Inactivation of Pathogenic Microorganisms in Food by High Pressure Processing

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Inactivation of Pathogenic Microorganisms in Foods by High-Pressure Processing

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10.1 Introduction

Foodborne outbreaks continue to be reported presently around the world, and more than 250 different foodborne diseases have been described (CDC 2015a). Pathogenic bacteria are the main cause of the reported problems, although viruses and parasites have also been associated with the foodborne outbreaks. Pathogens are a problem in low-acid foods (pH > 4.6). It is known that the acidity of high-acid foods (pH < 4.6) inhibits the germination and growth of microbial spores and the growth of vegetative pathogens. Therefore, low-acid pasteurized foods are generally stored and distributed

under refrigeration (Silva and Gibbs 2009). For pasteurized foods, at least a 5–6 log reduction (Betts and Gaze 1992; FDA 2001) in the key pathogen or spoilage organism is recommended. Thermal processing is the primary method used by the food industry to achieve this reduction. However, the heat can alter natural flavors and nutrients in the foods. Researchers and industry are developing and applying alternative methods for food processing with less impact on its sensory properties.

High-pressure processing (HPP) has emerged as an attractive pasteurization technology for food preservation. HPP is able to inactivate pathogenic and spoilage microorganisms in foods, while retaining their fresh or just prepared appearance, organoleptic characteristics, and nutritional quality. Guacamole (avocado with spices) is one example of successful HPP-treated food. A growing number of foods in the food market are HPP treated. The combination of HPP with heat, referred to as HPP-thermal or high-pressure thermal processing (HPTP), is possible when more severe process conditions are needed. This chapter reviews the updated knowledge dealing with the effects of HPP and HPTP on pathogenic microorganisms in foods, includ- ing spores and vegetative cells, and kinetic models to describe microbial inactivation behavior. Future perspectives of HPP and HPTP foods are also discussed.

10.2 Microbial Pathogens Contaminating Foods

10.2.1 Microbial Spores and Vegetative Cells

Microbial cells can exist in different states, vegetative and spores, depending on the conditions present. These states of microbes exhibit differences in their chemical composition, morphological structure, and physiology (Keynan 1969). Vegetative cells are actively growing, metabolizing, and dividing. Some microbial species can produce spores, the resting structures, which are formed from the vegetative cells. Bacterial spores (endospores, created inside the parent vegetative cell) are resistant structures able to survive under environmental stresses such as nutrient deprivation. Under favorable conditions (e.g., water, nutrients, and germinants), the spore breaks its dormancy through germination and outgrowth (initiating vegetative growth). Bacterial spores are well known for their resistance to various agents and stresses, such as radiation, high temperature, freezing, pressure, desiccation, extreme pH, and a wide variety of toxic chemicals (Tournas 1994; Setlow 2006; Black et al. 2007; Reineke et al. 2013), and thus are frequently used as targets in pasteurization processes. The resistance of spores is considered to be due to substantial structural specialization developed within a mother cell (Moir and Smith 1990).

10.2.2 Pathogenic Sporeformers Relevant for Food Safety

The outgrowth of pathogens in foods can cause contaminations, food spoilage, foodborne illnesses, and outbreaks. The main pathogenic sporeformers in foods are *Clostridium botulinum*, *Clostridium perfringens*, and *Bacillus cereus*. *C. botulinum* is the most dangerous sporeformer (Carlin et al. 2000a); it can produce potent and fatal neurotoxins (Brown 2000). *C. botulinum* types A, B, E, and F have been implicated in human foodborne botulism (FDA 2015a), and incidents from ingestion of the following contaminated foods have been reported (Lindström et al. 2006): hot-smoked fish (Pace et al. 1967), canned tuna fish in oil (Mongiardo et al. 1985), canned truffle cream or canned asparagus (Therre 1999), pasteurized vegetables in oil (Aureli et al. 1999), canned fish (Przybylska 2003), and canned eggplant (Peredkov 2004). Diagnosis of human botulism is usually from clinical symptoms (gastrointestinal and neurological) (Wells and Wilkins 1996), coupled with the detection of toxin in the patient's serum and/or feces as a standard method (Kautter and Solomon 1977).

C. perfringens has been identified as the most common cause of food outbreaks in ready-to-eat and partially cooked meat and poultry products as a result of improper handling and preparation of large quantities of foods (Evelyn and Silva 2015a, 2016a; Silva and Gibbs 2009), with type A toxin usually being involved in food poisoning (Scallan et al. 2011). In the United States, it causes nearly 1 million cases of foodborne illness each year (CDC 2015b), and it is ranked as an important cause of foodborne illness in the United Kingdom (Tam et al. 2012) and in several countries (Grass et al. 2013). An outbreak associated with vegetables (spinach and fried bean curd dish) has also been reported with this species (Miwa et al. 1999). Diarrhea, severe abdominal cramps, and nausea are the most common symptoms reported after 8–24 h consumption of *C. perfringens* type A enterotoxin (*cpe*) in contaminated foods (Uzal et al. 2014). Based on the symptoms, detection for the illness involving *C. perfringens* (and generally *Clostridia* species) is usually a combination of isolation of *Clostridia* from feces, blood, or the wound, and toxin and serological assays (Berry et al. 1988).

B. cereus is another sporeforming and pathogenic bacterium commonly found in meat and in dishes containing meat, resulting in food poisoning, similar in many respects to *C. perfringens*. Diarrhea and emetic syndromes are two types of diseases caused by *B. cereus* (Schoeni and Lee Wong 2005). Rice, cereals, and spices are also food commodities often asscociated with *B. cereus*. Some strains have the ability to grow at low temperatures ($T < 8$°C) (Dufrenne et al. 1995; Garcia Armesto and Sutherland 1997; Choma et al. 2000), making them sporeformers frequently isolated from low-acid chilled foods (Silva et al. 2014; Silva and Gibbs 2010; Carlin et al. 2000b; Dufrenne et al. 1995). *Bacillus licheniformis* is another *Bacillus* species often contaminating dairy products. Foodborne outbreaks have been registered in cooked meats and vegetables, raw milk, and commercial baby foods (Salkinoja-Salonen

et al. 1999). *Bacillus pumilus* human infection is rare, although a few cases of food poisoning from rice were reported. The symptoms include dizziness, headache, chills, back pain, stomach cramps, and diarrhea (From et al. 2007). One of several methods used for the diagnosis of human illness caused by *B. cereus* and other *Bacillus* spp. is the isolation of *B. cereus* from suspect food and determining its enterotoxigenicity by serological (diarrheal toxin) or biological (diarrheal and emetic) tests (FDA 2014).

