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**Highlights**

- The increase of HPP temperature from 38 to 70°C doubled *B. cereus* log reductions in beef slurry.
- HPP technology allowed lower temperatures for *B. cereus* spore inactivation.
- Weibull was an appropriate model for the 600 MPa HPP-thermal inactivation of *B. cereus* in beef slurry.
Modeling the inactivation of psychrotrophic *Bacillus cereus* spores in beef slurry by 600 MPa HPP combined with 38-70°C: comparing with thermal processing and estimating the energy requirements

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Abstract

The growth of psychrotolerant *Bacillus cereus* in pre-prepared cooked chilled foods and refrigerated processed foods of extended durability (REPFED) is a concern. High pressure processing (HPP) is an established food processing technology that retains the flavor and nutrients in the processed food. In this study, the efficacy of 600 MPa HPP in combination with 70°C for the inactivation of *B. cereus* ICMP 12442 spores in beef slurry was investigated and compared with 70°C thermal processing alone. The HPP-70°C process enhanced the *B. cereus* spore thermal inactivation in beef slurry, resulting in 4.9 log reductions after 20 min vs. 0.5 log for thermal processing. Then, the effect of temperature at 600 MPa on the spore inactivation up to 40 min was studied, and the log survivors vs. time were modeled. Weibull model was appropriate to characterize the inactivation. Increasing the HPP temperature from 38 to 70°C, increased the spore inactivation in beef slurry up to 3 log. Lastly, the thermal inactivation of the spores was investigated. The spore thermal inactivation
was linear and first order kinetic parameters were determined. The results of this study confirm the advantage of HPP technology for the inactivation of \textit{B. cereus} spores in beef slurry at moderate temperatures.

**Keywords** High pressure processing; HPTP; energy; heat; bacteria; beef safety

1. Introduction

Increasing consumer demand for pre-prepared cooked chilled foods (including refrigerated processed foods of extended durability or REPFEDs) has led tremendous research in the area of food safety microbiology to reduce the risk associated with this type of convenience foods (Afchain et al., 2008; Carlin et al., 2000a, 2000b; Choma et al., 2000; Daelman et al., 2013; Evelyn and Silva, 2015a; Guinebretiere, 2003; Lopez-Pedemonte, et al. 2003; Malakar et al., 2004; Membré et al., 2006; van Opstal et al., 2004). Spore forming and psychrotolerant bacteria has been linked to the safety and stability of these foods because of its ability to survive the normal heat treatment (pasteurization and/or cooking) and grow at low temperature during the chilled storage (Membré et al., 2006; Silva and Gibbs, 2010; Silva et al., 2014). As a result, the foods are spoiled and reduced in their shelf life, and there is a risk of foodborne diseases caused by the outgrowth of pathogenic spore formers such as psychrotolerant \textit{Bacillus cereus} and non-proteolytic strains of \textit{Clostridium botulinum} (Silva and Gibbs, 2010).

\textit{B. cereus} is a Gram-positive, rod-shaped, spore-forming facultative anaerobic bacterium which is able to grow over a wide range of temperatures (4–55 °C), pH (4.9–9.3), and water activities values (0.92–1.0) (EFSA, 2005). The psychrotrophic strains of \textit{B. cereus} are able to
regenerate to large numbers at refrigerated temperatures (Choma et al., 2000; Christiansson et al., 1989; Valero et al., 2007), produce toxins in foods (Samapundo et al., 2011), and cause diarrhea or emesis food poisoning and fatal meningitis (Dierick et al., 2005; Evreux et al., 2007; Luby et al., 1993; Schoeni, 2005; Slaten et al., 1992). Prevalence (16.8% population per package) and concentrations at the time of consumption (2.5% of the packages contaminated with >6.7 log cfu/g) of psychrotolerant B. cereus group II in REPFED foods indicate this group as a high food poisoning risk (Hendrickx, 2011). The following contaminated foods have been reported in outbreaks of B. cereus around the world: meat (Luby et al., 1993; Slaten et al., 1992), raw and pasteurized milk (Ahmed, 1983; Røssland et al., 2005), starchy foods (for instance rice, potato, pasta) and cheese products (USFDA, 2012), vegetable puree as well as other chilled-foods containing vegetables (Carlin et al., 2000a, 2000b; Jenson et al., 2003), and cake and other desserts (Granum and Lund 1997; Ghelardi et al., 2002). With respect to thermal resistance, the decimal reduction time (D-value) of psychrotrophic B. cereus spores can range from 0.22 to 3.1 min at 100°C, depending on the strain and heating menstrum (Evelyn and Silva, 2015a, 2015b; Fernández et al. 2001; Wimalaratne 2009).

