

# 2

## Food Sterilization by Combining High Pressure and Thermal Energy

G.V. BARBOSA-CÁNOVAS AND P. JULIANO

### 2.1. High Pressure Processing: An Industrial Reality

High pressure processing (HPP) is an industrially tested technology that offers a more natural, environmentally friendly alternative for pasteurization or shelf life extension of a wide range of food products (Welti-Chanes et al., 2005). Commercial high pressure, low temperature methods achieve inactivation of vegetative microorganisms by subjecting vacuum-sealed food in flexible packaging to treatment at hydrostatic pressures of 600 MPa (or less) and initial temperatures lower than 40°C for one to fifteen min depending upon the product application. The use of lower temperatures has allowed better retention of sensory attributes characteristic of “fresh” or “just prepared,” as well as food nutritional components (Cano and de Ancos, 2005). As a result, HPP has become a post packaging technology convenient for foods whose quality would otherwise be altered by heat pasteurization.

Among many advantages, HPP can add significant value to low-cost or heat-sensitive raw materials and other prepared foods. Furthermore, similar quality levels can be reached when processing large volumes or larger samples. Different from heat penetration, hydrostatic pressurization allows “instant” pressure transmission in fluids and semisolids within the pressure vessel, thereby achieving reduced product damage from lower temperatures. Moreover, HPP can add significant shelf life to an existing refrigerated product (Hjelmqwist, 2005). In fact, it has the potential to deliver chemical- or additive-free products with minimum impact on shelf life.

Like any other food preservation processes, HPP is product specific, making shelf life extension dependent on food composition, the presence of enzymes, and on the actual bacterial species/strains present in a given food factory. Another use of HPP is in texture modification of foods with high protein content.

Tenderized meats and modified dairy or egg-based ingredients of varied functionality are some examples of the benefits observed in texture modification (Montero and Gómez-Guillén, 2005; Guamis et al., 2005).

The US Food and Drug Administration (FDA) and Department of Agriculture (USDA) have approved HPP as a post package pasteurization technology for

manufacture of shelf-stable high-acid foods and pasteurized low-acid food products, and developed guidelines and regulations for those products (21CFR114 and 21CFR113). Furthermore, the European Commission on food regulations has adapted existing legislation on novel foods to products processed by HPP (EC258/97; European Commission, 2002; Barbosa-Cánovas et al., 2005a).

The term “pasteurization,” originally specifying the destruction of non-spore-forming foodborne pathogens by heat, has been extended to HPP and other validated pathogen-lethality technologies. Members of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, an advisory group chartered by the USDA’s Food Safety and Inspection Service, the FDA, the Centers for Disease Control and Prevention, the National Marine Fisheries Service, and the Department of Defense Veterinary Service Activity) have jointly established this revised definition of pasteurization, providing specific considerations for a number of thermal (cooking, microwave processing, ohmic/inductive heating, steam and hot water treatments) and nonthermal (HPP, UV radiation, pulsed electric fields, etc.) preservation processes (NACMCF, 2004; 2005).

Moreover, the FDA and USDA have promoted the implementation of Good Manufacturing Practices (GMP) as well as procedures for Hazard Analysis and Critical Control Point (HACCP) in HPP production facilities for refrigerated or pasteurized foods to ensure product safety. A food safety management standard that can be implemented when designing an HPP factory has also been developed by the International Standardization Organization (ISO) (Tapia, et al. 2005; Surak, 2006).

High pressure processing provides new opportunities for the food industry to develop new products for consumers. About 80 full scale HPP units in the world are run by 55 companies worldwide (Leadley, 2005; Hendricks, 2005). Most applications in Europe are in Italy, Spain, and Portugal, whereas others are spread around the United States, Mexico, and Japan. A number of companies are forming consortia to develop new products and new applications using high pressure technologies, such as low-acid food sterilization (de Heij et al., 2005). Among these companies are Kraft, Hormel, Unilever, Basic American Foods, Stork Food and Dairy Systems, Washington Farms, ConAgra, and Avomex.

HPP has demonstrated strong potential for the delivery of a wide range of high quality chilled products with extended shelf life. Among these, rare and cooked meats, fruits, vegetables, fresh herbs, and a variety of products prepared with these ingredients can be mentioned. Some of the products already commercialized in the worldwide market (Table 2.1) are fresh-like foods (e.g., new varieties of avocado products and juices) and ready-to-eat (or heat and serve) meat/meals. The food industry has shown interest in using HPP technology for the development of modified dairy-based items (desserts, puddings), sauces and savory foods, whole muscle meats (partially precooked), and for the improvement of shucking efficiency and seafood safety.

HPP technology, as commercially defined today, is unable to produce low-acid shelf stable products, since bacterial spore inactivation requires high pressures of at least 800–1700 MPa at room temperature, far in excess of what is commercially

TABLE 2.1. High pressure commercial chilled products (adapted from NC Hyperbaric, 2004)

Product types	Countries*	Shelf life achieved (4°C to room temperature)
Juices and beverages	Japan, France, Mexico, USA, Lebanon, UK, Portugal, Italy, Ireland, Czech Republic	21 d. to 12 mo.
Vegetable products	Japan, USA, Italy, Canada	1 to 6 mo.
Meat products	Japan, Spain, USA, Italy	21 d. to 2 mo. (cooked products)
Seafood products	Japan, USA, Australia, Canada, Spain	10 d. to 2 mo.

\*Countries are listed in order of product appearance in the market

feasible (Farkas and Hoover, 2000; Leadley, 2005). Even foods with pH lower than 4.5 require refrigerated storage and other preservation hurdles to prevent enzymatic degradation reactions and to inhibit spore germination. It was not until the early 1970s when studies on *Clostridium* species demonstrated the need to combine pressure and heat to achieve spore inactivation (Sale et al., 1970; Heinz and Knorr, 2001). Furthermore, inactivation data on *C. botulinum* spores date from the late 1990s (Rovere et al., 1998; Reddy et al., 1999; Heinz and Knorr, 2001), where pressures in the range of 690 to 900 MPa were combined with initial temperatures between 50 and 70°C. The following section will provide an in depth description of the potential application of high pressure combined with heat in commercial sterilization.

## 2.2. High Pressure Thermal Sterilization

High pressure high temperature (HPHT) processing, or pressure-assisted thermal processing (PATP), involves the use of moderate initial chamber temperatures between 60°C and 90°C in which, through internal compression heating at pressures of 600 MPa or greater, in-process temperatures can reach 90°C to 130°C. The process has been proposed as a high-temperature short-time process, where both pressure and compression heat contribute to the process's lethality (Leadley, 2005). In this case, compression heat developed through pressurization allows instantaneous and volumetric temperature increase, which, in combination with high pressure, accelerates spore inactivation in low-acid media. Several recently developed patents show a number of approaches for the attainment of commercial food sterility in selected low-acid foods (Meyer et al., 2000; Wilson and Baker, 2000; van Schepdael et al., 2002; März, 2002, 2003; Wilson and Baker, 2003; Cooper et al., 2004). Some of these microbial spore inactivation approaches proposed combining (de Heij et al., 2003; Leadley, 2005): (a) two low pressure pulses at 200–400 MPa (the first one for spore germination and the second for germinated cell inactivation); (b) a low pressure pulse at 200 to 400 MPa for spore germination followed by a thermal treatment at 70°C for 30 min for vegetative cell inactivation; (c) package preheating above 75°C and pressurization at 620 to 900 MPa for 1 to

20 min; and (d) package preheating above 70°C and applying two or more pulses at 400 to 900 MPa for 1 to 20 min.

Three of the above-mentioned approaches have proven inconvenient from either a microbiological or an economical perspective. When applying low pressures between 200 and 400 MPa, combined with moderate temperature [cases (a) and (b)], residual dormant spores have been detected after treatment (van Opstal et al., 2004; Leadley, 2005), making this option unlikely for a commercial process. Moreover, a high pressure multiple pulse approach [case (d)] is not recommended, as additional cycles decrease equipment lifetime and increase maintenance costs (de Heij et al., 2003). Hence, application of a single pulse above 600 MPa for 5 min or less [case (c)], combined with initial temperatures above 60°C, would be more cost-effective and a safer approach for industrial purposes (de Heij et al., 2005). As will be explained later, success of this processing approach depends on the efficient use of compression heat in achieving nearly adiabatic conditions.

### 2.2.1. Advantages of HPHT Processing

Recent publications claim that the main advantage of HPHT treatment is its shorter processing time compared to conventional thermal processing in eliminating spore-forming microorganisms (Fig. 2.1; Matser et al., 2004).

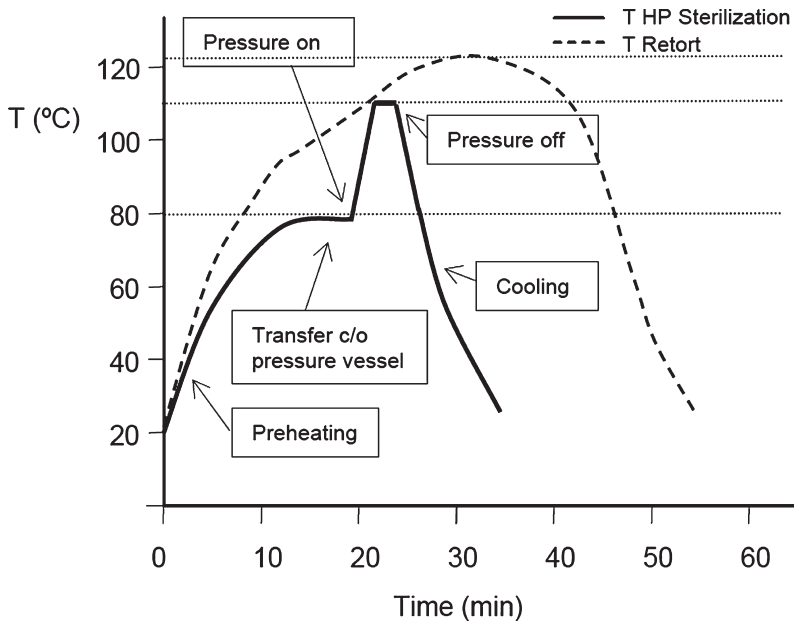


FIG. 2.1. Typical product temperature profiles in a retort and HPHT process. Processing steps during pressurization

This shorter process time and ultimate pressurization temperatures lower than 121°C have resulted in higher quality and nutrient retention in selected products. For example, better retention of flavor components in fresh basil, firmness in green beans, and color in carrots, spinach and tomato puree have been found after HPHT processing (Krebbbers et al., 2002; Krebbbers et al., 2003). Nutrients such as vitamins C and A have also shown higher retention after HPHT processing in comparison to retort methods (Matser et al., 2004).

One more benefit of HPHT processing is its use to process non-pumpable foodstuffs like soups containing solid ingredients such as noodles, barley, and/or cut-up vegetables and meat (de Heij et al., 2005).

As mentioned earlier, high pressure low temperature processing provides direct product scale-up and higher efficiency for larger volumes of food, compared to thermal processing, due to “instant” hydrostatic pressure transmission. Similarly, HPHT processing is suitable for larger sizes, as compression heating to high temperatures is instantly achieved throughout the entire package volume. Nevertheless, the preheating step, or the period of time necessary to reach initial product temperature before pressurization, needs to be considered when evaluating overall processing time. A long preheating time, especially in a large container, may lower product quality retention at the end of the HPHT process.

Although the HPHT process can be seen as advantageous due to its shorter time, lower processing temperatures cannot yet be assured for *C. botulinum* inactivation until optimal temperature/pressure/time combinations are identified. Section 2.5 will highlight some of the latest findings in terms of HPHT processing conditions required for *C. botulinum* and surrogate inactivation.

### 2.2.2. *Potential HPHT Processed Foods*

Production of shelf stable foods intended for outdoor, military, or humanitarian use has shown a tremendous increase since the late 1970s, when the concept of Meals Ready-to-Eat (MRE) packed in flexible plastic retort pouches was introduced (Mermelstein, 2001; Hirsch et al., 2005). However, the quality and nutrition challenges encountered in the development of certain foods have led to considering alternative manufacturing processes. There are a number of foods that cannot be turned into shelf-stable products by means of retort processing due to the non-acceptable or low quality values obtained after long exposure to high heat. Nonetheless, some of these foods show potential for commercial sterilization using HPHT treatment.

Products stabilized using HPHT processing can be categorized as long-life, chill- stable, and shelf-stable. The chill-stable category includes meat snacks, vegetables, and ready-to-eat meals, or heat and serve meats, among many products (Franceschini et al., 2005).

Potential HPHT shelf-stable products may include egg-based breakfast items, meat joints, pot roasts/stews, high quality soups, ready-to-drink teas/coffees, dairy desserts/smoothies, cheese/cream sauces, low-acid pasta sauces, high quality fruits/vegetables, and liquid flavors/herbs (Stewart, 2005).

The quality, acceptability, and nutritional value of these products will not only depend on the developed formulation, but also on the design of the process, i.e., the preheating equipment, the high pressure system, and the packaging material chosen.

## 2.3. Developing a High-Pressure High-Temperature System

A high-pressure system designed for commercial sterilization purposes must at least be able to withstand high pressures within the range of 600–800 MPa, chamber temperatures up to 98°C, and retain product temperatures created during compression up to 130°C. This can mainly be accomplished by building a pressure chamber of appropriate thickness, adapting an insulated polymeric liner with a sample carrier, and a pumping system that rapidly injects preheated compression fluid. Sections below will describe in more detail requirements for existing pressure systems working at HPHT conditions.