Other than the main pathogenic sporeformers mentioned above, the following bacteria can also cause diseases in humans after consumption of contaminated foods: *Clostridium baratii* (infant botulism), *Clostridium butyricum* (infant botulism), *Clostridium difficile* (diarrhea to fulminant colitis), *Bacillus thuringiensis* (gastroenteritis), and *Bacillus anthracis* (gastrointestinal illness and diarrhea) (Aureli et al. 1986; Jackson et al. 1995; Barash et al. 2005; Pavic et al. 2005; Rupnik and Songer 2010; CDC 2000).

10.2.3 Vegetative Pathogens Relevant for Food Safety

Staphylococcus aureus, enterohemorrhagic *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Vibrio* spp. are vegetative pathogens important to food safety. The toxins of these enteric pathogens (diarrheal caused syndrome) can be transmitted via a wide range of foods, causing outbreaks of illness following consumption. Food poisoning outbreaks in milk-based products caused by *S. aureus* enterotoxins have been frequently reported due to ingestion of foods containing staphylococcal enterotoxins (SEs) (Evenson et al. 1988; Asao et al. 2003; Schmid et al. 2009; Ostyn et al. 2010; Hennekinne et al. 2012). Commonly described symptoms of *S. aureus* foodborne infection other than diarrhea are nausea, vomiting, abdominal cramping, dizziness, and sometimes moderate fever (Hennekinne et al. 2012). Diagnosis of staphylococcal food poisoning is generally confirmed by either recovery of *S. aureus* ($\geq 10^5$/g food) or by the detection of SEs in food remnants (Hennekinne et al. 2012).

Outbreaks of *E. coli* O157:H7 have been associated with a wide range of foods, including beef, vegetables (e.g., lettuce, spinach, and sprouts), raw milk (CDC 2014; Armstrong et al. 1996), and very recently, flour (CDC 2016a). The ability of this strain to survive and grow at very acidic conditions and low temperature (Weagant et al. 1994; Conner and Kotrola 1995; Hsin-Yi and Chou 2001) may explain the occurrence of outbreaks in the following high- acid foods: fruit juices (CDC 1996; Cody et al. 1999), apple cider (Miller and Kaspar 1994), mayonnaise (Weagant et al. 1994), mustard and ketchup (Tsai and Ingham 1997), and yogurt (Morgan et al. 1993). Commonly described symptoms of *E. coli* O157:H7 food infection include diarrhea and abdomi- nal cramping, which in some cases progresses to bloody diarrhea (Ibrahim 2015). Human illness is usually diagnosed through laboratory testing of stool specimens (feces) (CDC 2015c).

Listeria monocytogenes infection (listeriosis) has been a great concern until now due to its capability to cause severe illness with a high morbid- ity, hospitalization, and mortality rate in vulnerable populations, such as pregnant women and the elderly (Henriques and Fraqueza 2015). Flu-like or gastrointestinal illnesses, miscarriage, stillbirth, septicemia, meningitis, and encephalitis are symptoms detected for listeriosis-infected persons (Gillespie et al. 2010). Disease can be confirmed by isolation of the bacteria from blood, spinal fluid, or amniotic fluid or the placenta (for pregnant women) (CDC 2014). For the years 2014–2016, multistate outbreaks caused by *L. monocytogenes* were recorded in the United States, linked to foodstuffs such as packaged caramel apples and salads, cheese, raw milk, and frozen vegetables (CDC 2016b).

Salmonellosis, especially from *Salmonella enteritidis* and *Salmonella typhimurium*, is the most frequently reported foodborne disease worldwide, and outbreaks have been associated with a diverse range of food vehicles. Food from animal origins, that is, eggs, meat, poultry, and milk, are the main food commodities reported (CDC 1990; Perales and Audicana 1989), although an increasing number of outbreaks was reported in contaminated green vegetables (Doyle and Erickson 2008; Hanning et al. 2009; WHO 2013). The most common clinical symptoms are diarrhea and abdominal cramps; however, illness may be accompanied by a fever of 38°C–39°C (Giannella 1996). Stool, blood, urine, and sometimes tissues can be used for the diagnosis of *Salmonella* bacteria (Behravesh et al. 2008).

Vibrio parahaemolyticus and *Vibrio vulnificus* are two major foodborne bacterial pathogens of great concern in raw foods such as oysters, sushi, and sashimi, or undercooked seafood, since they are naturally distributed in water (Jay 2000). Large outbreaks of *V. parahaemolyticus* (O3:K6 serotype) occurred during 1997–1998 in Washington, Texas, and New York, and on the West Coast of the United States (Daniels et al. 2000; DePaola et al. 2000), whereas an outbreak of *V. vulnificus* was reported occur in coastal states from the Gulf of Mexico region (Shapiro et al. 1998). It is estimated that there are 35,000 foodborne infections caused by *V. parahaemolyticus* in the United States annually, while *V. vulnificus* is reported to have the highest mortality rate (up to 50%) among other foodborne pathogens (Scallan et al. 2011).

Other pathogenic vegetative bacteria, such as *Streptococcus faecalis, Campylobacter jejuni, Yersinia enterocolitica, Citrobacter freundii,* and *Aeromonas hydrophila,* also pose a health risk due to their association with meat products, and outbreaks are sometimes reported (Deming et al. 1987; Hussain et al. 1988; Tsai and Chen 1996; Gaibani et al. 2013; Grahek-Ogden et al. 2007). Among vegetative pathogens described previously, *L. monocytogenes, Y. enterocolitica, Salmonella, V. parahaemolyticus,* and *A. hydrophila* are of great concern because they are able to grow at refrigerated or low temperatures (D'Aoust 1991; Penfield et al. 1990), and thus can be a problem in HPP chilled foods.