High pressure processing (HPP) is a commercially non thermal food preservation technology with less adverse effects on food quality (Cullen et al., 2012). HPP relies on the use of high pressures (typically 400-600 MPa) to process liquid and solid foods (with or without heat) between 5 and 10 min, to inactivate spoilage/pathogenic microorganisms and to extend food shelf-life. High pressure treatment at room temperature is not sufficient for reduction of bacteria and mould spores (Evelyn and Silva, 2015b, 2015c, 2016; Evelyn et al., 2016; Silva et al., 2012), in which some may resist pressures higher than 1000 MPa. HPP at ambient temperature can also have limited effectiveness for the inactivation of endogenous food spoilage enzymes, namely polyphenoloxidase (Sulaiman and Silva, 2013; Sulaiman et al.,
Therefore, combination of HPP with a mild thermal process is required (Evelyn and Silva, 2015b, 2015c, 2016; Evelyn et al., 2016; Patterson 2005; Silva et al., 2012). This process is commonly referred as HPP-thermal or high pressure thermal process (HPTP). With respect to *B. cereus* spores, the efficacy of HPP between 100-900 MPa (pulsed or continuous) in conjunction with mild heat, antimicrobial agents (e.g. nisin and sucrose laurate), or an additional control hurdle (e.g. olive powder) to inactivate *B. cereus* spores has been investigated (Aoyama et al., 2005; Arroyo et al., 1997; Daryaei et al., 2013; Evelyn and Silva, 2015b; Fornari et al., 1995; Gola et al., 1996; Ju et al., 2008; Lopez-Pedemonte et al., 2003; Luu-Thi et al., 2014; Marco et al., 2011; McClements et al., 2001; Meyer, 2000; Raso et al., 1998; Robertson et al., 2008; Rovere et al., 1998; Scurrah et al., 2006; Shearer et al., 2000; Shigeta et al., 2007; van Opstal et al., 2004). *B. cereus* spore log reductions between 1 and \(\geq 7\) were achieved, depending on the conditions applied and the spore resistance. Among these results, Daryaei et al. (2013) and Luu-Thi et al. (2014) modeled the HPP inactivation kinetics with mesophilic *B. cereus* spores. McClements et al. (2001), Lopez-Pedemonte et al. (2003), and van Opstal et al. (2004) studied the inactivation of the psychrotrophic strain of *B. cereus* spores in milk and cheese however the kinetics was not modeled. We have previously modeled the inactivation of psychrotolerant *B. cereus* spores in milk by HPP-thermal and found that the Weibull model appropriately described survival curves (Evelyn and Silva, 2015b). This bacterium presents high variability in the spore resistance and is able to grow in minced beef stored under refrigeration. Furthermore, due to the increase of low acid chill stored foods market volume by 10% each year (Silva et al., 2014), there is an additional risk of foodborne infections by *B. cereus*. Therefore, HPP-thermal inactivation of these spores in beef slurry was investigated and the objectives were as follows: (i) to compare the 600 MPa HPP-thermal with thermal inactivation of psychrotrophic *B. cereus* spores in beef slurry at 70°C; (ii) to model the 600 MPa HPP-thermal inactivation of psychrotrophic *B. cereus* spores
in beef slurry; (iii) to model the thermal inactivation of psychrotrophic \textit{B. cereus} spores in beef slurry; and (iv) to compare the specific energy requirements for equivalent HPP-thermal and thermal processes.

2. Material and methods

2.1. Microbiology

2.1.1. Strain

Psychrotrophic \textit{B. cereus} ICMP 12442 (ATCC 9139, ATCC 21, BCRC 17036, CECT 5144, LMG 9005, NCCB 48010, NCIMB 11925, VTT E-96727) was obtained from the Landcare Research New Zealand. The psychrotrophic behavior of ICMP 12442 strain (growth at 8°C in cheese) was previously demonstrated by Lopez-Pedemonte et al. (2003). The strain was sourced freeze-dried and revived according to the suppliers.