### 2.3.1. *Available Equipment and System Requirements for HPHT Conditions*

A typical batch high-pressure machine system is made of a thick wire-wound cylindrical steel vessel with two end closures, a low-pressure pump, an intensifier that uses liquid from the low-pressure pump to generate high-pressure process fluid for system compression, and necessary system controls and instrumentation (Farkas and Hoover, 2000). For HPHT processing (or pressures over 400 MPa), pressure vessels can be built with 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. In some designs, cylinders and frame are prestressed by winding layer upon layer of wire under tension. The tension in the wire compresses the vessel cylinder so that the diameter is reduced (Hjelmqwist, 2005). This special arrangement allows an equipment lifetime of over 100,000 cycles at pressures of at least 680 MPa. The preferred practice is to design high-pressure chambers with stainless steel food-contacting parts so that filtered (potable) water can be used as the isostatic compression fluid (Farkas and Hoover, 2000).

During pressurization at high temperature conditions, a temperature increase is produced in both the compression fluid and food (Ting et al., 2002). However, since compression heating in the system steel vessel is almost zero (Ting et al., 2002; de Heij et al., 2003), there is heat loss toward the chamber wall. In theory, heat generated by compression is dissipated by a combination of conduction and convection within the pressurizing fluid in the chamber and transfer of heat across the chamber wall into the surroundings (Carroll et al., 2003). Heat dissipation may cause cooling down of the sample during both come up and holding time, which may thereby decrease spore inactivation effectiveness (de Heij et al., 2002; Ardia et al., 2004). Thus, it is important to avoid heat loss through the chamber system.

A number of high-pressure systems specified for high pressure (600–1000 MPa) and high temperature (130°C) have been developed. There are several designs for vessel volumes ranging from micro/laboratory scale (0.02–2 L) to pilot scale (10–35 L). However, not all systems fulfill equal requirements in terms of pumping speed, compression-heat retention, and type of compression fluid used (Balasubramaniam et al., 2004). In most cases, vessels are heated to initial temperature by means of an internal heater (jacket or coils), which also controls the temperature. However, this is not enough to retain compression heat generated during pressurization.

Modern systems are required to use several features for heat loss prevention by mainly: (a) adapting a dense polymeric insulating liner with a free moving piston at the bottom or valve to allow adequate pressure transmission; (b) preheating the inflowing pressurization fluid and pipes; and (c) preheating the vessel at a temperature higher than the initial fluid/sample temperature. Successful installation of these features can make the system close to adiabatic and, in this way, maximize preservation efficacy at chosen HPHT conditions.

High pressure vessels insulated from the interior by means of a cylindrical liner can prevent heat losses through the steel structure. In this case, a material with low thermal conductivity (less than 1 W/m/K) is required as part of the vessel design (de Heij et al., 2003). This product container (5 mm or more in wall thickness) can be made of polymeric materials such as dense polypropylene, polyoxymethylene, polyetheretherketone, or ultra-high-molecular-weight polyethylene to provide intended heat retention (de Heij et al., 2003). Compression heating rates of these materials have not yet been determined; however, empirical testing has proven their benefit for heat loss prevention. Fig. 2.2 shows the

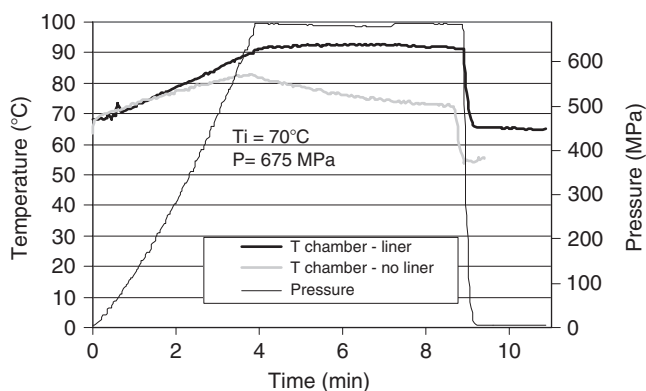


FIG. 2.2. Temperature profiles of pressurized water at 70°C initial temperature and 680 MPa when: a) compressed water is in pressure vessel; b) compressed water is in preheated polypropylene liner. Data was extracted from a cylindrical liner (internal diameter 75 mm, external diameter 100 mm, height 21.5 mm) made with a movable lid inserted into a 1.7 L high pressure chamber (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA)

temperature profile of water in a white polypropylene liner (25 mm wall thickness) during pressurization in a 1.7L vessel.

Some laboratory scale systems require using compression fluid mixtures such as food-approved oil or water containing FDA- and USDA-approved lubricants, and anti-corrosion agents. Water solutions of castor oil, silicone oil, and sodium benzoate are sometimes used as pressure-transmitting fluids (Ting et al., 2002). For HPHT processing of foods, typical fluids used in pressure vessels include water with glycerol, edible oils, and water/edible oil emulsions (Meyer et al., 2000). High compression heating of oil-added compression fluids can be of aid for additional compression heating retention. However, for commercial purposes, the use of potable water is the most recommended compression medium.

Pressure vessel size also plays an important role in the compression heating retention (Ting et al., 2002), since larger size non-insulated vessels have been shown to retain more heat during holding time (Hartmann and Delgado, 2003). At least three 35L pilot sterilization units have been built in the world, each designed by Avure Technologies (USA, The Netherlands, Italy, and Australia), and no machine has yet been designed for industrial use. The latest 35L vessel design receives compression water from the intensifier after being passed through an ultra-high pressure heater to reach initial target temperature. Another heat retention aid is the addition of preheated water from a fill tank once the product carrier, which is inserted into a cylindrical polymeric liner, is placed inside the vessel to avoid residual air after chamber closure. A series of automatic controls recirculate water prior to starting pressurization to assure that initial target temperature is reached in both the compression fluid and samples.

Ideally, a proper HPHT system requires thermocouples that provide reliable in-package temperature readings during the pressurization process. For this purpose, unpublished research has been performed by high pressure equipment manufacturers, who have screened thermocouples for types K (chromel/alumel), J (iron/constantan), and T (copper/constantan) in terms of accuracy, precision, and signal response, when exposed to HPHT conditions. Furthermore, systems that hermetically fix thermocouples inside the pouches are being tested. There are also pH measurement devices being developed to work under HPHT conditions.

The stage after pressure release is also an important part of the process. Assuming that spore inactivation depends on preheating and pressurization steps, it is important to optimize the cooling phase in order to prevent overheating and to maximize quality retention. In this case, samples can immediately be removed and transported into a turbulent low temperature water bath at the end of the pressure cycle.

### 2.3.2. *HPHT Processing and Critical Steps*

A single pulse HPHT process involves six main process time intervals: (i) sample vacuum packaging and product loading, (ii) preheating to target temperature, (iii) product equilibration to initial temperature, (iv) product temperature increase to pressurization temperature by means of compression heating, (v) product temperature



decrease during decompression, and (vi) product cooling to ambient temperature. Each of these steps (illustrated in Fig. 2.3) marks the temperature evolution of the process. However, reaching the preheating target temperature inside the food, maintaining it up to the pressure pump starts, achieving constant target pressure, and retaining heat inside the product during pressure come up and holding time are all critical to achieving consistent product sterility.

When looking at the overall process flow chart (Fig. 2.3), two main control points can be distinguished as critical to safety: preheating and pressurization. As observed before, target pressurization temperature in all pouches inside the chamber or liner, as well as in all parts of the food, depends on the target preheating temperature before pressure is initiated. As a result, process variables must be controlled in different sections of the preheating/pressure system to assure that conditions required for spore inactivation are met.

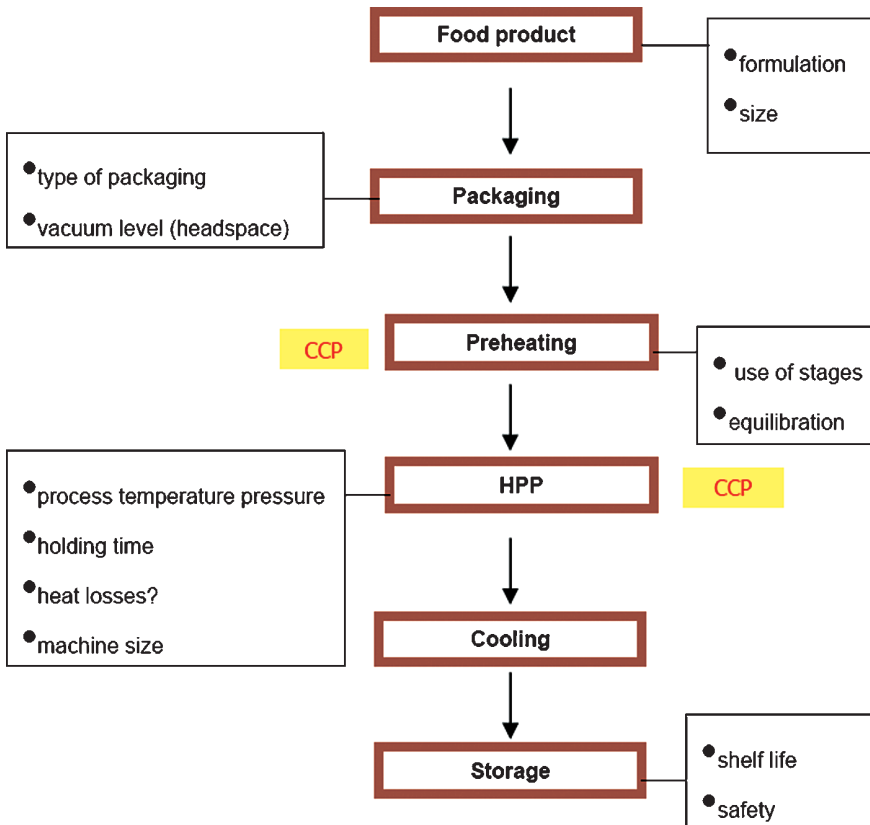


FIG. 2.3. Flow chart of HPHT process showing critical control points (CCP) for food safety as well as other process variables

### 2.3.2.1. Critical Factors

During an HPHT process, the amount of heat received is determined by three main conditions: target preheating/equilibration temperature, selected pressure, and pressure/temperature holding time. Furthermore, there are other inherent factors such as the presence of lower temperature sites in the vessel, pressure come up rate, pressure holding, decompression rate, and food/package properties. All need to be accounted for when evaluating sterilization performance.

*2.3.2.1a. Temperature Distribution.* In terms of temperature distribution within a non-insulated system, “cold spots” are located close to the vessel wall. This may cause the product fraction located near the wall to cool down and not match the final temperature obtained at the center of the vessel (de Heij et al., 2002; Ting et al., 2002; Otero and Sanz, 2003; Ardia et al., 2004).

Another factor influencing vessel temperature distribution is the (preheated) liquid entering the vessel (2-30 mL, depending on vessel dimensions), which can cool down the transmission fluid located inside the vessel.

Three-dimensional numerical simulations have illustrated temperature gradients created by incoming fluid at the entrance of the vessel (Hartmann and Delgado, 2002).

In order to decrease cooling caused by the inflowing pressurizing fluid, the system can be modified according to the following (de Heij et al., 2003; Balasubramaniam et al., 2004): (a) An internal pressure intensifier can be incorporated to decrease the amount of liquid entering the machine; (b) the pressurizing fluid, the high-pressure pipes, and the external intensifier system in the high-pressure pump can be preheated to a higher initial temperature; and (c) insulation with a special liner can be added to prevent contact between packages and entering fluid. These solutions can be executed according to (i) the pressure vessel dimensions, (ii) the initial temperature specified for the preheating system, (iii) the compression fluid used by the specific machine, and (iv) the intensifier system, including the incoming fluid. If all processing aids are simultaneously applied, temperature loss during come up and holding times can reach a minimum. However, up until now there has been no existing equipment that can guarantee a heat retention efficiency of 100%.

The difference between the temperature of the fluid/package system before and after high-pressure processing can indicate the extent of heat loss during processing (Ting et al., 2002). Thus, based on what was discussed above, the following parameters can be used to account for the overall process performance concerning product safety:

- Target preheating/equilibration temperature
- Target temperature at maximum (constant) pressure
- Temperature at the end of holding/pressurization time
- Temperature at the end of pressure release

Rates of preheating, compression (pressure come-up time), and cooling can be associated with the process performance in terms of processing time, or time of exposure of the food package to high heat.

*2.3.2.1b. Pressure come-up time.* The pressure rise period has proven important concerning spore inactivation, since bacterial spores can be inactivated at zero holding time when combined with temperature (Koutchma et al., 2005). In this case, come-up time, or compression rate, is determined by setting the power of the low-pressure pump (driving the intensifier) and the target process pressure (Farkas and Hoover, 2000; Balasubramaniam et al., 2004). For a specific process, variability in the compression rate must be determined and tolerance should be specified for the inactivation process. Moreover, effects on compression rate variation in spore inactivation are still unknown, and its determination could help establish a compression rate tolerance.