10.3 HPP and HPTP of Foods

10.3.1 HPP and HPTP Fundamentals and Effect on Microbes

HPP is a commercial nonthermal food pasteurization technology with less adverse effects on food quality than conventional thermal processes (Cullen et al. 2012). Industrial HPP typically operates at pressures of 400–600 MPa to process liquid and solid foods between 5 and 10 min either chilled or at temperatures of ≤40°C. According to Le Chatelier's principle, hydrostatic pressure reduces the volume of the pressurized material without chang- ing its shape. Covalent bonds from primary structures of proteins are unaltered by pressure (Mozhaev et al. 1994), making this the central hypoth- esis behind the preservation of biological activity of functional compounds (Balasubramaniam et al. 2015). The main goal of HPP is the nonthermal pasteurization of foods through the inactivation of pathogenic and spoilage vegetative microorganisms, usually by 5 or 6 D. After processing, the foods are usually cold stored and distributed, and the shelf life is influenced by the intensity of the treatments, storage conditions, and other factors, such as packaging characteristics. The common shelf life of HPP-treated foods is 3–10 times that of untreated HPP foods (Hiperbaric 2013). Fruit juices and smoothies, fruit jams and sauces, yogurt, jelly, guacamole, dips and salsas, ready-to-eat meals, meat and poultry products, and seafood are examples of commercially available HPP food products worldwide, with manufacturers located in Japan, the United States, and Europe (Hogan et al. 2005).

Room temperature HPP standard treatment does not inactivate most microbial spores and enzymes. For example, spores of *B. subtilis* species were found to survive up to 1200 MPa at ambient temperature (Larson et al. 1918). Thus, as mentioned, HPP foods require a cold chain for distribution. While high-acid fruit products (pH < 4.6) do not allow the germination and growth of pathogenic and spoilage sporeformers (Silva and Gibbs 2009; Silva et al. 2014), low-acid foods (pH ≥ 4.6), such as meat products, fish, vegetables, milk, and cheeses, pose significant health hazards associated with pathogenic sporeforming bacteria and need to be stored and distributed below 7°C. In addition to cold storage conditions, pressures between 400 and 800 MPa, combined with temperatures higher than 50°C, appear to have potential to reduce spore numbers in foods. However, the resistance of spores varies highly among species, and is also affected by the food matrix. A combination of HPP with moderate heat or HPTP is inevitably needed to inactivate these resistant spores, and past works have shown that HPTP pressure and temperature synergistically enhance the spore inactivation (Akhtar et al. 2009; Silva et al. 2012; Daryaei et al. 2013; Evelyn and Silva 2015b,c, 2016a,b; Evelyn et al. 2016). Until now, considerable efforts have been made for full HPTP inactivation of microbial spores, to produce shelf-stable sterilized food products with high quality. However, the HPTP sterilization technology has not

yet been successfully demonstrated; thus, the technology is used industrially for food pasteurization and the production of refrigerated but not yet shelf-stable foods.

10.3.2 Mechanism Inactivation of Spores and Vegetative Cells

Many researchers have thoroughly investigated and reported the mechanism of vegetative and spore cell inactivation by high (Mathys 2008; Smelt et al. 2001; Lado and Yousef 2002; Patterson 2005; Knorr et al. 2010; Black et al. 2007; Reineke et al. 2013). Generally, microbial cell death occurs when there are considerable alterations in the cellular structure or physiological functions of microorganisms after their exposure to pressure (and heat). Regarding vegetative microorganisms, various HPP inactivation mechanisms suggested that result in cell death are the inactivation of essential enzymes (Smelt et al. 2001; Ardia 2004), changes in intracellular pH (Smelt et al. 2001), disintegration of ribosomes in their subunits (Smelt et al. 2001), and rupture of the cell membrane due to membrane phase transition and fluidity changes (Smelt et al. 2001; Ananta et al. 2005), leading to morphological changes in HPP-treated cells (Patterson 2005).

With respect to spores, two step processes have been widely accepted for the mechanism of spore inactivation, which are mostly based on mechanism inactivation of *Bacillus* spores in the buffer: (1) release of dipicolinic acid (DPA) during germination (direct or through activation of nutrient germinant receptors) due to disruption to the spore inner membrane, causing a loss of spore resistance, and (2) subsequent inactivation by pressure and heat as vegetative cells (Black et al. 2007; Heinz and Knorr 2001; Mathys et al. 2009; Reineke et al. 2013). Spore germination and inactivation pathways depend on the pressure–temperature combinations (Reineke et al. 2013; Mathys et al. 2009); however, more research is needed to elucidatethe mechanisms of the spore inactivation in food products. Various tools, such as flow cytometry, fluorescence anisotropy measurement, and electron microscope, have been used to observe cell membrane damage and morphological changes of spores and vegetative cells (Mathys et al. 2007; Abe 2013; Ananta et al. 2005).

10.3.3 Kinetic Models for HPP and HPTP Microbial Inactivation

Mathematical models allow many researchers to describe, predict, and optimize microbial behavior in foods after exposure to certain lethal treatment to ensure the microbiological quality and safety of food products. According to Whiting (1995), microbial modeling in foods can be classified as primary, secondary, and tertiary. The use of basic models (the primary followed by the secondary models) has been frequently reported after HPP and HPTP treatments in foods, as they are the most straightforward methods for the users (FDA 2015b).

10.3.3.1 Primary Models

Primary models aim to describe microorganisms' response as a function of time under specific conditions, in which the main goal is to estimate kinetic parameters such as the inactivation rate. Linear, concave, or sigmoidal trends are frequently observed in HPP and HPTP treatments based on the shape of their survival curves. Two common primary models reported to describe microbial log survivors in foods after HPP and HPTP are the simple first-order kinetic (Bigelow) and Weibull models.

The simple first-order kinetic model was established to define safe thermal processes for canned foods and it has also been successfully applied to other food processes, in which a plot of the logarithm of the surviving fraction against time yields a straight line as a constant intensity of heat or a lethal factor is applied. With respect to HPP and HPTP, the main kinetic parameter of the model is decimal reduction time, or the $D_{P,T}$ value, which is the time in minutes at a certain pressure and/or temperature necessary to reduce the microbial population by 90% (and is calculated from the reciprocal of the slope of Equation 10.1):

$$\log \frac{N}{N_0} = \frac{t}{D_{PT}} \tag{10.1}$$

where N_0 is the initial or untreated cell population in food (cfu/g), and N is the number of survivors after being exposed to HPP or HPTP treatment for a specific time t (min).

The Weibull model (Equation 10.2), perhaps the most prominent nonlinear model used for pressure treatments, is based on the principle of heterogeneity in the resistance distributed among individual cells within a population (vitalistic approach) (Pin and Baranyi 2006).

$$\log \frac{N}{N_0} = -bt^n \tag{10.2}$$

Two parameters obtained from this primary model are b, the scale factor, and n, the survival curve shape factor. b is a rate parameter that is related to the velocity of the inactivation of the microorganism. n describes the degree of curvilinearity, where $n < 1$ and $n > 1$ correspond to concave- upwards (tailings) and concave-downwards (shoulders) survival curves, respectively. When $n = 1$, the Weibull model becomes the simple first-order kinetics.