2.1.2. Sporulation and spore enumeration

The same sporulation and enumeration procedure for this strain was described previously (Evelyn and Silva, 2015a, 2015b). The \textit{B. cereus} spore concentration in beef slurry before and after HPP-thermal and thermal processing was determined by spread plating onto trypticase soy agar (TSA) and aerobic incubation at 37°C for 48 h. Beef slurry samples were first homogenized in a stomacher (Masticator stomacher, IUL Instruments, Germany) with equal volume of 0.1% (w/v) sterile buffered peptone water as dilution fluid for 2 min (this dilution was considered in the calculation of final spore concentration). Samples were further decimal diluted ten times in 0.1% (w/v) sterile buffered peptone water, mixed
repeatedly with a vortex mixer, and plated twice. Average colony counts (± standard deviation) were calculated and results were expressed in colony forming units per gram (cfu/g) of beef slurry. For enumeration of spore in a suspension, the same procedure was followed without the 2 fold predilution and homogenization in the stomacher. The spores were stored in sterile distilled water and maintained at 2°C until use.

2.1.3. Beef slurry preparation and inoculation

Pasteurised beef was chosen since it is prone to contamination by *B. cereus* (Thippareddi et al., 2009). The beef was made into slurry by mixing the pasteurised sirloin beef mince with sterile distilled water (SDW) in a sterile laboratory scale blender (100 mL of SDW was added to every 100 g of minced meat). The major composition was determined by an accredited laboratory in New Zealand: moisture (76%), protein (14%), fat (7%), carbohydrate (2.6%), and ash (0.4%). The spore suspension was inoculated in beef slurry to yield a final concentration of approximately ~10^7 cfu/g. For HPP-thermal and thermal experiments, a portion (*ca.* 0.1 mL) of spore suspension was inoculated into 3 g of beef slurry placed inside 8 × 8 cm food grade sterile pouches (Cas-Pak, New Zealand).

2.2. High pressure processing of beef slurry

2.2.1. High pressure equipment and operation

A high pressure food processing system (QFP 2L-700, Avure Technologies, Columbus, Ohio, USA) was used for pressure and pressure combined heat treatment of *B. cereus* spores. The maximum pressure and temperature supported by the Avure HPP machine were 690 MPa and
75°C, respectively. For combined pressure–heat treatment applications, the 2 L stainless steel pressure chamber was immersed in a temperature controlled bath, in which propylene glycol acted as the heating medium (heating with propylene glycol was not carried out for the treatment at room temperature). The temperature of the external glycol bath was set prior the combined treatment to achieve the target temperature. Two internal thermocouples were used to monitor the temperature in the distilled water contained in the pressure chamber and another internal thermocouple was used to measure the glycol bath temperature during the processing. Fig. 1 illustrates one example of the pressure and temperature histories obtained during the 600 MPa-thermal process cycle. Pressure come up times were \( \leq 1.5 \text{ min} \) and depressurization took less than 30 s. During the pressurization phase, adiabatic heating occurred. Then during the pressure–holding time phase of the HPP cycle, the chamber temperature dropped steadily toward the initial set temperature. The HPP temperature was approximately the average temperature registered during the constant pressure phase of the HPP cycle, which was obtained after preliminary trials to select the initial temperature for the HPP treatment.

### 2.2.2. High pressure and high pressure combined with thermal experiments

The pouches containing 3 g of inoculated beef slurry samples were submitted to 600 MPa high pressure at room temperature (38 °C [no additional heat]), or 600 MPa high pressure combined with moderate temperatures (50°C, 60°C, and 70°C) for times between 1 and 40 min. The long processing times were needed to reduce the number of spores significantly and to model the inactivation kinetics of *B. cereus* spores by HPTP processes. The process hold-times did not include the pressure come-up (\( \leq 1.5 \text{ min} \)) or the depressurization times (\(< 30 \text{ s}\)), and change in the spore population during the come-up time was not accounted. The spore
inactivation is higher for higher pressure and temperatures and therefore we worked with the maximum pressure of 600 MPa and temperatures (38-70°C) below the maximum allowed by the equipment. For this range of temperatures, the initial temperatures of beef slurry before HPP were between 30 and 67°C, since adiabatic heating due to compression caused a temperature increase of 2-3°C per 100 MPa from the initial temperature. After HPTP samples were submerged in ice water bath prior to spore enumeration. Two samples were processed for each processing time and three survival experiments were carried out for each temperature.

2.3. Thermal processing of beef slurry

Thermal inactivation of *B. cereus* spores in beef slurry were carried out at 70, 80 and 90°C. The pouches containing the inoculated spores in 3 g of beef slurry were thermosealed (Multivac C200, Germany) and placed in the stomacher (Masticator Stomacher, IUL Instruments, Germany) for 2 min to ensure good spore mixing with the beef slurry and uniform distribution. The pouches were then compressed into a very thin layer (approximately 1‒2 mm thick) and processed for up to 20 min (depending on the treatment temperature) in a thermostatic water bath. Treated samples were taken out at different time intervals and kept in an ice water bath until microbial enumeration. Two survival experiments were carried out with duplicate samples for each processing time.
2.4. Modeling the inactivation of psychrotrophic B. cereus spore in beef slurry

Based on the aspect of the survival curves (log microbial numbers vs. time), two mathematical models (Weibull and first order kinetics) were fitted to the inactivation of the psychrotrophic B. cereus spores in beef slurry.