*2.3.2.1c. Constant Pressure.* Maintaining a constant pressure during pressurization is also essential for compression heat retention. In modern high-pressure systems, pressure intensifiers and automatic pressure control maintains pressure constant during the holding time. High-pressure equipment allows controlling pressure within  $\pm 0.5\%$  (e.g.,  $\pm 3.4$  MPa at 680 MPa) and recording it to the same level of accuracy (Farkas and Hoover, 2000).

*2.3.2.1d. Decompression Rate.* It is unclear whether the decompression rate is critical for spore inactivation. However, from a process efficiency perspective, it would be desirable to achieve ambient pressure recovery in the shortest possible time. Control of decompression rate should be recommended based on the results of the effect of pressure-release rate on spore inactivation, which is still unknown. Single stroke intensifiers may be used to control the decompression rate of a system (Farkas and Hoover, 2000).

*2.3.2.1e. Food Package-related Factors.* Factors that are food-related include the package dimensions and weight, food molecular properties (pH, composition, water activity), and thermophysical/structural properties (volume, shape, density/porosity, compressibility, specific heat, thermal expansion coefficient). Variation of these properties may affect temperature uniformity in the treated food. Uniform initial target temperature of the food sample is desirable to achieve a uniform temperature increase in a homogenous system during compression (Farkas and Hoover, 2000; Meyer et al., 2000). Non-homogeneities in terms of composition, presence of food pieces, or uneven preheating (e.g., from microwaves) may affect the temperature distribution during pressurization. Hence, it is important to analyze and quantify the effect of each factor on the overall process performance and to evaluate the most critical ones.

### 2.3.3. *Compression Heating in HPHT Processing*

In general, compression/decompression temperature and pressure curves are nearly linear and, therefore, compression rate can be assumed constant for a given time interval. Once target temperature is fixed, a constant compression rate should provide a constant compression heating. However, as indicated by the

compression heating equation (Eq. 2.1), this will depend on how the volumetric expansion coefficient  $\alpha_p$  (1/K), the density  $\rho$  (kg/m<sup>3</sup>), and the isobaric heat capacity  $C_p$  (J/kg.K) of both the food and liquid will change during pressurization time (Carrol et al., 2003).

$$\frac{dT}{dP} = \frac{T\alpha_p}{\rho C_p} \quad (2.1)$$

where  $T$  is the temperature (K) of the food or compression fluid.

Both the temperature of the product and compression fluid may rise 20–40°C during high-pressure treatment, whereas, as stated before, the steel pressure vessel is not subjected to significant compression heating (de Heij et al., 2002; Ting et al., 2002). As shown in Fig. 2.4, several food components provide variable compression heating rates. Therefore, temperature increase may vary in foods with relatively complex composition. In fact, the compression heating rates of fats and oils can be up to three times higher than in water (Ting et al., 2002; Rasanayagam et al., 2003).

Not much data has been reported on the compression heating rate for food products at HPHT conditions. Balasubramaniam et al. (2004) reported compression heating of water at 4.0, 4.6, and 5.3°C/100MPa and initial temperatures of 60, 75, and 90°C, respectively. However, Rasanayagam et al. (2003) observed little or no increase in compression heating in oils and fats due to higher initial temperature.

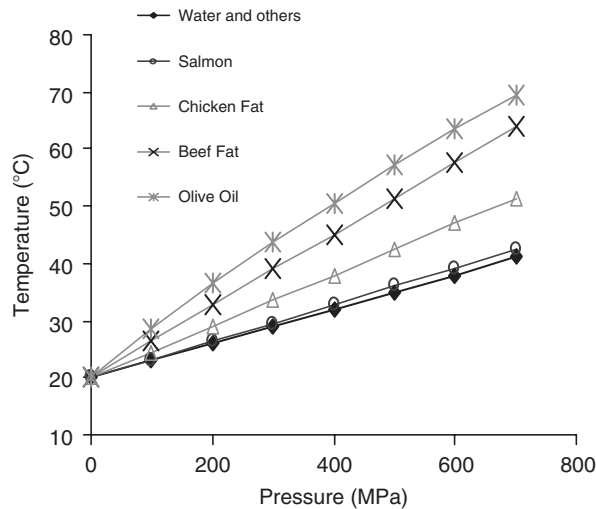


FIG. 2.4. Temperature elevation from room temperature due to pressurization up to 700 MPa (modified from Barbosa-Cánovas and Rodríguez, 2005)

Equation (2.1) has been applied using small pressure intervals of  $\Delta P$  (around 10 MPa) to predict compression heating of water in the range of 0.1 up to 350 MPa and initial temperatures of 22 and 62°C (Otero et al., 2000). The authors reported the decompression cooling rate (rather than compression heating) after holding time, and good agreement was found with experimental data when using values for  $\rho$ ,  $\alpha$ , and  $C_p$  taken from the literature (Ter Minassian et al., 1981). Moreover, Ardia et al. (2004) predicted temperature rise in water, sugar solutions, and orange juice by comparing data from the National Institute of Standards and Technology (NIST) (Harvey et al., 1996) and experimental measurements done in a high pressure machine. They found no significant deviations between NIST and experimental results for water obtained from the Multivessel Model U111 (Unipress, Warsaw, PL) for distilled water, even at sterilization conditions (initial liquid temperature of 80°C at 600 MPa).

For predictions in sugar solutions and orange juice, Eq. (2.1) was rewritten using  $\alpha$  for water and implementing NIST formulations for the regressive calculation of each property (Eq. 2).

$$\Delta T = \int_{P_0}^{P_1} \frac{\alpha}{\rho_{mixture} \cdot C_{p\ mixture}} \cdot T \cdot dP \quad (2.2)$$

For sugar solutions, mixing rules (Eqs. 2.3 and 2.4) were used to express density and the specific heat of a pure solid/water mixture,  $\rho_{mixture}$  and  $C_{p\ mixture}$ , as a function of temperature:

$$\rho_{mixture} = \left[ \frac{[W]}{\rho_{water}} + \frac{[S]}{\rho_{solid}} \right]^{-1} = \left[ \frac{[W]}{\rho_{water}} + \frac{[S]}{\left[ \frac{1587.9}{1 + 0.000107(T - 15)} \right]} \right]^{-1} \quad (2.3)$$

$$C_{p\ mixture} = [W] \cdot C_{p\ water} + [S] \cdot C_{p\ solid} = [W] \cdot C_{p\ water} + [S] \cdot \xi \cdot (1622 + 7.125 \cdot T) \quad (2.4)$$

where [W] and [S] are the relative amounts of water and solid (in mass or volume percentage, respectively). Since mixing rules equations are applicable at ambient pressure, an empirical correction factor was used to correctly fit the experimental results for  $C_{p\ mixture}$ :

$$\xi = \frac{C_{p\ water}(T, 0.1\ MPa)}{C_{p\ water}(T, p)^{0.75}} \quad (2.5)$$

where the numerator is represented by  $C_p$  of water calculated at atmospheric pressure and the denominator denotes  $C_p$  of water at higher pressure conditions.

Ardia et al. (2004) found no significant deviations for sugar solutions in the range of 0.1 to 600 MPa, while good continuity was found when extrapolating between 600 and 1400 MPa. When simulating the heat of compression in orange juice with a 9% solid content, no evident deviations from measured temperature were detected. Thus, the model produced satisfactory and reproducible compression temperature rise even at sterilization conditions. This means that the  $\alpha$ ,  $\rho$ , and  $C_p$  values of pure water determined from the NIST database were also accurate for predicting the increase in temperature, even at sterilization conditions (Eq. 2.2).

### 2.3.4. *Packaging for HPHT Processing*

Packaging materials selected for shelf-stable products must meet a number of requirements in terms of seal strength, overall integrity, and barrier properties to oxygen and water vapor. However, little is known about the effect of HPHT conditions on polymeric laminates. Recent studies have found that aluminum foil laminated retort pouches designed to withstand a temperature of 121°C may delaminate and blister after a thermal pressure process, particularly at chamber temperatures >90°C and >200 MPa (Schauwecker et al., 2002; Caner et al., 2004).

Delamination during pressurization has been mainly observed in areas in contact with trapped air (undergoing higher compression heating) and in folded parts (suffering higher localized stress at hydrostatic compression). Some researchers claim that HPHT conditions decompose portions of the adhesive layer, causing other layers (e.g., polyethylene and foil laminate in retort pouches) to separate. Research on several laminates to identify packaging materials suitable for HPHT conditions is ongoing. Special attention is being given to the effect of HPHT treatment on packaging components and the contribution of air retained in packages (or headspace) to delamination.

Another aspect to consider about a packaging material is its behavior as a barrier to heat and pressure transfer. During preheating and cooling steps, the container material constitutes an intermediate barrier in the transfer of heat from the heating medium into the product and from the product to the cooling medium. The thickness of the material, as well as its thermal diffusivity, can affect the rate of heat conduction to (or from) the product during preheating (or cooling). During the pressurization stage, packaging material such as polypropylene has been shown to act as an insulating barrier, preventing heat loss from the product (Hartmann and Delgado, 2003). Other than the laminate composition and thickness, the headspace left inside the package as well as internal stresses from the vacuum created are additional factors to consider in the transfer of pressure and heat.

## 2.4. Preheating Step: Design and Quality Optimization

Correct performance of the preheating step is critical to ensure that the temperature of the food product matches the target initial temperature. As already mentioned, a uniform initial target temperature throughout the food sample is desirable to

achieve a uniform temperature increase in a homogenous system during compression (Farkas and Hoover, 2000; Meyer et al., 2000). If “cold spots” are present within the food, some areas in the product will not reach the target process temperature during pressurization, thereby posing a safety risk in those areas with lower temperature (Meyer et al., 2000). However, an extended preheating time can also affect the quality characteristics of the food product, so in order to maximize quality retention, it is desirable to minimize the duration of preheating needed to reach the target initial temperature (Barbosa-Cánovas et al., 2005b; Juliano et al., 2006b).

#### *2.4.1. Preheating Design for Optimal Quality Retention*

Several alternatives have been proposed to save preheating time, for example, the use of still water baths set at temperatures higher than the target temperature, and other heat transfer aids such as steam, steam injection in water, water circulation pump systems, or dielectric heating (Hoogland et al., 2001; Juliano et al., 2006b). However, care must be taken when adopting faster preheating methods, as they can affect initial temperature distribution within the food package. Since faster preheating methods provide less uniformity, they require a longer time for equilibration to achieve temperature homogeneity.

In practice, carrier operation time can be reduced by using a two-stage preheating approach, i.e., by having samples preheated at a lower temperature in a separate vessel. In this case, the sample should undergo two equilibration steps: (1) equilibrating in a still water bath at a lower temperature (e.g., 60°C) and (2) placing samples in a carrier, preheating up to target temperature, and equilibrating at target temperature. An alternative approach in step 2 is to preheat the food to a temperature greater than the target temperature. This will shorten equilibration time and assure that all parts of the food reach the initial temperature (Barbosa-Cánovas et al., 2005b). In both cases, two-stage preheating could result in improved productivity, especially in larger packages. Choosing this approach will depend on product composition and its sensitivity to long-time exposure to moderate temperatures. As will be discussed further, the dimensions and thermal properties of the test sample and vessel determine equilibrium time (step 2) inside the chamber (Balasubramaniam et al., 2004).

#### *2.4.2. Factors Affecting Preheating*

Factors related to package geometry, product characteristics, and heating system can affect preheat time (NFPA 1985) and are organized in Table 2.2 for each element in the preheating system.

In thermal processing, pouches usually have a slab geometry design to achieve a more rapid heat penetration due its thin profile and high surface-area-to-volume ratio (NFPA 1985). Therefore, container thickness can be considered the main factor affecting heating rate. If containers are placed in hot water, the water vapor released and residual gas retained inside may expand the container, altering its

TABLE. 2.2. Factors affecting heat transfer during preheating of packaged foods

Process variable	System element	Process factors	Parameters
Fluid temperature	Preheating system	<ul style="list-style-type: none"> <li>• Type of system</li> <li>• Ratio of fluid mass:product mass (number of packages)</li> <li>• Racking system (separation between container, circulation between layers, package restraint to specified thickness)</li> <li>• Heat transfer aids (steam, steam/vapor, microwaves, radiofrequencies, circulation pumps)</li> </ul>	<ul style="list-style-type: none"> <li>• Heat transfer coefficient</li> <li>• Heating rate</li> </ul>
	Container geometry	<ul style="list-style-type: none"> <li>• Packaging material (composition, thickness)</li> <li>• Package thickness</li> <li>• Fill weight</li> <li>• Sample confining system (racks, trays, cassettes)</li> <li>• Container headspace (amount of air in the package)</li> <li>• Container shape</li> <li>• Distribution of food particulates</li> </ul>	<ul style="list-style-type: none"> <li>• Package thermal diffusivity*</li> <li>• Time to reach target temperature</li> <li>• Temperature equilibration time</li> </ul>
Target preheating temperature	Product characteristics	<ul style="list-style-type: none"> <li>• Composition of ingredients</li> <li>• Particulate size</li> <li>• Soluble solids</li> <li>• Physical state (fresh/cooked, liquid, semisolid, frozen)</li> <li>• Food structure (homogeneity)</li> <li>• Occluded gases</li> <li>• Viscosity</li> </ul>	<ul style="list-style-type: none"> <li>• Product thermal diffusivity*</li> </ul>

\*Thermal diffusivity  $\gamma$  is known as the ratio of the heat conducted to the heat stored and is calculated using the following expression:  $\gamma = k/(\rho C_p)$ , where  $k$  is the thermal conductivity,  $\rho$  is the specific density, and  $C_p$  is the specific heat

thickness. In retort processing, thickness is controlled with overpressure, which also ensures seal integrity. In an HPHT process, internal product temperatures can be as high as 90°C in preheating systems, in which case, package thickness can be controlled by other physical means. For example, polymeric racks, grid trays, and cassettes can be specially designed to hold the container in place, avoiding sample mobility and maintaining pouch thickness.