The nonlinear models are often more appropriate than first-order kinetics for HPP microbial inactivation. However, due to their complexity and more difficult application, they are not commonly used by microbiologists to fit log microbial survivor data.

10.3.3.2 Secondary Models

The secondary model is an extension of the primary model, in which the parameters of the primary model (e.g., D value and inactivation rate k) are related to the environmental variables/or conditions, such as pressure or temperature. In the area of inactivation and with respect to first-order linear kinetics, Bigelow uses the z_T value (°C) to model the inactivation rate dependence on the applied temperature. The z_T value (°C), or the temperature coefficient, is the temperature increase for constant pressure, which results in a 10-fold decrease in the D value. This is estimated from the negative reciprocal of the slope of Equation 10.3:

$$\log\left(\frac{D}{D_{Tref}}\right) = \frac{T_{ref}-T}{z_T} \tag{10.3}$$

where D_{Tref} is the D value at the reference temperature T_{ref} (can be any reference temperature, °C), and T is the temperature of the isothermal treatment (°C).

Likewise, a similar equation can be used to relate the inactivation D value with the HPTP pressure, for a fixed temperature, or room temperature HPP. The pressure coefficient, or z_P value (MPa), can also be estimated as follows (Equation 10.4):

$$\log\left(\frac{D}{D_{Tref}}\right) = \frac{P_{ref}-P}{z_P} \tag{10.4}$$

In the same way, the Weibull model can also relate the inactivation rate parameter (b value) to environmental variables, particularly temperature, and it can be used to predict the parameter value outside the range of variables tested (Evelyn and Silva 2015a–c, 2016a,b; Evelyn et al. 2016), ideally by validating the secondary model used and understanding the fundamental mechanisms involved.

The polynomial model or response surface model (RSM) is another secondary model that has been developed to predict the effect of multiple environmental factors on the inactivation parameters (Ross and Dalgaard 2004). Second-order polynomial equations are generally used, involving first-order, second-order (quadratic), and interaction terms, as shown in Equation 10.5 (Pérez-Rodríguez and Valero 2013):

$$Y = B_0 + \sum_{i=1}^{n} B_i X_i + \sum_{i=1}^{n} B_{ii} X_i^2 + \sum_{j\neq 1}^{n} B_{ij} X_i X_j + \varepsilon \tag{10.5}$$

where Y is the predicted response (microbial log survivors); B_0, B_i, B_{ii}, and B_{ij} are the estimated regression coefficients; X_i and X_j are the

independent variables (environmental factors); and ε is the error term. Pressure, temperature, and pressure holding time are three main variables frequently investigated under HPTP treatments. RSM can be graphically translated; thus, food operators can find the operating conditions that optimize the response. In Sections 10.4 and 10.5, the effects of HPP and HPTP on the main foodborne sporeforming and vegetative pathogens in foods and their kinetic models are reviewed.

10.4 HPTP and HPP Inactivation of Pathogenic Spores in Foods

Overall, spores show great resistance to inactivation requiring the combination of high pressure with thermal processing (HPTP). The genus *Clostridium* has the greatest degree of resistance, followed by *Bacillus*, and then lastly, vegetative cells, in which the inactivation is achieved with relatively mild process conditions (see Section 10.5). Simple nonlinear models were used to describe the spore inactivation after HPTP, whereas the first-order kinetic model was frequently applied for the inactivation of vegetative cells after HPP or HPTP.

10.4.1 Log Reductions

Tables 10.1 through 10.3 show the spore log reductions obtained for *C. botulinum*, *C. perfringens*, *B. cereus*, and other *Bacillus* spores in food products after high pressure in the range of 345–827 MPa, combined with tempera- tures of 25°C–86°C. HPTP at 827 MPa and 84°C for 15 min for *C. botulinum* and HPP at 600 MPa and 75°C for 15 min for *C. perfringens* were not suffi- cient to inactivate some strains of these spores (≤2 log), indicating high resis- tance to the HPTP treatments (Table 10.1). With the exception of *C. botulinum* strains ATCC 19397, ATCC 25765, and KAP8-B with >5.5 log after ≥600 MPa and ≥80°C for 16 min (Margosch et al. 2004a; Reddy et al. 2006), all others reported modest to almost no clostridial spore reductions in foods (0.4–4.1 log) after high pressures at 345–827 MPa combined with temperatures of 60°C–86°C for 5–16 min (Kalchayanand et al. 2003; Margosch et al. 2004a; Reddy et al. 2003, 2006; Evelyn and Silva 2016a).

Regarding *Bacillus*, HPTP in the range of 500–600 MPa and temperatures of 60°C–85°C between 1 and 17 min had been used (Tables 10.2 and 10.3). Van Opstal et al. (2004) reported generally similar spore inactivation (>5 log) for four *B. cereus* strains in milk after 500 MPa, 60°C, and 15 min (Table 10.2). Evelyn and Silva (2015b) obtained 3–4 log reductions of two strains of *B. cereus* spores in skim milk after 600 MPa, 70°C, and 15 min (Table 10.2). Scurrah et al. (2006) obtained ≈4 log for five *B. cereus* strains also in skim milk after 600 MPa, 72°C (initial temperature), and 1 min, with the

TABLE 10.1

Inactivation of *Clostridium* Spores in Foods by HPTP

Spores	Strains	Food Products	pH	Pressure (MPa)	Average Temperature (°C)	Time (min)	Log Reduction	References
Clostridium botulinum								
Proteolytic type A	62-A	Crabmeat blend	nr	827	86	15	2.7	Reddy et al. 2003
	B5-A						3.0	
Nonproteolytic type B	2B	Crabmeat blend	7.2–7.4	827	84	15	<1.0	Reddy et al. 2006
	17B						1.6	
	KAP9-B						2.0	
	KAP8-B						>5.5	
nr	TMW 2.357	Mashed carrot	5.2	600	80[a]	16	1.2	Margosch et al. 2004a
	TMW 2.356						2.6	
	TMW 2.359						2.6	
	TMW 2.358						4.1	
	ATCC 19397						>5.5	
	ATCC 25765						>5.5	
Clostridium perfringens								
	NZRM 2621 (ATCC 12917)	Beef slurry	6.5	600	75	15	1.5	Evelyn and Silva 2016a
	NZRM 898 (ATCC 14809)						2.0	
	1027	Roast beef	nr	345	60	5	0.4	Kalchayanand et al. 2003

Note: nr, not reported.