2.4.1. Weibull model

The Weibull equation written in the decimal logarithmic form was used to model as function of processing time the log survivors by HPTP and also thermal alone, to enable the comparison of both processes (Peleg and Cole 1998; Weibull, 1951):

\[ \log \frac{N}{N_0} = -bt^n \]

(1)

where \( b \) (the scale factor) is a rate parameter which is related to the velocity of inactivation of the microorganism. \( n \) is the survival curve shape factor: \( n<1 \) and \( n>1 \) correspond to survival curves with concave-upwards (tailings) and concave-downwards (shoulders), respectively. When \( n = 1 \), the Weibull model becomes the simple first-order kinetics. \( t \) is the treatment time in min.

2.4.2. First order kinetics

First order kinetics was used to model the thermal inactivation results and did not suit the HPP-thermal log survivors. In this model, decimal reduction time (\( D_{10} \)-value) is the time in min at a certain temperature necessary to reduce microbial population by 90% and was calculated from the reciprocal of the slope as follows (Bigelow, 1921):

\[ D_{10} = \frac{1}{\text{slope}} \]
\[ \log \frac{N}{N_0} = -\frac{t}{D_T} \]  \hspace{1cm} (2)

where \( N_0 \) is the initial or untreated spore concentration in the beef slurry (cfu/g), \( N \) is the concentration of spore survivors after being exposed to the heat treatment for a specific time \( t \) (min). The temperature coefficient, \( D_T \)-value (°C) is the temperature increase that results in a 10-fold decrease in the \( D_T \)-value and was estimated from the negative reciprocal of the slope:

\[ \log \left( \frac{D}{D_{T\text{ref}}} \right) = \frac{T_{T\text{ref}} - T}{D_T} \]  \hspace{1cm} (3)

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

### 2.4.3. Model evaluation

TableCurve 2D version 5.01 (SYSTAT Software Inc., USA) was used to fit the models to the spore survival lines and estimate the model parameters. Mean square error (MSE), coefficient of determination (\( R^2 \)), and accuracy factor (\( A_F \)) were used to compare the performance of different models. \( R^2 \) and \( A_F \) values close to 1 and a relatively small MSE, indicated the adequacy of the model to describe the data. For each temperature two or three survival experiments were carried out and the model parameters (\( D \)-value, \( b \), \( n \)) were estimated by regression of logarithmic number of survivors (log \( N/N_0 \)) versus time. \( N_0 \) was the initial concentration of spores in untreated sample (control) and \( N \) was the spore concentration of beef slurry sample after a specific processing time at constant pressure and/or temperature. Then, the parameters’ mean ± standard deviation (SD) was calculated for each temperature. Additionally, the parameter’s temperature dependence was characterized with an appropriate secondary model.
2.5. Specific energy calculations for HPP and thermal processes

Equation 4 was used to determine the sensible heat to warm up the temperature of the beef slurry before HPP and thermal processes:

\[ Q = m \cdot C_p \cdot \Delta T \]  

(4)

where \( Q \) is the heat energy needed to raise the beef slurry temperature (J); \( m \) is the mass of the beef slurry sample (kg); \( C_p \) is the beef slurry heat capacity (3440 J/(kg°C)); \( \Delta T \) is the increase of beef slurry temperature (°C). Temperature of 2°C of the ice water bath/fridge was considered as the initial beef slurry temperature in the calculations of sensible heat.

Regarding HPTP, in addition to sensible heat, energy is also required for compression (Smith et al., 2005):

\[
W_{\text{compression}} = \int_{P_1}^{P_2} PV \left( \frac{dP}{dV} - \frac{\alpha}{V} \right) dP = \frac{1}{2} \beta V (P_2^2 - P_1^2) - P_2 V \alpha (T_2 - T_1)
\]

(5)

where \( W_{\text{compression}} \) is the compression work (J); \( P_1 = 0.1 \times 10^6 \) and \( P_2 = 600 \times 10^6 \) are the initial and final/target pressure during compression, respectively (Pa); \( V = 0.002 \) is the volume of the chamber (m³); \( \alpha \) is the volume expansivity of water (K⁻¹ or °C⁻¹); \( \beta \) is isothermal compressibility of water (Pa⁻¹); \( T_1 \) and \( T_2 \) are the initial and final temperature during the compression phase of the HPP cycle (°C).