In laboratory scale HPHT processes, samples are generally preheated in selected baths in a carrier and immediately transferred inside the liner for product equilibration. This liner can later be located inside the high pressure machine for



subsequent pressurization. Another option, currently used in pilot scale vessels, is to directly perform preheating inside the liner, while having samples stacked in the carrier. Liners should include steam/air injection and circulation pumps for stirring of the heating media. If higher preheating fluid temperature is used, the liner system needs a means of decreasing fluid temperature at the end of preheating near to the target product temperature (e.g., from 100 to 80°C). Temperature decrease of the preheating fluid inside the liner will avoid temperature overshooting during product equilibration and pressurization steps while the liner is located inside the pressure chamber. One alternative would be to partially remove the heating fluid and mix it with water preheated at initial pressurization temperature. In this case, highly controlled automatic devices that regulate the inflow/outflow of fluid until the system reaches initial target pressurization temperature need to be designed.

In order to ensure proper temperature exposure to all packages in the system, the racking system should be designed in a way that water circulation is parallel to the container length or width. Furthermore, the separation between each container layer should be calculated to permit water/steam/air mixture circulation and assure temperature uniformity. Given the importance of this critical step, a reference temperature device, similar to the mercury-in-glass thermometer used in retorts, should be added to the system.

Other factors related to the container geometry, e.g., fill weight, container size, and product characteristics (Table 2.2), need to be identified when adapting a preheating system to a particular product and processing. Container geometry is defined by the package shape and distribution of the food and its particulates, which can affect preheating rate. As previously mentioned, heating rate is also a function of the food's physical and chemical properties, and variation of these properties mainly depends on product formulation. If the product is a semisolid, minor variations in formulation might not significantly change the rate of heat penetration. Moreover, heat transfer rate can be associated with the thermal diffusivities of the package and food components of the semisolid (Palazoglu, 2006). On the other hand, foods containing sufficient free liquid to promote convection may change heat transfer drastically according to their composition (e.g., due to added starch or other thickening agents), thereby changing heat penetration rate.

### 2.4.3. *Heat Penetration Evaluation*

Evaluation of heat penetration during preheating is important for comparative purposes, particularly when characterizing a system modified for faster temperature rise, during scale-up studies using multiple pouch stacks, or when testing larger size products. Primarily, an adequate temperature log-in system must be installed to provide reliable temperature profiles used to determine preheating efficiency. Plastic stuffing boxes can help fixing the thermocouples at the center of the pouch (or at the slowest heating position) for accurate determination of heat penetration profiles. Other thermocouple positioning devices shown in guidelines from the US National Food Processors Association can also be adapted (NFPA, 1985).

Heat penetration can be evaluated by using the heating rate index  $f_h$  and heating lag factor  $j_h$  (Holdsworth, 1997). The heating rate index  $f_h$  and heating lag factor  $j_h$  can be determined by using Eqs. (2.6) and (2.7) (Holdsworth, 1997):

$$\log u = \log \left( \frac{T_R - T}{T_R - T_0} \right) = -\frac{t}{f_h} + \log j \quad (2.6)$$

where  $u$  is the reduced temperature,  $T$  is the temperature at the geometric center of the package,  $T_0$  is the initial product temperature,  $T_R$  is the reference temperature of the heating medium, and  $j$  is the extrapolated lag factor. The corrected heating lag factor  $j_h$  can be determined from the 58% come-up time ( $t_{58}$ ), which corresponds to an additional 42% of come-up time needed to reach the target temperature.

$$j_h = 10^{\frac{t_{58}}{f_h} + \log j} \quad (2.7)$$

The lag factor  $j_h$  is related to the lag time needed to reach uniform heating rate values. The accuracy of heat penetration determination depends, among other factors, on the headspace inside the pouch, as well as the internal vapor pressure created due to increased temperature, which can influence the  $j_h$  value due to convective currents in contact with the product surface. Accuracy of temperature readings may also be affected by the type of thermocouples used, thermocouple entry system into the package, and use of connectors and extension wires (NFPA, 1985).

When comparing different preheating methods, the heat transfer coefficient can provide additional information on preheating efficiency. It can be calculated either by empirical correlations between dimensionless numbers (e.g., Nussell and Reynolds number) or by fitting simplified Fourier balances to experimental temperature histories. The instant heat transfer coefficient  $h$  can be determined by solving the following overall heat balance equation (Varga and Oliveira, 2000):

$$\rho C_p V \frac{dT_{ave}^t}{dt} = h_1 A (T_{\infty}^t - T_{surf}^t) \quad (2.8)$$

where  $T_{\infty}$ ,  $T_{surf}$ , and  $T_{ave}$  are the heating medium and product surface and volume average temperatures, respectively. Since this expression defines an instant value, the superscript and subscript  $t$  indicate that these variables are a function of time.

Varga and Oliveira (2000) showed that more reliable results can be obtained from the derivatives of average heat transfer coefficient  $\bar{h}_t$ , instead of calculating  $h_t$  values for short time steps in the preheating process.

The average external heat transfer coefficient  $\bar{h}_t$  between zero and time  $t$  was defined as:

$$\bar{h}_t = \frac{\int_0^t h_t dt}{t - t_0} \quad (2.9)$$

where  $t_0$  is the initial time for the preheating stage (depending on whether a one- or two-stage preheating is considered). Varga and Oliveira (2000) offered two solutions to determine the average external heat transfer coefficient from  $t_0$  to several values of  $t$  considered: (a) integrating the heat balance (Eq. 2.8) or (b) using the residual sum of the square method to minimize the difference between experimental and model temperatures. A detailed description of this methodology escapes the scope of this manuscript and can be found in the mentioned reference.

## 2.5. Microbial Engineering and Regulatory Implications

Bacterial spore inactivation depends on pressure applied and initial temperature of the food and vessel. Most thermal baro-resistant spores are inactivated at pressures 600 MPa or greater, in combination with initial temperature above 60°C. However, relatively low pressures (below 200 MPa) can trigger spore germination (Patterson, 2005; Leadley, 2005). Furthermore, studies on target microorganisms for inactivation and related safety assurance of canned food products, such as for *Clostridium botulinum*, have shown large variation in the pressure resistance of different spore strains (Margosch et al., 2004; Margosch, 2005; Gola and Rovere et al., 2005).

### 2.5.1. Inactivation of *C. botulinum* Strains by HPHT Processing

Until now, the resistance to pressure and temperature has been studied with at least five *C. botulinum* strains in varied conditions and media (Reddy et al., 1999; Reddy et al., 2003; Margosch et al., 2004; Gola and Rovere, 2005). *C. botulinum* spores type E in pH 7 buffer, for example, have been reduced 4.5 logs at 50°C/758 MPa/5 min and 5 logs at 40°C/827 MPa/10 min (Reddy et al., 1999). Furthermore, *C. botulinum* type A was reduced by more than 3 log units in pH 7 buffer and crab meat following treatment at 75°C/827 MPa/20 min (Reddy et al., 2003). Other studies on several *C. botulinum* strains (types A, B, F proteolytic, B non-proteolytic) in mashed carrots were carried out by Margosch et al. (2004) and Margosch (2005). They found that treatment at 80°C/600 MPa/1 s reduced strains by more than 5.5 log cycles to none at all. In particular, non-proteolytic *C. botulinum* type B was the least resistant strain and was reduced by more than 5.5 log cycles after 80°C/600 MPa/1 s. In comparison, proteolytic *C. botulinum* type A had more than 5 log reductions after 80°C/600 MPa/12 min treatment, and proteolytic *C. botulinum* type B spores were inactivated by less than three orders of magnitude at 80°C/600 MPa/60 min.

Since spore resistance also proved to be product dependent, more research is needed to find the optimum inactivation pressure/temperature/time conditions for *C. botulinum* strains in particular products, as the type of medium may influence spore germination rates (Margosch, 2005). Moreover, understanding how HPHT conditions affect bacterial neurotoxins produced is worth of consideration (Margosch et al., 2005).

### 2.5.2. *Inactivation of Microbial Spore Surrogates by HPHT Processing*

Regarding other spore-forming microorganisms, microbial studies have proven that an initial chamber/product temperature of 75-85°C and pressure 600-827MPa can effectively inactivate target heat resistant spore-forming bacteria commonly used as indicators of food safety and shelf stability (Heinz and Knorr, 2001). In particular, *Bacillus stearothermophilus* spores were reduced 5 log cycles in phosphate buffer and beef broth at 70°C/700MPa/3 min (Gola et al., 1996; Rovere et al., 1998), and at least 4.5 log cycles in meat balls in tomato puree at 90°C/700MPa/30s (Krebbbers et al., 2003). Furthermore, *B. stearothermophilus* spores were inactivated in egg patties at 700 MPa and 105°C (Rajan et al., 2006a; Rajan et al., 2006b; Koutchma et al., 2005). Koutchma et al. (2005) also showed that 700MPa/105°C/4 min was sufficient to destroy 6 logs of *B. stearothermophilus* in spore strips in egg patties, whereas 6 logs of *C. sporogenes* PA 3679 at 700MPa/110°C/5 min were destroyed in the same media.

Other spore genii such as *Bacillus licheniformis* spores suspended in pH 7.0 buffer were also inactivated at 60°C/600MPa/20 min (Taki et al., 1991), and reduced 6 logs in pH 7 buffer and beef broth at 70°C/700MPa/5 min (Gola et al., 1996; Rovere et al., 1998). *Bacillus cereus* spores were also reduced 8 logs in pH 7 buffer at 60°C/690MPa/1 min with previous sporulation at 37°C (Raso et al., 1998), and 5 logs in beef broth at 70°C/700MPa/5 min (Rovere et al., 1998). Similarly, *Bacillus subtilis* spores were inactivated at 827MPa and a process temperature ranging from 102 to 107°C, yielding up to a 6 log reduction (Balasubramaniam and Balasubramaniam, 2003).

Moreover, *B. amyloliquefaciens* was higher in HPHT resistance than *C. botulinum* strains (Margosch, 2005), becoming a potential surrogate for future research in HPHT sterilization (Margosch et al., 2004). Ahn et al. (2005) obtained up to 7-8 log reductions with *B. amyloliquefaciens* after treatment at 700MPa and 121°C for less than 1 min.

It is worth mentioning that data reported by several authors on spore inactivation were not obtained at the same conditions due to heat loss experienced in the chamber using machines of different sizes, the compression fluids, and heat retention configurations (de Heij et al., 2002; Balasubramaniam and Balasubramaniam, 2003; Ardia et al., 2004; Balasubramaniam et al., 2004). Thus, in many cases, data may not be comparable. Previous research has proven by means of numerical simulation the difference in inactivation reduction of *Alicyclobacillus acidoterrestris* spores at 50°C/800MPa (Ardia et al., 2004) and *B. stearothermophilus* (de Heij et al., 2002) at 700MPa/121°C/2-90s pulses between the center and inner side-wall of pressure vessel. Particularly, Ardia et al. (2004) predicted that a difference of 3-4°C between these two crucial points would result in a difference of approximately 6 log reductions between the center and inner side-wall of a polyethylene container located inside the pressure vessel. De Heij et al. (2002) predicted a 15°C difference between the center and vessel side-wall, giving a rough difference of 5 log cycles.

### 2.5.3. Regulatory Perspective

During the last five years, high pressure sterilization initiatives have been started by two consortia, one in the US and one in Europe, offering a new technology that will rival or complement conventional canning. Although 21CFR113 was not intended for pressure-processed low-acid vegetables and seafood, it is likely to be applicable since heat is included during compression (Sizer et al., 2002). Other parts in the US Code of Federal Regulations (9CFR318.300 and 9CFR381.300) applicable to the sterilization of food products containing 3 percent or more raw (2 percent cooked) meat or poultry could also be used for this technology.

A standard scenario for commercial sterilization using high pressure processing can be defined as the combination of 700 MPa and a final process temperature of 121°C (initial temperature 90°C) with a holding time of 3 min. In this case, an  $F_0$  value of approximately 3 min (corresponding to a 12D reduction of *C. botulinum* spores) is obtained by only accounting for the thermal component and neglecting the pressure effect on microbial inactivation (Sizer et al., 2002). This standard scenario would allow filing HPHT treatment as a thermal process according to 21CFR113 as a first approach, avoiding the use of large volumes of microbial inactivation data and special kinetic models to account for the combined effect of temperature and pressure.

On the other hand, various researchers are gathering safety data on microbial inactivation, as well as process data, to model the kinetics of *C. botulinum* inactivation that will allow identifying process performance criteria for process validation and process filing with regulatory agencies such as the FDA.