[a] Initial temperature.

TABLE 10.2

Inactivation of *Bacillus cereus* Spores in Foods by HPTP and HPP Alone

Strains	Food Products	pH	Pressure (MPa)	Average Temperature (°C)	Time (min)	Log Reduction	References
ATCC 9818	Cooked rice	6.0	600	85	4	7.0	Daryaei et al. 2013
NZ 4 (NCTC 8035)	Skim milk	nr	600	72[a]	1	4.1	Scurrah et al. 2006
NZ 6						4.3	
NZ 3/NZ 5/NZ 7						4.4	
ERR 82603						6.1	
NZRM 984 (ATCC 11778	Skim milk	nr	600	70	15	3.0	Evelyn and Silva 2015b
ICMP 12442 (ATCC 9139						3.5	
As 1.1846	Milk buffer	7.0	540	71	17	6.0	Ju et al. 2008
LMG 6910 (ATCC 7004)	Milk	6.7	500	60	15	5.4	Van Opstal et al. 2004
INRAAV P21S						5.6	
INRAAV Z4222						5.6	
INRAAV TZ415						6.6	
nr	Pork slurry	nr	600	RT	10	<1.0	Shigehisa et al. 1991
NCFB 578	Milk	nr	400	RT	15	<0.5	McClements et al. 2001
NCFB 1031						<0.5	
ATCC 9139	Cheese	5.5	400	RT	15	<0.5	Lopez-Pedemonte et al. 2003

Note: RT, room temperature HPP; nr, not reported.

[a] Initial temperature before compression.

TABLE 10.3

Inactivation of Other *Bacillus* Spores in Foods by HPTP

Species	Strains	Food Products	pH	Pressure (MPa)	Temperature[a] (°C)	Time (min)	Log Reduction	References
HPTP								
Bacillus licheniformis	TMW 2.492	Mashed carrot	5.2	600	80[a]	16	>7.0	Margosch et al. 2004b
B. licheniformis	NZ 23/Werribee 260	Skim milk	nr	600	72[a]	1	1.6	Scurrah et al. 2006
	NZ 24/NZ 25						2.0	
	FRR B2653 (ATCC 9789)						2.6	
	NZ 22						2.7	
	NZ 21(NCTC 6346)						3.4	
	Werribee 229						3.6	
	Werribee 207						4.3	
Bacillus pumilus	NZ 33	Skim milk	nr	600	72[a]	1	1.8	Scurrah et al. 2006
	NZ 32 (NCTC 10327)						2.9	
	NZ 27						3.5	
	NZ 31						3.7	
	NZ 29						4.3	
	NZ 28						4.7	

Note: nr, not reported.

[a]Initial temperature before compression.

exception of FRR B2603 with 6 log (Table 10.2). These authors also found large differences in the resistance of seven strains of *B. licheniformis* (1.6–4.3 log) and six strains of *B. pumilus* (1.8–4.7 log) spores in skim milk after the same treatment (Table 10.3). Margosch et al. (2004b) reported >7 log for *B. licheniformis* spores in mashed carrot. These results suggest that species, strain, and food play significant roles in spore resistance to HPTP; thus, investigat- ing the most resistant spores for each species–strain–food combination is needed to ensure food safety and quality. HPTP at ≥600 MPa and ≥85°C for ≥4 min seems to be needed to achieve ≥7 log inactivation of *B. cereus* spores in cooked rice (Daryaei et al. 2013). HPP treatments (400–600 MPa) at room temperatures (≤30°C) for 10–15 min generally showed a negligible effect on *Bacillus cereus* spores (Table 10.2).

10.4.2 Kinetic Models

The *Clostridium* and *Bacillus* spore inactivation in foods after HPTP was nonlinear; thus, the Weibull model was reported (Table 10.4). For 600 MPa processes at 70°C–75°C, the Weibull shape factors (n) for *C. perfringens* and *B. cereus* spores were between 0.39 and 0.74, indicating that the log survival curves have upward concavity with more pronounced tailings, as processing times increase in some cases (Evelyn and Silva 2015b, 2016a). Response surface methodology was also used to investigate the effects of pressure, temperature, and time on spore inactivation, and to predict the processing conditions to achieve a desired log reduction of spores. For example, for a 6 log cycle reduction on *B. cereus* in milk buffer, HPTP process parameters of at least 540 MPa and 71°C and a holding time of 16.8 min were required, according Ju et al. (2008).

10.5 HPTP and HPP Inactivation of Vegetative Pathogens in Foods

10.5.1 Log Reductions

In the studies of foodborne vegetative pathogens, with the exception of García-Graells et al. (1999) with resistant mutant *E. coli* strains (LMM 1020, LMM 1030, and LMM 1010) in skim milk, HPTP (345–600 MPa, 5–15 min) at >50°C (initial food temperature) generally resulted in large viability losses (5.5 to >8.0 log) in the food products (Patterson and Kilpatrick 1998; Gervilla et al. 1999; Ponce et al. 1998; Alpas and Bozoglu 2000; Bayındırlı et al. 2006) (Tables 10.5 through 10.9). *S. aureus* appeared to have the highest resistance to the treatments among the foodborne vegetative species reported (Table 10.5), followed by *E. coli* (Tables 10.5 and 10.6), and then *L. monocytogenes* and

TABLE 10.4

Modeling the Microbial Spore Inactivation in Foods after HPTP

	FoodProducts	pH	Model	Pressure (MPa)	Average Temperature (°C)	Time (min)	Model Parameters/ Equations[a]	References
Clostridium perfringens								
NZRM 2621 (ATCC 12917)	Beef slurry	6.5	Weibull	600	75	—	$b = 0.20$, $n = 0.74$	Evelyn and Silva 2016a
NZRM 898 (ATCC 14809)							$b = 0.68$, $n = 0.39$	
Bacillus cereus								
NZRM 984 (ATCC 11778)	Skim milk	nr	Weibull	600	70	—	$b = 0.55$, $n = 0.59$	Evelyn and Silva 2015b
ICMP 12442 (ATCC 9139)							$b = 0.67$, $n = 0.57$	
As 1.1846	Milk	7.0	Second-degree	400–600	60–80	10–20	$Y = 5.42 + 1.54P + 0.30T + 0.25t$	Ju et al. 2008
	Buffer		polynomial (RSM)				$-0.11P^2 + 0.17T^2 - 0.23t^2 - 0.23Pt$	

Note: nr, not reported.

