressure on Living Organisms. New York. Academic Press Inc. 103.

Tanaka, T. and Hatanaka, K. 1992. Application of hydrostatic pressure to yoghurt to prevent its after-acidification. Nippon Shokuhin Kogyo Gakkaishi (J Japanese Society for Food Science and Technology). 39(2):173-177

Tauscher, B. K. 1998. Effect of high pressure treatment to nutritive substances and natural pigments. Fresh Novel Foods by High Pressure. VTT Symposium 186. Technical Research Centre of Finland. Helsinki, Finland.

Tauscher, B. 1999. High pressure and chemical reactions: effects on nutrients and pigments. Emerging Food Science and Technology. Tempere, Finland. November 22-24, 1999. 58.

Timson, W. J. and Short, A. J. 1965. Resistance of microorganisms to hydrostatic pressure. Biotechnol and Bioeng. VII:139-159

Ting, E. 1999. Personal Communication. Flow International, Kent, WA.

Wei, C. I., Balaban, M. O., Fernando, S. Y. and Peplow, A. J. 1991. Bacterial effect of high pressure CO2 treatment of foods spiked with Listeria or Salmonella. J Food Protect. 54(3):189-193

Weiss, K. F. and Strong, D. H. 1967. Some properties of heat-resistant and heat-sensitive strains of clostridium perfringens. I. Heat resistance and toxigenicity. J Bacteriol. 93:21-26

Wilson, D. C. 1974. High pressure sterilization. 34th Annual Meeting of the Institute of Food Technologists. New Orleans, LA. May 12-15.

Wouters, P. C., Glaasker, E. and Smelt, J. P. P. M. 1998. Effects of high pressure on inactivation kinetics and events related to proton efflux in Lactobacillus plantarum. Appl Environ Microbiol. 64(2):509-514

Wuytack, E. Y., Boven, S. and Michiels, C. W. 1998. Comparative study of pressure-induced germination of Bacillus subtilis spores at low and high pressure. A Environ Microbiol. 64:3220-3224

Yano, Y., Nakayama, A., Kishihara, S. and Saito, H. 1998. Adaptive changes in membrane lipids of barophilic bacteria in response to changes in growth pressure. Appl Environ Microbiol. 64:479-485

ZoBell, C. E. 1970. Pressure effects on morphology and life process of bacteria. A. M. Zimmermann. High pressure effects on cellular processes. New York. Academic Press Inc. 85-130.

Zook, C. D., Parish, M. E., Braddock, R. J. and Balaban, M. O. 1999. High pressure inactivation kinetics of Saccharomyces cerevisiae ascospores in orange and apple juice. J Food Sci. 64(3):533-535

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