#### 2.5.3.1. FSO Concept in High-Pressure Sterilization

The concept of Food Safety Objective (FSO) has been introduced by the Codex Committee on Food Hygiene (Codex, 2004) as the “maximum frequency or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)” (NACMFS, 2005). This concept is also emerging as a regulatory parameter for evaluating the efficacy of novel technologies to inactivate target pathogenic microorganisms. An inactivation performance criterion can be expressed as follows (NACMFS, 2005):

$$H_0 - \sum R + \sum I \leq \text{FSO (or PO)} \quad (2.10)$$

where FSO is the food safety objective, PO is the performance objective,  $H_0$  is the initial level of the hazard,  $\sum R$  is the total (cumulative) reduction of hazard on a  $\log_{10}$  scale, and  $\sum I$  is the total (cumulative) increase of hazard on a  $\log_{10}$  scale.

Alternatively, the Performance Objective (PO), or “the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain prior to consumption that provides or contributes to an FSO or ALOP, as applicable” (Codex, 2004), can be used in Eq. (2.10) to establish process performance. In the context of risk analysis, an FSO would be based on a public health goal that provides an ALOP or “reasonable certainty of no harm.” Since the most resistant

microorganism of public health significance in HPHT sterilization of low-acid foods is *C. botulinum*, an FSO could be defined as the achievement of a <100 cfu *C. botulinum*/g in a food at the point of consumption.

#### 2.5.4. *Process Performance Criteria Accounting for Temperature History and Inactivation Kinetics*

As mentioned before, the 21CFR113 primarily stipulates minimum temperature requirements for commercially sterilizing low acid foods. However, inactivation of *C. botulinum* has not been validated for thermally pressurized foods, not only due to the lack of inactivation data for several strains, but also because the microbial performance or process outcome criterion has not yet been established for comparison purposes (Koutchma et al., 2005). This process performance criterion should include inactivation kinetic parameters of *C. botulinum*, along with other parameters that account for the pressure and temperature profile during the established HPHT sterilization process.

The following paragraphs will describe approaches to determining parameters that simultaneously account for uniformity and the sterility of an HPHT process and that can therefore be applied in process validation.

##### 2.5.4.1. Conventional Thermal Processing Approach

Koutchma et al. (2005) showed that the HPHT process may be validated by applying concepts such as decimal reduction time ( $D_T$ ,  $D_p$ ) and temperature sensitivity ( $Z_T$ ,  $Z_p$ ), traditionally used in conventional thermal processing of low-acid foods. The authors based their research on evidence reported on the linearity of microbial inactivation curves (semilog scale) of classical surrogates, namely *B. stearothermophilus* and *C. sporogenes* PA 3679, at a pressure range of 600–800 MPa and process temperature range of 91–108°C. They found that thermal sensitivity of PA 3679 spores ( $Z_p$  values) did not vary with pressure, nor did pressure sensitivity ( $Z_T$  values) vary with temperature at these ranges. They were able to calculate  $F_0$  values for the HPHT sterilization process by adapting a concept established by Pflug (1987) to calculate the process lethality, which included the initial microbial load. Nevertheless, research is ongoing since  $Z$  values of *C. botulinum* spores at a reference sterilization temperature 121°C are necessary for quantifying the additional contribution of pressure in an HPHT process in terms of lethality and overall thermal death time. In order to separate the pressure effect, care must be taken when comparing an HPHT process with a conventional thermal process, since processing factors (i.e., package geometry, food media, temperature measurement, etc.) should be reproducible at only-thermal and thermal pressurization conditions. Furthermore,  $F_0$  values accounting for the thermal components should coincide in both processes. Due to the asymmetric reduced temperature semilog scale profile obtained in an HPHT process, the General Method (Holdsworth, 1997) could most accurately determine  $F_0$  values from temperature profiles in commercial software packages.

### 2.5.4.2. Weibullian Approach

Peleg et al. (2005) identified a theory to redefine the concept of “thermal death time” for non-isothermal heat treatments like HPHT processing that was an alternative to the  $F_0$  value concept, by describing *C. botulinum* inactivation kinetics using a power law or “Weibullian” model with temperature dependent parameters:

$$\log \left[ \frac{N(t)}{N_0} \right] = -b(T)t^{n(T)} \quad (2.11)$$

where  $N_0$  is the initial number of microorganisms and  $N(t)$  is the number of surviving microorganisms at time  $t$ . The parameters  $b(T)$  and  $n(T)$  are a function of the applied temperature. A similar model can be used to describe the HPHT process using model parameters,  $b$  and  $n$ , as functions of both pressure and temperature (Campanella and Peleg, 2001). Thus, the combined effect of pressure and temperature of the process can be accounted for in this expression:

$$\log \left[ \frac{N(t)}{N_0} \right] = -b(P,T)t^{n(P,T)} \quad (2.12)$$

The relationship between parameters  $b$  and  $n$  with pressure and temperature can be determined from experimental survival curves obtained at combined pressures and temperatures. In order to know the effects of pressure and temperature on parameters  $b$  and  $n$ , the temperature [i.e.,  $T(t)$ ] and pressure [i.e.,  $P(t)$ ] histories must be known. Thus, an analytical heat transfer model to express  $T(t)$  and  $P(t)$  needs to be incorporated into Eq. (2.12). Once parameters  $b(t)$  and  $n(t)$  are known, inactivation of microorganisms at selected pressure and temperature can be predicted. Peleg et al. (2003) and Peleg et al. (2005) proposed a methodology to estimate survival parameters  $b$  and  $n$  for conditions of variable pressure and temperature. However, as said before, no sufficient *C. botulinum* data exists that validates the model. Once data is collected for a tolerable number of *C. botulinum* strains, these parameters could eventually be used to validate an established HPHT sterilization process.

The advantage of this approach is that parameters will depend on the actual thermal pressurization history; therefore, more accurate conditions to reach sterilization can be defined to establish a given HPHT process. This approach allows process optimization to minimize over processing, and increases the chances of yielding higher quality foods with increased nutritional content (Peleg et al., 2005).

### 2.5.4.3. Alternative Performance Criteria

Design of thermal process operations requires the use of heat transfer models to determine process parameters that account for temperature in terms of its distribution within the food or equipment during treatment (Nicolai et al., 2001).

This concept can be extended to build models that not only account for temperature distribution, but also for microbial survivor distribution. Numerical heat transfer models that incorporate inactivation constants as a function of pressure and temperature have been proposed to account for the extent of combined temperature and pressure inside the pressure vessel (Denys et al., 2000; Hartmann and Delgado, 2003).

For instance, the temporal and spatial distribution of activity of *Bacillus subtilis*  $\alpha$ -amylase and cfu-concentration of *E. coli* (Hartmann et al., 2003) was described with the first order inactivation kinetics equation in terms of continuum mechanics and scalar transport (Ludikhuyze et al., 1997):

$$\frac{\partial A}{\partial t} + u \frac{\partial A}{\partial x} + v \frac{\partial A}{\partial y} + w \frac{\partial A}{\partial z} = -K(P,T)A \quad (2.13)$$

where  $A$  is the relative activity (actual activity related to the initial activity, varying between 100% with values close to zero),  $K(P,T)$  is the inactivation rate constant, and  $u$ ,  $w$ , and  $v$  are the components of the fluid velocity vector in the  $x$ -,  $y$ -, and  $z$ -directions, respectively. Velocity vectors were taken from thermal and fluid dynamic conservation equations of mass, momentum and energy (Hartmann and Delgado, 2003). Eq. (2.13) represents the coupling between the activity  $A$  and the flow field (i.e., the velocity of the vessel fluid and fluid inside packages) and the coupling of  $A$  and the temperature distribution. In this way, the pressure-temperature-time profiles, calculated through the model, were integrated through a numerical scheme, and the activity retention could be evaluated at any point in time and vessel space.

This model allowed obtaining inactivation distribution per package located at specified volumetric regions in the vessel. For this purpose, a volume-weighted averaging can be carried out for each package, and arithmetic averaging of all packages can provide an idea of global inactivation effectiveness (Hartmann and Delgado, 2003). Furthermore, a process uniformity parameter,  $\Lambda$ , has been defined in terms of the average relative activity retention, i.e.,

$$\Lambda = \frac{A_{ave\_min}}{A_{ave\_max}} \quad (2.14)$$

where  $A_{ave\_min}$  is the minimum average activity retention and  $A_{ave\_max}$  is the maximum average activity retention (Hartmann and Delgado, 2003). A similar inactivation distribution analysis can be performed by means of thermal and fluid dynamic models that include inactivation kinetic equations for pressure/temperature resistant spore-forming bacteria such as *C. botulinum*. In this case, microorganisms should be assumed as a transport quantity varying over space and time (Hartmann et al., 2003). This would allow calculations of temperature uniformity during HPHT sterilization processes, of special use in scale-up studies. A few works have already integrated thermodynamic, heat transfer, and spore inactivation kinetic models to predict the effect of temperature evolution at



selected points in the pressure vessel on spore inactivation (de Heij et al., 2002; Ardia et al., 2004). A mathematical model integrating thermodynamics and inactivation kinetics of *Bacillus stearothermophilus* was built to predict temperature distribution in the high-pressure vessel (de Heij et al., 2002). In this case, an axi-symmetric one-dimensional finite element model based on heat conduction was developed, which predicted temperature at the vessel wall and at the center. A first order kinetic constant  $k$  was found from spore inactivation data and fit into a modified Arrhenius-Eyring equation (Eq. 2.15), expressed as follows as a function of pressure and temperature:

$$k = k_{ref} e^{\frac{E_a}{R} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)} - V_a (P - P_{ref}) \quad (2.15)$$

where volume  $V_a$  is the activation volume,  $E_a$  is the activation energy, and  $R$  is the universal gas constant (8.314 J/mol/K). Although this contribution does not clearly specify results in terms of microbial reduction, as already mentioned, the model could predict temperature profiles at selected vessel points and illustrate the lower log cycle reduction achieved near the vessel walls after a two-cycle 700 MPa/121°C/90s process.

Similarly, Ardia et al. (2004) modeled compression heating using a finite element method, also based on heat conduction in radial coordinates. The model, based on Eq. (2.1), included  $dT/dP$  as a time dependent heat source during the compression or de-compression phase. The numerical routine implemented NIST formulations (Harvey et al., 1996) for regressive calculation of the thermal expansion coefficient  $\alpha$ , density  $\rho$ , and specific heat  $C_p$ . To account for temperature increase due to compression, a microbial inactivation model was included into the finite difference scheme, yielding the degree of inactivation (log cycle reductions) for any radial position. In this case, the time dependent inactivation was mathematically expressed assuming  $n^{\text{th}}$  order inactivation kinetics:

$$\frac{dN}{dt} = -k \cdot N^n \quad (2.16)$$

The inactivation rate constant was then expressed as a function of pressure and temperature using the Arrhenius-Eyring equation:

$$\ln(k) = \frac{1}{RT} (E_a - \Delta V^* P) \quad (2.17)$$

where the activation volume  $\Delta V^*$  is the characteristic parameter for the pressure dependence of the rate constant. This modeling approach predicted the inactivation of *Alicyclobacillus acidoterrestris* spores at 50°C/ 800 MPa in geometrically defined locations, finding (as mentioned before) a difference of 6 log cycles inactivation at the center of the pressure chamber and closer to the chamber walls (due to a 3–4°C difference at the end of pressurization).

### 2.5.5. Synergistic Approach

A number of foods composed of heat-labile components, relevant in terms of product quality and nutrition, can be affected by HPHT conditions necessary for sterilization (Matser et al., 2004; van Loey et al., 1998). In this case, additional synergies can be tried by using food additives and preservatives, such as bacteriocins (inactivation by membrane pore formation), surfactants like sucrose esters (affecting both proteins and biomembrane structure and function), acidulants (providing specificity in antimicrobial inactivation), humectants, chelating agents, and others.

Kalchayanand et al. (2003) showed that, at moderate pressure, bacterial spores can be induced to germinate and outgrow, at which stage they can be killed by bacteriocin-based biopreservatives. In this study, inactivation of *Clostridium laramie* spores or a mixture of 4 clostridial spores (*C. sporogenes*, *C. perfringens*, *C. tertium*, and *C. laramie*) in roast beef with added preservative mixtures (pediocin, nisin, lysozyme, Na-EDTA, and BPy) were subjected to treatment at 60°C/345 MPa/5 min. It was found that a combination of HP with bacteriocins extended the shelf life of inoculated roast beef up to 7 days at 25°C and up to 84 days at 4°C. Stewart et al. (2000) showed that *B. subtilis* and *C. sporogenes* ATCC 7958 were especially sensitive to nisin addition. A marked synergistic decrease in spore count was detected with pressurization at 250–300 MPa combined with 45°C and pH less than or equal to 6.0 in the presence of sucrose laurate fatty ester.

Moreover, Shearer et al. (2000) investigated the addition of sucrose laurate and combined treatment at 45°C/392 MPa/10–15 min, finding 3–5.5 log reductions for *B. subtilis* in milk, *B. cereus* in beef, *B. coagulans* in tomato juice (pH 4.5), *Alicyclobacillus* sp. in tomato juice (pH 4.5), and *Alicyclobacillus* sp. in apple juice. In this study, sucrose laurate appeared to be inhibitory rather than lethal to spores. However, its application to food as an extra hurdle may allow the use of lower temperatures in combination with pressure.