[a] b and n are the Weibull scale and shape factors (Equation 10.2), respectively; Y, P, T, and t are the log reductions and pressure, temperature, and holding time variables of the RSM polynomial equation (Equation 10.5), respectively.

TABLE 10.5

Inactivation of *Staphylococcus aureus* in Foods by HPTP and HPP Alone

Strains	Food Products	pH	Pressure (MPa)	Average Temperture (°C)	Time (min)	Log Reduction	References
NCTC 10652	Poultry meat	nr	600	50[a]	15	6.0	Patterson and Kilpatrick 1998
(ATCC 13565)	UHT milk	nr	500	50[a]	15	6.0	
CECT 534 (NCTC 4163)	Ovine milk	6.7	500	50	15	>7.0	Gervilla et al. 1999
485	Milk	6.7	345	50[a]	5	5.5	Alpas and Bozoglu 2000
765	Milk					>8.0	
nr	Dry-cured ham	nr	600	RT	6	0.6	Hugas et al. 2002
	Cooked ham					1.1	
	Marinated beef					2.7	
ATCC 25923	Pork slurry	nr	600	RT	10	6.0	Shigehisa et al. 1991
NCTC 10652	Milk	nr	600	RT	15	2.0	Patterson et al. 1995
(ATCC 13565)	Poultry meat					3.0	
ATCC 6538	Cheese slurry	5.2–5.4	600	RT	20	4.5	O'Reilly et al. 2000

Note: RT, room temperature HPP; nr, not reported; UHT, ultrahigh temperature.

[a] Initial temperature before compression.

TABLE 10.6

Inactivation of *Escherichia coli* in Dairy and Poultry Foods by HPTP and HPP Alone

	Strains	Food Products	pH	Pressure (MPa)	Average Temperature (°C)	Time (min)	Log Reduction	References
K12	LMM 1020	Skim milk	6.6	550	50	15	2.4	Garcia-Graells et al. 1999
K12	LMM 1030						2.4	
K12	LMM 1010						4.7	
K12	MG 1655 (ATCC 47076)						7.0	
—	CECT 405 (ATCC 10536)	Liquid whole egg	8.0	450	50	10	5.5	Ponce et al. 1998
O157:H7	NCTC 12079	UHT milk	nr	500	50*	15	8.0	Patterson and Kilpatrick 1998
		Poultry meat					7.5	
O157:H7	933	Milk	6.7	345	50*	5	>8.0	Alpas and Bozoglu 2000
	931						>8.0	
O157:H7	NCTC 12079	Milk	nr	600	RT	15	1.5	Patterson et al. 1995
		Poultry meat					3.0	
K12	ATCC 29425	Cheese slurry	5.2–5.4	500	RT	20	>6.0	O'Reilly et al. 2000
—	ATCC 25922	Pork slurry	nr	400–500	RT	10	>6.0	Shigehisa et al. 1991
—	CECT 405 (ATCC 10536)	Fresh goat cheese	6.5	450–500	RT	5	>8.5	Capellas et al. 1996

Note: RT, room temperature HPP; nr, not reported; UHT, ultrahigh temperature.
*Initial temperature before compression.

TABLE 10.7

Inactivation of *Escherichia coli* in Fruit Juices by HPTP and HPP Alone

	Strains	Fruit Juices	pH	Pressure (MPa)	Average Temperature (°C)	Time (min)	Log Reduction	References
O157:H7	931	Orange juice	3.8	345	50[a]	5	>8.0	Alpas and Bozoglu 2000
	933						>8.0	
O157:H7	933	Apricot juice	3.8	350	40[a]	5	>8.0	Bayındırlı et al. 2006
		Orange juice	3.8				>8.0	
		Sour cherry juice	3.3				>8.0	
		Apple juice	3.5				>8.0	
O157:H7	SEA 13B88	Apple juice	3.7	615	RT	2	0.4	Teo et al. 2001
	ATCC 43895, 932[b]	Orange juice	3.7				2.2	
		Carrot juice	6.2				6.4	
		Grapefruit juice	3.0				8.3	
O157:H7	NCTC 12079	Orange juice	3.4–4.5	550	RT	5	>7.0	Linton et al. 1999
O157:H7	ATCC 43894	Mango juice	4.5	550	RT	5	>8.0	Hiremath and Ramaswamy 2012
O157:H7	C9490	Orange juice	3.8	500	RT	5	>7.0	Jordan et al. 2001
		Apple juice	3.5				>7.0	
		Tomato juice	4.1				>7.0	
O157:H7	nr	Orange juice	3.7	250	RT	20	5.0	Noma et al. 2004
		Apple juice	3.8				>7.0	
K12	LMM 1010	Mango juice	4.0	500	RT	2	>5.0	Garcia-Graells et al. 1998

(*Continued*)

TABLE 10.7 (CONTINUED)

Inactivation of *Escherichia coli* in Fruit Juices by HPTP and HPP Alone

Strains	Fruit Juices	pH	Pressure (MPa)	Average Temperature (°C)	Time (min)	Log Reduction	References
Resistant mutant LMM 1010	Apple pieces in 24° Brix GLUCOSE syrup	3.5	600	RT	10	>6.0	Vercammen et al. 2012
ATCC 11775	Orange juice	3.4	414	RT	0.03	>7.0	Guerrero-Beltrán et al. 2011a
ATCC 11775	Mango nectar	3.6	414	RT	1	>8.0	Bermúdez-Aguirre et al. 2011
ATCC 11775	Pineapple juice	3.8	300	RT	5	1.0	Buzrul et al. 2008
	Kiwifruit juice	3.3				4.0	
ATCC 11775	Pear nectar	4.2	241	RT	3	4.0	Guerrero-Beltrán et al. 2011b
ATCC 25922	Cashew apple juice	4.1	400	RT	3	6.5	Lanhas et al. 2008

Note: RT, room temperature HPP; nr, not reported.

[a] Initial temperature before compression.

[b] Cocktail of strains.

TABLE 10.8

Inactivation of *Listeria monocytogenes* in Foods by HPTP and HPP Alone

	Food Products	pH	Pressure (MPa)	Initial Temperature (°C)	Time (min)	Log Reduction	References
CA	Milk	6.7	345	50	5	>8.0	Alpas and Bozoglu 2000
Ohio2						>8.0	
nr	Goat cheese	nr	500	RT	5	>5.6	Gallot Lavallee 1998
NCTC 11994	Milk	nr	375	RT	15	0.5	Patterson et al. 1995
(DSM 15675)	Poultry meat					2.0	
Scott A	UHT milk	6.5	340	RT	20	1.5	Styles et al. 1991
	Raw milk					2.0	

Note: RT, room temperature HPP (for HPTP, the temperature was the initial one before compression); nr, not reported; UHT, ultrahigh temperature.

TABLE 10.9

Inactivation of *Salmonella* in Foods by HPTP and HPP Alone

Species	Strains	Food Products	pH	Pressure (MPa)	Initial Temperature[a] (°C)	Time (min)	Log Reduction	References
Salmonella enteritidis	FDA	Milk	6.7	345	50	5	>8.0	Alpas and Bozoglu 2000
Salmonella typhimurium	E 21274	Milk	6.7	345	50	5	>8.0	Alpas and Bozoglu 2000
S. enteritidis	nr	Liquid whole egg	8.0	450	50	15	>7.8	Ponce et al. 1999
					RT	15	5.1	
S. enteritidis	SE-4	Liquid whole egg	nr	400	RT	10	6.0	Bari et al. 2008
S. typhimurium	ATCC 7136	Strained chicken baby food	nr	340	RT	15	2.0	Metrick et al. 1989
S. *typhimurium*	ATCC 14028	Pork slurry	nr	400	RT	10	6.5	Shigehisa et al. 1991
Salmonella senftenberg	775 W	Strained chicken baby food	nr	340	RT	15	2.5	Metrick et al. 1989

Note: RT, room temperature (20°C–25°C); nr, not reported.
[a] Initial temperature before compression.

Salmonella spp. (Tables 10.8 and 10.9). Thus, the minimum processing conditions of 600 MPa and 15 min with an initial temperature of 50°C before compression enabled large reductions of *S. aureus*, and should actually be used to ensure the HPP inactivation of the most resistant vegetative cells in foods. *Vibrio* spp. and other vegetative pathogens, such as *C. jejuni*, *Y. enterocolitica*, *C. freundii*, and *A. hydrophila*, generally required milder processing conditions (170–586 MPa, 0–20 min, and room temperature) to achieve the same viability losses, >5.0 to 8.0 log (Tables 10.10 and 10.11).

Similar inactivation of vegetative pathogens by room temperature HPP between pathogenic strains belonging to the same species has also been observed in most of the past works of many authors compiled. Forexample, the following ranges of processing conditions were recorded: 400–500 MPa and 5–20 min for *E. coli* in cheese and pork with >6.0 to >8.5 log (Shigehisa et al. 1991; Capellas et al. 1996; O'Reilly et al. 2000) (Table 10.6), 500–550 MPa and 2–5 min for *E. coli* O157:H7 in fruit juices with >5.0 to >8.0 log (Garcia-Graells et al. 1998; Linton et al. 1999; Jordan et al. 2001; Hiremath and Ramaswamy 2012) (Table 10.7), 340–375 MPa and 15–20 min for *L. monocytogenes* in milk and meat with 0.5–2.0 log (Styles et al. 1991; Patterson et al. 1995), and 500 MPa and 5 min process resulted in >5.6 log (Gallot Lavallee 1998) (Table 10.8); 400–450 MPa and 15 min for *Salmonella* spp. in liquid whole egg with 5.1 to >7.8 log (Ponce et al. 1999; Bari et al. 2008) (Table 10.9), 586 MPa and 0 min for *Vibrio* spp. in oyster with >5.5 to >6.5 log (Koo et al. 2006) (Table 10.10), and 375–400 MPa and 10 min for *C. jejuni* and *Y. enterocolitica* in meat products with >6.0–8.0 log (Shigehisa et al. 1991; Solomon and Hoover 2004) (Table 10.11). However, on the other hand, few researchers observed large resistance differences among *S. aureus* in the food products under the same conditions of room temperature HPP, for example, log reductions in the range of 0.6–6.0 log for *S. aureus* after 600 MPa for 6–20 min (Shigehisa et al. 1991; Patterson et al. 1995; O'Reilly et al. 2000; Hugas et al. 2002) (Table 10.5), which poses a significant concern of this most resistant vegetative pathogen. Due to a few outbreaks registered in raw unpasteurized acidic fruit juices, *E. coli* inactivation by HPP and HPTP was also investigated in this class of beverages, since it might grow in this environment. Some researchers have shown that different fruit juices resulted in large variations in the room temperature HPP resistance of *E. coli* O157:H7 at the same processing conditions (Teo et al. 2001; Buzrul et al. 2008). However, Bayındırlı et al. (2006) worked with a cocktail of resistant *E. coli* strains and showed that 350 MPa HPTP with an initial temperature of 40°C for 5 min achieved very high reduction (>8.0 log) of the pathogenic *E. coli* in four fruit juices (Table 10.7).

10.5.2 Kinetic Models

Until 2012, modeling studies of vegetative pathogens after HPP in various food products were carried out using first-order kinetics (Table 10.12). However, note that although first-order kinetic parameters were determined

TABLE 10.10

Inactivation of *Vibrio* in Oysters and Clam Juice by Room Temperature HPP

Species	Strains	Food Products	pH	Pressure (MPa)	Time (min)	Log Reduction	References
Vibrio vulnificus	MO-624	Oyster	nr	586	0	>6.5	Koo et al. 2006
V. vulnificus	MLT 403	Oyster	nr	300	2	>7.0	Ye et al. 2012
V. vulnificus	nr	Homogenized oyster	nr	275	3	>7.0	Cook 2003
Vibrio parahaemolyticus	TX-2103, serotype O3:K6	Oyster	nr	586	0	>5.5	Koo et al. 2006
V. parahaemolyticus	10 different strains	Homogenized oyster	nr	300	3	>6.0	Cook 2003
V. parahaemolyticus	ATCC 43996	Oyster	nr	300	2	7.0	Ye et al. 2012
V. parahaemolyticus	T-3765-1	Clam juice	7.5	170	10	>5.0	Styles et al. 1991

Note: nr, not reported.