## 2.6. Quality of Selected HPHT Processed Foods

Even though extensive research has been done on bacterial spore inactivation, quality validation studies of low-acid foods after HPHT treatment have rarely been performed. Furthermore, little consumer data on HPHT processed products have been reported, and few comparisons exist in the literature with retort processing on consumer acceptability. Information on appearance, texture, and flavor/aroma mostly exists in terms of analytical evaluations of HPHT treated products such as broccoli juice, green beans, tomato puree, and meat sauce (van Loey et al., 1998; Rovere et al., 2000; Krebbers et al., 2003; Matser et al., 2004). Indeed, a number of high pressure, high temperature (HPHT) treated low-acid foods such as meat, milk, and vegetable products showed more desirable texture, color, and flavor/aroma retention in comparison to retorted products and, in some cases, to

frozen products (Hoogland et al., 2001; Krebbers et al., 2002; Krebbers et al., 2003; Matser et al., 2004).

Color, it has been shown, can be retained in selected vegetables (Matser et al., 2004). For instance, spinach has been found to retain higher color intensity than conventionally sterilized spinach after two pulses at 90°C/700MPa/30s. Furthermore, tomato puree treated at 90°C/700MPa/30s also retained color, whereas color degradation was greater after conventional retort treatment. This data was supported by a sensory panel that found higher color acceptability in tomato puree treated at HPHT conditions, in comparison to retorted samples. Lycopene content was maintained in this case, while a lower content was found after retort treatment (Krebbers et al., 2003). Moreover, Juliano et al. (2006a, b) indicated that HPHT treatment at 70°C/700MPa/5 min can maintain color and appearance of selected scrambled egg patty formulations (as evaluated by a trained panel).

Regarding texture, firmness of HPTS-treated green beans after 75°C/1000MPa/80s was much higher than those conventionally retorted, dried, or frozen (Krebbers et al., 2002). HP treated tomato puree (90°C/700MPa/30s/121°C) displayed lower serum separation and higher viscosity than the retorted product. Previous studies have also identified that utilization of lower pressurization temperatures can significantly improve the texture and water retention of scrambled egg patties (Juliano et al., 2006b). In fact, previous studies on high pressure formation of gels from whole liquid eggs, egg white, egg yolk, and egg yolk/white (Ma et al., 2001; Ahmed et al., 2003; Lee et al., 1999) have shown that pressures greater than 600MPa not only increase apparent viscosity, but also provide instantaneous gelation of egg yolk and egg white.

Flavor components in fresh basil were best retained after HPHT processing (two pulses of 85°C/700MPa) in comparison to freezing, conventional retorting, and drying. Egg flavor retention has also been reported in high pressure high temperature treated egg patties at 700MPa/105°C/5 min (Juliano et al., 2006b).

### *2.6.1. Shelf-Stable Egg-Based Products Processed by HPHT: A Case Study*

Manufacturing of acceptable shelf-stable egg products using conventional thermal processing remains a challenge, as retort processing yields undesirable flavors, greenish-gray discoloration, and detrimental changes in texture and syneresis (Baliga, et al., 1969; Wesley et al., 1982; Luechapattanaporn et al., 2005). In fact, the US Army recently stopped the production of retorted scrambled eggs in plastic institutional trays due to the dissatisfaction found by military consumers with respect to the quality of this benchmark product (Dunne, 2005).

At present, Washington State University (WSU), in conjunction with The Ohio State University (OSU) and the National Center for Food Safety and Technology (NCFST) has been directing a short-term Combat Rations Network (CORANET, US Defense Logistics Agency) project for the development of shelf-stable egg products using HPHT processing. The project, run under the guidance of the US

Natick Soldier Systems Center, has been part of a series of efforts carried out to identify processing alternatives for the manufacture of acceptable shelf-stable egg-based products. Alternatives include the use of high-temperature short-time retort processing, radiofrequency heating, freeze-drying, and refractance windows drying. The HPHT processing project had the active collaboration of experts from industry in various fields, including the high pressure equipment design team from Avure Technologies, the egg product development team from Michael Foods Egg Products Company, the high pressure processing packaging development team from ALCAN, and military ration developers from Wornick, Ameriqua, and Sopakco.

Among existing precooked egg products, scrambled egg patties were identified as an adequate product for high-pressure processing, especially during HPHT processing, due to their semisolid homogeneous structure (Juliano et al., 2006b). During the first phase of this project, several efforts have been directed in terms of formulation development, microbial challenge studies, identification of adequate processing conditions, as well as packaging studies.

The team focused on identifying the effects of HPHT conditions on commercial and modified egg patty formulations. Two processing strategies were initially proposed for egg-based product commercial sterilization and *C. botulinum* inactivation: (a) standard thermal sterilization treatment at process temperature 121°C accelerated by the application of 700 MPa with holding time at 3 min, and (b) thermal HPHT treatment using lower temperature than in (a) with increased holding time. Option (a) was considered with the purpose of facilitating FDA approval as a thermal process. In both cases, microbial challenge studies have helped in the selection of processing conditions. Furthermore, inactivation kinetics studies, incubation tests, packaging identification, and compression heating studies gave a deeper understanding of the process. Results of these endeavors, after iterative steps, are highlighted in the following sections.

#### 2.6.1.1. Egg Patty Formulation Identification for HPHT Conditions

Michael Foods has played a key role in the development and production of 6 egg patty formulations that were supplied to WSU, NCFST and OSU for testing. Trained and consumer sensory panels at WSU, along with additional physical tests (texture profile analysis, quantification of syneresis, and instrumental color measurements) have helped identify a formulation suitable in flavor, aroma, and appearance.

A six-member descriptive panel trained at WSU analyzed a basic formulation (whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid) and other formulations with added water, cheddar cheese particles, xanthan gum, EDTA and flavors. Formulations with added xanthan gum, EDTA and flavors, treated at 700 MPa/105°C/5 min, showed higher tones of butter flavor as well as lower tones of rancid, unclean and retort flavor than the basic formula after pressure (Juliano et al., 2006b).

Texture was initially identified as one of the most challenging problems in the quality of the egg products after pressurization at process temperatures greater

than 100°C (Juliano et al., 2006b). It was found that the addition of xanthan gum and water significantly decreased the hardness of high pressure treated patties by 30–55% after 700 MPa/105°C/5 min (Juliano et al., 2006a). Furthermore, addition of xanthan gum reduced the water loss (or syneresis) after pressure.

Results of a 40-member consumer panel verified previous studies on the effects of process temperature on product quality after HPHT processing. It was found that decreasing the high pressure process temperature from 121 to 105°C at 700 MPa improved the overall acceptability of HPHT treated formulations with added xanthan gum. A formulation containing 20% Cheddar cheese and treated at 105–110°C and 700 MPa obtained ratings similar to the untreated egg patty controls (Juliano et al., 2007).

Testing in a DUST 35L machine (QUINTUS Food Autoclave Type 35L-600, Avure Technologies, Kent, WA) showed that texture can be improved by using lower vacuum packaging conditions, whereas texture of the patty was not affected when processed in smaller 1.5 L chambers (Quintus Food Processor-6, Flow Autoclave systems, Columbus, OH) and 1.7 L chambers (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA). Furthermore, the three centers (WSU, NCFST and OSU) studied the effect of HPHT processing on the pH of the basic egg formulation and formulation with added xanthan gum, detecting no significant changes after processing.

#### 2.6.1.2. Preheating Studies

By working with different preheating systems, trials were conducted to reduce preheating times and to minimize decreased quality and excessive use of heat due to long preheating periods (Juliano et al., 2006b). Comparisons were made between preheating with an electrical heater, a water kettle with steam jacket, a water kettle with steam injection, or microwaves, showing that microwaves and steam injection elevated patty temperatures up to 75°C most quickly.

Considering that microwave heating showed significant temperature gradients within the patties (proven by means of infrared imaging), direct steam injection was shown to be advantageous in reducing preheating time. One other effect found was that the type of packaging material influenced preheating times. Aluminum foil-based laminates provided better penetration rates than non-foil ones.

#### 2.6.1.3. Compression Heating of Egg-Based Products

Determination of compression heating properties of egg mixtures gave no significant differences with water. The compression heating factor of egg patties ranged from 3.3°C/100 MPa to 4.8°C/100 MPa at the initial process temperatures of 25°C and 80°C, respectively. Therefore, no temperature gradients between the compression fluid and the egg patties are expected due to different compression heating.

#### 2.6.1.4. Microbial Challenge Studies

Microbial inactivation studies were carried out by NCFST and OSU. Inactivation of *B. stearothermophilus* was studied after different stages in the process: (a) baking of the egg mix to form patties, (b) after preheating and (c) after HPHT

processing. *B. stearothermophilus* spore inoculated into the egg mix showed a one log cycle reduction after baking. After treatment at 700 MPa/105°C, inactivation of *B. stearothermophilus* (ATCC 7953) spores in egg patties was accelerated (Rajan et al., 2006b). The resistance of *B. stearothermophilus*, given by its D-values, also proved to be much lower when using pressure. Inactivation of *B. stearothermophilus* in spore strips located between two egg patties can be reduced by at least 6 log cycles at 688 MPa/ 105°C/5 minutes (Koutchma et al., 2005). However, *Clostridium sporogenes* (PA 3679) bioindicator spores were more resistant than *Bacillus stearothermophilus* and needed a process temperature of at least 110°C.

#### 2.6.1.5. Identification of Packaging Materials for HPHT Processing

Throughout the first phase, project partners worked with different packaging companies to identify suitable individual flexible pouches (clear and foil laminates). Selected plastic and foil-laminated pouches from Kapak, Pyramid, ALCAN, and Smurfit-Stone manufacturers were screened for their ability to withstand HPHT and retort treatments. Overall packaging integrity, oxygen permeability (determined from a Mocon-Oxtran unit), and seal strength (determined from tensile tests using an Instron texture analyzer) were evaluated before and after treatments at NCFST facilities. In addition, pouches were tested for delamination, flex damage, or other treatment-related anomalies.

It was proven that foil laminates from Pyramid and Smurfit-stone (48 ga. polyethylene/ adhesive/ 0.0005" aluminum foil/adhesive/ 4 mL polyolefin) retained their barriers during steam injection preheating and HPHT treatment. However, a statistically significant loss of barrier was found in clear plastic pouches from Pyramid without aluminum foil. Seal strength of all packaging materials was not significantly affected by HPHT and HP low temperature treatments, while retort at 121°C decreased seal strength. Furthermore, WSU, in partnership with ALCAN Packaging, identified a pouch made of coextruded laminates that provided almost no blistering and low oxygen permeability after pressure under a worst-case scenario condition (700 MPa/121°C/3 min). ALCAN packaging material composition was a 60 ga. biaxial nylon/adhesive/5.0 mL ethylene vinyl alcohol (EVOH) coextruded sealant.

#### 2.6.1.6. Incubation Tests – Shelf Stability Studies

Egg patties (basic formulation) were treated in WSU's 1.7L machine at pressures between 200 and 700 MPa, with holding time of 5 min and initial temperature of 90°C for storage testing. Treated and untreated packages were stored at room temperature and 37°C for one year. Results indicated that at pressures above 400 MPa, no pouches showed production of gas or decomposition. HPHT treated patties stored at room temperature for one year maintained initial color, hardness and aroma. Moreover, other non-inoculated formulations tested in the NCFST/ Avure 35 L pilot machine also remained stable (no gas formation) for six months when stored at 37°C after 700 MPa/105°C treatment.

Another set of samples were inoculated with *B. stearothermophilus* spores ( $7.5 \times 10^6$  spore/g) at OSU laboratories and treated at 700 MPa/105°C/5 min. The product remained stable after at least two months storage at 37°C.

## 2.7. Final Remarks

The concept of combining high hydrostatic pressure and heat to commercially sterilize low-acid foods emerged in the early 1970s and is scaling up from the laboratory bench to the pilot plant. At least four pilot 35L high pressure vessels located around the world are being used today as part of various industrial/government consortia projects to identify the benefits of HPHT processing for several products.

Patents have been published proposing different approaches, among which the application of a single pressure pulse of 600 MPa or greater, combined with temperature between 90 and 130°C, seems most appropriate from a food safety and economic point of view. This approach defines a high temperature short time sterilization process, which has been proven to provide improved flavor, texture, color and nutrient retention in selected food components, in comparison to retort. At this stage of development, HPHT technology can be claimed advantageous for its shorter processing time. However, lower processing temperatures than 121°C cannot yet assure sterilization. For this purpose, additional microbial inactivation data on many *C. botulinum* strains as well as surrogate spore-forming microorganisms of higher resistance (to be identified) are greatly needed. Hence, based on the current knowledge, regulatory approval can only be obtained by filing this technology as a thermal process, following the guidelines established in the 9CFR318.300, 9CFR381.300, and 21CFR113.

Once *C. botulinum* inactivation data on several strains is gathered (together with process data) kinetic models can be developed for HPHT conditions. In fact, microbial kinetic models in combination with heat transfer models could be used to express overall process performance in terms of energy use. Overall parameters obtained for these models would completely account for (or be directly related to) individual performance parameters such as preheating rate, pressure come-up time, target preheating/equilibration temperature, target temperature at maximum pressure, temperature at the end of holding/ pressurization time, and temperature at the end of pressure release.

Synergistic approaches through the addition of natural antimicrobial preservatives such as bacteriocins can help reduce the HPHT conditions needed to reach sterilization, providing opportunities for the development of products with more heat labile components. The FSO concept can help establish optimal conditions and ingredient addition for sterilization from a regulatory standpoint.

Attainment of optimal sterilization conditions is also related to the efficient use of compression heat developed during pressurization. Equipment modification with heat retention aids such as an insulating polymeric liner at chamber walls, preheated pressurization fluids, and an internal pressure intensifier to decrease

the amount of inflowing pressurization fluid, can yield more uniform temperature distribution across the chamber volume. If a nearly adiabatic state is achieved inside the liner, pressure holding times may be decreased, as temperature will be uniform, even near the steel chamber walls.

The preheating step has been identified as a critical control point in the overall process since it determines the achievement of the target pressurization temperature in all food packages. A number of factors intervening in the preheating step have been listed, among which the preheating method used and package geometry seem the most relevant. Furthermore, the equilibration step after preheating is important to assure temperature homogeneity inside the food packages before pressure come-up time. From a quality perspective, minimization of preheating times, by selecting faster preheating methods, could help improve food attributes at the end of the HPHT process. A two-stage preheating approach has been proposed to save carrier operating time.

HPHT technology has the potential to manufacture shelf-stable egg, vegetable, meat and dairy products, but more information is needed in terms of the sensory quality of HPHT products and consumer preferences. Further studies on the effect of HPHT conditions on separate food components and developed formulations will allow identification of specific study cases. Once products are identified, characterization in terms of shelf life can be carried out. In this case, shelf stability of developed HPHT processed foods will not only depend on the treatment applied, but also on the barrier provided by the selected packaging material. Efforts are ongoing to identify packaging materials that meet the requirements for overall integrity, specific seal strength and gas permeability after HPHT treatment.

In conclusion, defining the conditions of *C. botulinum* inactivation is fundamental to establishing further product development studies on novel HPHT treated foods to satisfy the shelf-stable ready-to-eat markets. In addition, validation of process performance criteria related to *C. botulinum* inactivation, and pressure and temperature history will not only allow process filing with regulatory agencies, but will help establish a business case for transforming HPHT processing into an industrial reality.

## References

- 9CFR318.300, 2002, Animals and Animal Products. Entry into Official Establishments; Reinspection and Preparation of Products. Canning and Canned Products, in: *Food Safety and Inspection Service, US Department of Agriculture. Code of Federal Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC, Title 9, Chapter III, Part 318, Subpart G.
- 9CFR381.300, 2003, Animals and Animal Products. Poultry Products Inspection Regulations. Canning and Canned Products, in: *Food Safety and Inspection Service, US Department of Agriculture. Code of Federal Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC. Title 9, Chapter III, Part 381, Subpart X.
- 21CFR113, 2005, Food and Drugs. Food for Human Consumption, Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers, in: *US Food and Drug Administration, Department of Health and Human Services, Code of Federal*



- Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC, Title 21, Chapter I, Subchapter B, Part 113.
- 21CFR114, 2005, Food and Drugs. Food for Human Consumption. Acidified Foods, in: *US Food and Drug Administration, Department of Health and Human Services. Code of Federal Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC, Title 21, Chapter I, Subchapter B, Part 114.
- Ahmed, J., Ramaswamy, H.S., Alli, I., and Ngadi M., 2003, Effect of High Pressure on Rheological Characteristics of Liquid Egg, *Lebensm.-Wiss. U.-Technol.* **36**(5):517–524.
- Ahn J., Balasubramaniam, V.M., and Yousef A.E., 2005, Effect of Pressure-Assisted Thermal Processing on the Inactivation of Selected *Clostridium* and *Bacillus* Surrogate Spores [Abstract]. *Nonthermal Processing Workshop*, September 15-16, Philadelphia, PA, USDA Eastern Regional Research Center, USDA ARS Eastern Regional Research Center, Wyndmoor, PA, Abstract no.# p. 24.
- Ardia, A., Knorr, D., and Heinz V., 2004, Adiabatic Heat Modeling for Pressure Build-Up During High-Pressure Treatment in Liquid-Food Processing, *Food Bioprod. Process.* **82**(C1):89–95.
- Balasubramaniam, S., and Balasubramaniam V.M., 2003, Compression Heating Influence of Pressure Transmitting Fluids on Bacteria Inactivation During High Pressure Processing, *Food Res. Int.* **36**(7):661–668.
- Balasubramaniam, V.M., Ting, E.Y., Stewart, C.M., and Robbins J. A., 2004, Recommended Laboratory Practices For Conducting High-Pressure Microbial Inactivation Experiments, *Innov. Food Sci. Emerg. Technol.* **5**(3):299–306.
- Baliga, B.R., Rao, A.S., and Lahiry N.L., 1969, Prevention of Browning in Hard Boiled Eggs During Canning. *J. Food Sci. Technol.* **6**(3):200–204.
- Barbosa-Cánovas, G.V., Juliano, P., and Keener L., 2005a, Legislative Issues with Respect to Processed Food, in: *Global Harmonization of Legislation of Food Products and Processes*, Institute of Food Technologists Conference. New Orleans, July 2005, pp. 27–3.
- Barbosa-Cánovas, G.V., Juliano, P., Koutchma, T., Balasubramaniam, V.M., Mathews, J.W., and Dunne C.P. 2005b, High Pressure Thermal Sterilization of Precooked Egg Patties: Factors Affecting Preheating Efficiency, in: *High Pressure Processing*, American Institute of Chemical Engineers Annual Meeting, Cincinnati, p. 572c.
- Barbosa-Cánovas, G.V., and Rodríguez J.J., 2005, Thermodynamic Aspects of High Hydrostatic Pressure, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano (eds.), CRC Press, New York, pp. 183–206.
- Campanella, O.H., and Peleg M., 2001, Theoretical Comparison of a New and the Traditional Method to Calculate *Clostridium Botulinum* Survival During Thermal Inactivation, *J. Sci. Food Agr.* **81**:1069–1076.
- Caner, C., Hernandez, R.J., Pascall, M., Balasubramaniam, V.M., and Harte B.R., 2004, The Effect of High-Pressure Food Processing on the Sorption Behavior of Selected Packaging Materials, *Packaging Technol. Sci.* **17**(3):139–153.
- Cano, M.P., and De Ancos B., 2005, Advances in Use of High Pressure Processing and Preservation of Plant Foods, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano (eds.), CRC Press, New York, pp. 283–310.
- Carroll, T., Chen, P., and Fletcher A., 2003, A Method to Characterize Heat Transfer During High-Pressure Processing, *J. Food Eng.* **60**:131–135.
- Codex, 2004, *Procedural Manual of the Codex Alimentarius Commission*, 14 Ed., Codex Alimentarius Commission. Rome.
- Cooper, K.L., Call, M.K., and Meyer R.S. (Inventors), 2004, Ultra-High Pressure Vegetable Sterilization Method and Product, patent: U.S. 20040191382.

- De Heij, W.B.C., van den Berg, R.W., van Schepdael, L., Hoogland, H., and Bijmolt H., 2005, Sterilization—only better, *New Food* **8**(2):56,58–61.
- De Heij, W., van Schepdael, L., van den Berg, R., and Bartels P., 2002, Increasing Preservation Efficiency and Product Quality Through Control of Temperature Profiles in High Pressure Applications, *High Pressure Res.* **22**(3–4):653–657.
- De Heij, W.B.C., van Schepdael, L.J.M.M., Moezelaar, R., Hoogland, H., Matser, A.M., and van den Berg R.W. 2003. High-Pressure Sterilization: Maximizing the Benefits of Adiabatic Heating. *Food Technol.* **57**(3):37–42.
- Denys, S., van Loey, A.M., and Hendrickx M.E. 2000. A Modeling Approach for Evaluating Process Uniformity During Batch High Hydrostatic Pressure Processing: Combination of a Numerical Heat Transfer Model and Enzyme Inactivation Kinetics, *Innov. Food Sci. Emerg. Technol.* **1**(1):5–19.
- Dunne, C.P., 2005, *Personal Communication*, U.S. Army Natick Soldier Center, Department of Defense, August 5.
- EC258/97, 1997, Novel Foods and Novel Food Ingredients, Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997, Official Journal L 043, 14/02/1997, pp. 0001–0006.
- European Commission, 2002, Implementation of Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 Concerning Novel Foods and Novel Food Ingredients, Directorate General Health and Consumer Protection (SANCO D4), European Commission. (March 10, 2006); [http://europa.eu.int/comm/food/food/biotechnology/novelfood/initiatives\\_en.htm](http://europa.eu.int/comm/food/food/biotechnology/novelfood/initiatives_en.htm).
- Farkas, D.F., and Hoover D.G., 2000, High Pressure Processing, in: *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies*, *J. Food Sci.* Supplement, pp. 47–64.
- Franceschini, B., Gola, S., Rovere, P.P., and Frustoli M., 2005, Application of High Hydrostatic Pressure to Increase the Safety and the Shelf-Life of Ready-To-Eat (RTE) Traditional Meals, *Ind. Conserve.* **80**(4):391–409.
- 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 with High Pressure, in: *High Press Biosci. Biotechnol.*, R. Hayashi and C. Balny (eds.), Elsevier Science B.V., Amsterdam, pp. 253–259.
- Gola, S., and Rovere, P.P., 2005, Resistance to High Hydrostatic Pressure of Some Strains of *Clostridium Botulinum* in Phosphate Buffer, *Ind. Conserve.* **80**(2):149–157.
- Guamis, B., Pla, R., Trujillo, A.J., Capellas, M., Gervilla, R., Saldo, J., and Yuste J., 2005, High Pressure Processing of Milk and Dairy and Egg Products, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano (eds.), CRC Press, New York, pp. 343–360.
- Hartmann, C., and Delgado, A., 2002, Numerical Simulation of Convective and Diffusive Transport Effects on a High-Pressure Induced Inactivation Process, *Biotechnol. Bioeng.* **79**:94–104.
- Hartmann, C., and Delgado, A., 2003, The Influence of Transport Phenomena During High-Pressure Processing of Packed Food on The Uniformity of Enzyme Inactivation, *Biotechnol. Bioeng.* **82**(6):725–735.
- Hartmann, C., Delgado, A., and Szymczyk J., 2003, Convective and Diffusive Transport Effects in a High Pressure Induced Inactivation Process of Packed Food, *J. Food Eng.* **59**(1):33–44.
- Harvey, A.H., Peskin, A.P., and Sanford A.K., 1996, NIST/ASTME – IAPSW Standard Reference Database 10, version 2.2.

- Heinz, V., and Knorr D., 2001, Effect Of High Pressure On Spores, in: *Ultra High Pressure Treatment of Foods*, M.E.C. Hendrickx and D. Knorr (eds.), Kluwer Academic/Plenum Publishers, New York, pp. 77–116.
- Hendricks, M., 2005, Pathways to Commercialization: From Academia to the Marketplace Academic Incubators and Innovation (European Model), in: *Commercializing Nonthermal Technologies*, Institute of Food Technologists Continuing Technical Education Committee. New Orleans, pp. 15–16.
- Hirsch, E.S., Kramer, F.M., and Meiselman H.L., 2005, Effects of Food Attributes and Feeding Environment on Acceptance, Consumption and Body Weight: Lessons Learned in a Twenty-Year Program of Military Ration Research. US Army Research (Part 2). *Appetite* **44**(1):33–45.
- Hjelmqvist, J., 2005, Commercial High Pressure Equipment, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M P. Cano (eds.), CRC Press, New York, pp. 361–374.
- Holdsworth, S. D., 1997, *Thermal Processing of Packaged Foods*, Blackie Academic & Professional, New York, pp.112–120.
- Hoogland, H., de Heij, W., and van Schepdael L., 2001, High Pressure Sterilization: Novel Technology, New Products, New Opportunities, *New Food*, **4**(1):21–26.
- Juliano, P., Clark, S., Koutchma, T., Ouattara, M., Mathews, J.W., Dunne, C.P., and Barbosa-Cánovas G. V., 2007, Consumer And Trained Panel Evaluation Of High Pressure Thermally Treated Scrambled Egg Patties, *J. Food Qual.* **30**(1):57–80.
- Juliano, P., Li, B., Clark, S., Mathews, J.W., Dunne, P.C., and Barbosa-Cánovas G.V., 2006a, Quality and Sensory Analysis of Precooked Egg Products After High Pressure Processing Combined with Low and High Temperatures, *J. Food Qual.* **29**(5):505–530.
- Juliano, P., Toldrà, M., Koutchma, T., Balasubramaniam, V.M., Clark, S., Mathews, J.W., Dunne, C.P., Sadler, G., and Barbosa-Cánovas G.V., 2006b, Texture and Water Retention Improvement in High Pressure Thermally Sterilized Scrambled Egg Patties. *J. Food Sci.* **71**(2):E52–61.
- Kalchayanand, N., Dunne, C.P., Sikes, A., and Ray, B., 2003, Inactivation of Bacterial Spores by Combined Action of Hydrostatic Pressure and Bacteriocins in Roast Beef, *J. Food Saf.* **23**(4):219–231.
- Koutchma, T., Guo, B., Patazca, E., and Parisi B., 2005, High Pressure–High Temperature Inactivation of *Clostridium Sporogenes* Spores: From Kinetics To Process Verification, *J. Food Process. Eng.* **28**(6):610–629.
- Krebbbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M., and van den Berg R.W., 2003, Combined High-Pressure and Thermal Treatments for Processing of Tomato Puree: Evaluation of Microbial Inactivation and Quality Parameters, *Innov Food Sci Emerg Technol.* **4**(4):377–85.
- Krebbbers, B., Matser, A.M., Koets, M., and van den Berg R.W., 2002, Quality and Storage-Stability of High-Pressure Preserved Green Beans, *J Food Eng.* **54**(1):27–33.
- Leadley, C., 2005, High Pressure Sterilisation: A Review. *Campden & Chorleywood Food Research Association* **47**:1–42.
- Lee, D.U., Heinz, V., and Knorr D., 1999, Evaluation of Processing Criteria for the High Pressure Treatment of Liquid Whole Egg: Rheological Study, *Lebensm.- Wiss. U.- Technol.* **32**(5):299–304.
- Ludikhuyze, L., van den Broeck, I., Weemaes, C.A., and Hendrickx M.E., 1997, Kinetic Parameters For Temperature-Pressure Inactivation Of *Bacillus Subtilis*  $\alpha$ -Amylase Under Dynamic Conditions, *Biotechnol. Prog.* **13**:617–623.