TABLE 10.11

Inactivation of Other Pathogenic Vegetative Cells in Meat Products by Room Temperature HPP

Vegetative Cells	Strain	Meat Products	pH	Pressure (MPa)	Time (min)	Log Reduction	References
Streptococcus faecalis	nr	Pork slurry	nr	600	10	>6.0	Shigehisa et al. 1991
Campylobacter jejuni	T1	Pork slurry	nr	400	10	>6.0	Shigehisa et al. 1991
C. jejuni	ATCC 35921	Chicken puree	nr	400	10	8.0	Solomon and Hoover 2004
		Milk	nr	375	10	8.0	
Yersinia enterocolitica	nr	Pork slurry	nr	400	10	>6.0	Shigehisa et al. 1991
Y. enterocolitica	9610	Ground pork	6.0	304	15	>7.0	Ellenberg and Hoover 1999
Citrobacter freundii	nr	Minced beef	5.6–5.8	300	20	>6.0	Carlez et al. 1993
Aeromonas hydrophila	ATCC 7965	Ground pork	6.0	253	15	>6.0	Ellenberg and Hoover 1999

Note: nr, not reported.

by the authors, most charts shown in the publications demonstrated a nonlinear log inactivation behavior (Metrick et al. 1989; Styles et al. 1991; Gervilla et al. 1999; Ponce et al. 1999; O'Reilly et al. 2000; Koo et al. 2006). The fitting carried out with the conventional first-order linear model simplified the analysis and comparison with the available results from other authors in past literature. *S. aureus* was confirmed to have the highest resistance (D_{450} = 16.7 min in milk) among other species (Gervilla et al. 1999), whereas *V. parahaemolyticus* was shown to be the least resistant vegetative pathogen, with a D_{136} value of 5.6 min in clam juice (Styles et al. 1991).

Using a secondary model parameter of the first-order kinetics, z_P values of 204 for *E. coli* and 359 MPa for *S. aureus* indicate low microbial susceptibility to small pressure changes (Table 10.12) (O'Reilly et al. 2000; Hiremath and Ramaswamy 2012). From this information, the estimated $D_{600\ MPa}$ values for 5 D of *S. aureus* and *E. coli* are 28.9 and 0.9 min, respectively.

10.6 Future Perspectives

High-pressure-treated foods have been categorized "novel foods" in countries in the European Union (EU) and in Canada, due to the capability to retain many of the qualities of the fresh food product, which would otherwise be altered by conventional thermal processing. Standard HPP treatments (400–600 MPa) can achieve 5.0–6.0 or more log reductions of most vegetative pathogens since they are susceptible to pressure at ambient temperature. However, a few studies demonstrated higher resistance of a few strains of microbial pathogens in the vegetative form in certain foods. Although >6.0 log reductions of *E. coli* are registered at room temperature HPP, Patterson et al. (1995) could only achieve a low level of inactivation (1.5–3.0 log) in milk and poultry meat after 600 MPa and 15 min, and Teo et al. (2001) has shown how difficult it is to inactivate a cocktail of *E. coli* strains in apple and orange juices. The survivors of *E. coli* may grow at low temperature during distribution, posing a human safety concern. Thus, HPP at a temperature of around >50°C is a possible solution to ensure complete inactivation of *E. coli*. Furthermore, more research is needed to obtain reliable inactivation models able to predict the nonlinear behavior of vegetative pathogens in HPP-treated foods at different pressure and temperature conditions.

HPP alone is not effective when the pasteurization aim is microbial spores, and HPTP at moderate temperatures or higher is required for spore inactivation. In addition to the food matrix being treated, the HPTP treatment conditions (pressure, average temperature, and processing time during the constant pressure phase of the HPP cycle) are important factors affecting spore inactivation. Some species of *Clostridium* and *Bacillus* are resistant sporeformers of public health concern that can only

TABLE 10.12

First-Order Kinetic Parameters for the Inactivation of Pathogenic Vegetative Microorganisms in Food Products after Room Temperature HPP

Pathogen	Strain	Food Products	pH	Pressure, P (MPa)	First-Order Parameters: D_P Value (min)	First-Order Parameters: z_P Value (MPa)	References
Staphylococcus aureus	CECT 534 (NCTC 4163)	Ovine milk	6.7	450	16.7	nr	Gervilla et al. 1999
S. aureus	ATCC 6538	Cheese slurry	5.2–5.4	400	20.0	359	O'Reilly et al. 2000
Escherichia coli	CECT 405 (ATCC 10536)	Liquid whole egg	8.0	400	14.1	nr	Ponce et al. 1998
E. coli O157:H7	ATCC 43894	Mango juice	4.5	450	0.72	204	Hiremath and Ramaswamy 2012
E. coli K12	ATCC 29425	Cheese slurry	5.2–5.4	350	19.0	nr	O'Reilly et al. 2000
Listeria monocytogenes	Scott A	UHT milk	6.5	340	13.2	nr	Styles et al. 1991
Salmonella enteritidis		Liquid whole egg	8.0	400	8.8	nr	Ponce et al. 1999
Salmonella typhimurium	ATCC 7136	Strained chicken baby food	nr	340	7.6	nr	Metrick et al. 1989
Salmonella senftenberg	775 W	Strained chicken baby food	nr	340	7.1	nr	Metrick et al. 1989
Vibrio parahaemolyticus	TX-2103 serotype O3:K6	Oyster	nr	345	2.0	nr	Koo et al. 2006
V. parahaemolyticus	T-3765–1	Clam juice	7.5	136	5.6	nr	Styles et al. 1991

Note: D_P and z values are the first-order kinetic parameters (Equations 10.1 and 10.2). Initial temperature, ≤25°C; nr, not reported; UHT, ultrahigh temperature. Note that although first-order kinetic parameters were determined by the authors, charts demonstrated a log nonlinear inactivation with the time in most of the studies reviewed.

be inactivated at high pressures and temperatures, not yet achievable by commercial HPP units. The nonlinear trend observed for spore inactiva- tion by HPTP (Evelyn and Silva 2015b, 2016a; Ju et al. 2008) should be fol- lowed up due to an increase of microbial spore resistance with processing time. More research is still required to standardize HPTP pasteurization conditions (process criteria, pressure–time–temperature combinations, etc.) in various food products to successfully introduce HPTP in the food industry.

As heat has detrimental effects on food quality, an alternative option is the simultaneous or sequential application of HPP and other nonthermal food preservation technologies to enhance the lethal effect of HPP (e.g., irradiation was investigated by Crawford et al. [1996]). Furthermore, the cold storage conditions should be topped up with other hurdles, such as modified atmospheres and the use of preservatives, to inhibit or slow down the growth of resistant sporeformers in HPP pasteurized food products.

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