- Luechapattanaporn, K., Wang, Y., Wang, J., Tang, J., Hallberg, L.M., and Dunne C.P., 2005, Sterilization of Scrambled Eggs in Military Polymeric Trays by Radio Frequency Energy, *J. Food Sci.* **70**(4):288–294.
- Ma, L., Chang, F.J., Barbosa-Cánovas, G.V., and Swanson B.G., 2001, Comparison Study of Pulsed Electric Fields, High Hydrostatic Pressure, and Thermal Processing on the Electrophoretic Patterns of Liquid Whole Egg, in: *Pulsed Electric Fields in Food Processing: Fundamental Aspects and Applications*, G.V. Barbosa-Cánovas and H. Zhang (eds.), Technomic Publishing Co., Lancaster, pp. 225–240.
- Margosch, D., 2005, *Behavior of Bacterial Endospores and Toxins as Safety Determinants in Low Acid Pressurized Food*, Doctoral Dissertation, Technischen Universität Berlin.
- Margosch, D., Ehrmann, M.A., Gaenzle, M.G., and Vogel R.F., 2004, Comparison of Pressure and Heat Resistance of *Clostridium Botulinum* and Other Endospores in Mashed Carrots, *J. Food Prot.* **67**(11):2530–2537.
- Margosch, D., Moravek, M., Gaenzle, M.C., Maertlbauer, E., Vogel, R F., and Ehrmann M.A., 2005, Effect of High Pressure and Heat on Bacterial Toxins, *Food Technol. Biotechnol.* **43**(3):211–217.
- März, A., 2002, Method for Inactivating Microorganisms Using High Pressure Processing. Patent number: EP 1 201 252 A1.
- März, A., 2003, Method for Inactivating Microorganisms Using High Pressure Processing. Patent number: US 6635223.
- Matser, A.M., Krebbers, B., van den Berg, R.W., and Bartels P.V., 2004, Advantages of High Pressure Sterilization on Quality of Food Products, *Trends Food Sci Technol.* **15**(2):79–85.
- Mermelstein, N.H., 2001, Military and Humanitarian Rations, *Food Technol.* **55**(11):73–75.
- Meyer, R.S., Cooper, K.L., Knorr, D., and Lelieveld H.L.M., 2000, High-Pressure Sterilization of Foods. *Food Technol.* **54**(11):67–68, 70–72.
- Montero, P. and Gómez-Guillén M.C., 2005, High Pressure Applications on Myosystems, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano (eds.), CRC Press, New York, pp. 311–342.
- NACMCF (National Advisory Committee on Microbiological Criteria for Foods), 2004, Requisite Scientific Parameters for Establishing the Equivalence Oof Alternative Methods of Pasteurization. (Washington; March 10, 2006) [http://www.fsis.usda.gov/About\\_FSYS/NACMCF/index.asp](http://www.fsis.usda.gov/About_FSYS/NACMCF/index.asp).
- NACMCF. 2005. Consideration for Establishing Safety-Based Consume-By Date Labels for Refrigerated Ready-To-Eat Foods, *J. Food Prot.* **68**(8):1761-1775.
- NC Hyperbaric, 2004, *High Pressure Processing. Technology That Makes Sense*. [Commercial booklet], Burgos.
- NFPA. 1985. *Guidelines for Thermal Process Development for Foods Packaged in Flexible Containers*. National Food Processors Association (NFPA), New York.
- Nicolai, M.B., Scheerlinck, N., Verboven, P., and Baerdemaeker J.D. 2001. Stochastic Finite-Element Analysis of Thermal Food Processes, in: *Food Processing Operations Modeling. Design and Analysis*, J. Irudayaraj (ed.), Marcel Dekker, New York, pp. 265–304.
- Otero, L., Molina-Garcia, A.D., and Sanz P.D., 2000, Thermal Effect in Foods During Quasi-Adiabatic Pressure Treatments, *Innov. Food Sci. Emerg. Technol.* **1**:119-126.
- Otero, L., and Sanz P.D., 2003, Modeling Heat Transfer in High Pressure Food Processing: A Review. *Innov. Food Sci. Emerg. Technol.* **4**(2):121–134.
- Patterson, M.F., 2005, A Review: Microbiology of Pressure-Treated Foods, *J. Applied Microbiol.* **98**:1400–1409.
- Palazoglu, K., 2006, Influence of Convective Heat Transfer Coefficient on the Heating Rate of Materials with Different Thermal Diffusivities, *J. Food Eng.* **73**(3):290–296.

- Peleg, M., Normand, M.D., and Campanella O.H., 2003, Estimating Microbial Inactivation Parameters from Survival Curves Obtained Under Varying Conditions—The Linear Case, *Bull. Math. Biol.* **65**:219–234.
- Peleg, M., Normand, M.D., and Corradini M.G., 2005, Generating Microbial Survival Curves During Thermal Processing in Real Time, *J. Appl. Microbiol.* **98**:406–417.
- Pflug, I.J., 1987, Using the Straight-Line Semi Logarithmic Microbial Destruction Model As an Engineering Design Model for Determining the F-Value for Heat Processes, *J. Food Prot.* **50**(4):342–246.
- Rajan, S., Ahn, J., Balasubramaniam, V.M., and Yousef A. E., 2006a, Combined Pressure-Thermal Inactivation Kinetics of *Bacillus Amyloliquefaciens* Spores in Mashed Egg Patty Mince, *J. Food Prot.* (In press.)
- Rajan, S., Pandrangi, S., Balasubramaniam, V.M., and Yousef A.E., 2006b, Inactivation of *Bacillus stearothermophilus* spores in egg patties by pressure assisted thermal processing, *LWT-Food Sci. Technol.* (In press.)
- Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., and Anderson C., 2003, Compression Heating of Selected Fatty Food Materials During High-Pressure Processing, *J. Food Sci.* **68**(1):254–259.
- Raso, J., Barbosa-Cánovas, G.V., and Swanson B.G. 1998. Sporulation Temperature Affects Initiation of Germination and Inactivation by High Hydrostatic Pressure of *Bacillus Cereus*. *J. Appl. Microbiol.* **85**:17–24.
- Reddy, N.R., Solomon, H.M., Fingerhut, G.A., Rhodehamel, E.J., Balasubramaniam, V.M., and Palaniappan S., 1999, Inactivation Of *Clostridium Botulinum* Type E Spores By High Pressure Processing, *J. Food. Saf.* **19**:277–288.
- Reddy, N.R., Solomon, H.M., Tetzloff, R.C., and Rhodehamel E.J., 2003, Inactivation of *Clostridium Botulinum* Type A Spores by High Pressure Processing at Elevated Temperatures, *J. Food Prot.* **66**(8):1402–1407.
- Rovere, P., Gola, S., Maggi, A., Scaramuzza, N., and Miglioli L., 1998, Studies on Bacterial Spores by Combined Pressure-Heat Treatments: Possibility to Sterilize Low-Acid Foods, in: *High Pressure Food Science, Bioscience and Chemistry*, N.S. Isaacs (ed.), The Royal Society of Chemistry, Cambridge, pp. 354–63.
- Rovere, P., Squarcina, N., Gola, S., Sandei, L., Iametti, S., and Carpi G., 2000, Effect of Thermal Treatment Under High Pressure on the Quality of a Meat Sauce, *High Pressure Res.* **19**:99–107.
- Sale, A.J.H., Gould, G.W., and Hamilton W.A., 1970, Inactivation of Bacterial Spores by High Hydrostatic Pressure, *J. Gen. Microbiol.* **60**:323–334.
- Schauwecker, A., Balasubramaniam, V.M., Sadler, G., Pascall, M.A., and Adhikari C., 2002, Influence of High-Pressure Processing on Selected Polymeric Materials and on the Migration of a Pressure-Transmitting Fluid, *Packaging Technol. Sci.* **15**:255–262.
- Shearer, A.E.H., Dunne, C.P., Sikes, A., and Hoover D.G., 2000, Bacterial Spore Inhibition and Inactivation in Foods by Pressure, Chemical Preservatives, and Mild Heat, *J. Food Prot.* **63**(11):1503–1510.
- Sizer, C.E., Balasubramaniam, V.M., and Ting E., 2002, Validating High-Pressure Processes for Low-Acid Foods, *Food Technol.* **56**(2):36-57.
- Stewart, C.M. 2005. HPP Safety and Quality Data, in: *Commercializing Nonthermal Technologies*, Institute of Food Technologists Continuing Technical Education Committee, New Orleans, pp. 15–16.
- Stewart, C.M., Dunne, C.P., Sikes, A., and Hoover D.G., 2000, Sensitivity of Spores of *Bacillus Subtilis* and *Clostridium Sporogenes* PA 3679 to Combinations of High Hydrostatic Pressure and Other Processing Parameters, *Innov. Food Sci. Emerg. Technol.* **1**(1):49–56.

- Surak, J.G., 2006, Global Harmonization: Foods Safety Management Standards, *Int. Rev. Food Sci. Technol.* Winter 2005/2006: 34–36.
- Taki, Y., Awano, T., Toba, S., and Mitsuura N. 1991. Sterilization of *Bacillus Sp.* Spores by Hydrostatic Pressure, in: *High Pressure Science for Food*, R. Hayashi (ed.), Sanei Pub. Co., Kyoto, pp. 217–24.
- Tapia, M.S., Arispe, I., and Martínez A., 2005, Safety and Quality in the Food Industry, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano (eds.), CRC Press, New York, pp. 669–680.
- Ter Minassian, L., Pruzan, P. and Soulard A., 1981, Thermodynamic Properties of Water Under Pressure up to 5 Kbar and Between 28 and 120°C. Estimations in the Supercooled Region Down to -40°C, *J. Chem. Phys.* **75**:3064–3072.
- Ting, E., Balasubramaniam, V.M., and Raghubeer E., 2002, Determining Thermal Effects In High-Pressure Processing. *Food Technol.* **56**(2):31–35.
- Van Loey, A., Ooms, V., Weemaes, C., van den Broeck, I., Ludikhuyze, L., Indrawati, Denys, S., and Hendrickx M., 1998, Thermal and Pressure-Temperature Degradation of Chlorophyll in Broccoli (*Brassica Oleraces L. Italica*) Juice: A Kinetic Study. *J. Agr. Food Chem.* **46**:5289–5294.
- Van Opstal, I., Bagamboula, C.F., Vanmuysen, S.C.M., Wuytack, E.Y., and Michiels C.W., 2004, Inactivation of *Bacillus Cereus* Spores in Milk by High Pressure and Heat Treatments, *Int. J. Food Microbiol.* **92**:227–234.
- Van Schepdael, L.J.M.M., de Heij, W.B.C., and Hoogland H., 2002, Method for high pressure preservation, patent: PCT WO 02/45528 A1.
- Varga, S., and Oliveira, J.C., 2000, Determination of the Heat Transfer Coefficient Between Bulk Medium and Packed Containers in a Batch Retort, *J. Food Eng.* **44**(4):191–198.
- Welti-Chanes, J., López-Malo, A., Palou, E., Bermúdez, D., Guerrero-Beltrán, J.A., and Barbosa-Cánovas G. V. 2005. Fundamentals and Applications of High Pressure Processing of Foods, in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, M.P. Cano, (eds.), CRC Press, New York, pp. 157–182.
- Wesley, R.D., Rousselle, J.R., Schwan, D.R., and Stadelman W.J., 1982, Improvement in Quality of Scrambled Egg Products Served from Steam Table Display, *Poultry Sci.* **61**(3):457–462.
- Wilson, M.J., and Baker R., 2000, High Temperature/Ultra-High Pressure Sterilization of Foods, Patent: US 6,086,936.
- Wilson, M.J., and Baker R., 2003, High Temperature/Ultra-High Pressure Sterilization of Low Acid Foods, Patent: EU 1 295 537 A2.



<http://www.springer.com/978-0-387-75429-1>

Food Engineering: Integrated Approaches

Gutiérrez-Lopez, G.F.; Welti-Chanes, J.; Parada-Arias, E.  
(Eds.)

2008, XXIV, 476 p., Hardcover

ISBN: 978-0-387-75429-1