Then, the specific energy (J/kg) for the HPP and thermal processes was obtained by dividing the total energy required by HPP or thermal processes by the mass (kg) of processed beef slurry.
3. Results and discussion

3.1. Comparing 600 MPa HPP-70°C vs 70°C thermal inactivation of psychrotrophic B. cereus spores in beef slurry

Fig. 2 shows the survival curve in logarithmic coordinates of B. cereus spores in beef slurry by 600 MPa HPP-70°C and 70°C thermal process. The HPP-thermal inactivation was much faster than thermal inactivation alone. For a 20 min process at 70°C, 4.9 log reduction for HPP-thermal vs. 0.5 log reduction for thermal alone were obtained, indicating a remarkable advantage when using HPP technology. The benefit of HPP-thermal vs. thermal was also documented by Daryaei et al. (2013) and Luu-Thi et al. (2014) for mesophilic B. cereus spores, and in our previous results carried out with the same strain in milk (Evelyn and Silva, 2015b). After 20 min 600 MPa at 70°C 4 log reductions were obtained in milk (Evelyn and Silva, 2015b) which was lower than 4.9 log obtained with beef slurry in our current study. This could be due to the baroprotective effect of milk components such as sucrose on the spore inactivation by HPP (Gervilla et al., 2000; Patterson et al., 1995; Simpson and Gilmour, 1997; Styles et al., 1991). Daryaei et al. (2013) reported similar log reductions (~ 3.5 log) for 600 MPa-75°C for 4 min holding time in cooked rice with B. cereus ATCC 9818. Luu-Thi et al. (2014) observed less than 1.5 log for B. cereus F4430/73 in ethanesulfonic acid (MES) buffer at the same process conditions. These results indicate that strain and spore suspending medium play an important role for the inactivation of B. cereus spores by HPP-thermal.
3.2. Modeling the 600 MPa HPP-thermal inactivation of psychrotrophic B. cereus spores in beef slurry

The log spore survivors as a function of time of B. cereus in beef slurry processed by 600 MPa HPP (38°C) and 600 MPa HPP-thermal (50, 60 and 70°C) are illustrated in Fig. 3. The spore counts dropped steadily at all temperatures since the beginning of the treatments, followed by slower inactivation at longer processing times. There could be minor inactivation of spores during the come-up time, however this was not accounted for. According to Reineke et al. (2011, 2012), at this threshold pressure (600 MPa) combined with temperatures above 50-60°C, germination can be bypassed with direct spore inactivation, similar to heat inactivation.

HPP temperature had a significant effect on the spore inactivation (Fig. 3), in which the higher the HPP process temperature the higher was the B. cereus spore inactivation. For example, increasing the temperature from 38 to 70°C at 600 MPa for 40 min process, increased the B. cereus spore inactivation in beef slurry by 3 log. In milk, the same increase in temperature at 600 MPa resulted in an additional 3.5 log reductions of these spores after 40 min (Evelyn and Silva, 2015b). An increase in the log reductions with temperature was also reported with spores of psychrotrophic B. cereus LMG 6910 (= ATCC 7004), mesophilic B. cereus ATCC 9818 and F4430/73, Alicyclobacillus acidoterrestris, Clostridium botulinum and Clostridium perfringens with 600 MPa HPP-thermal (Daryaei and Balasubramaniam, 2013; Evelyn and Silva, 2016; Luu-Thi et al., 2014; Margosch et al., 2006; Silva et al. 2012; van Opstal et al., 2004; Vercammen et al., 2012) and mould spores (Evelyn and Silva, 2015c; Evelyn et al., 2016).

The non linearity observed in the B. cereus spore survival curves (Fig. 3) was confirmed by the the low $R^2$ ($\leq 0.760$) obtained for first order kinetics, thus not being appropriate for HPP-
thermal process. On the contrary, Weibull model presented good performance indices ($0.020 \leq \text{MSE} \leq 0.036$, $0.983 \leq R^2 \leq 0.990$, $1.04 \leq A^R \leq 1.09$) (Table 1), and therefore was selected. The $n$ parameter in the Weibull model presents the shape factor of the survival curves and the deviation from linearity. All the $n$ values were less than 1 (Table 1), indicating that the spore survivor curves by HPP-thermal process were concave upward (Fig. 3), and confirming the non linearity. This type of concavity suggests that there is a mixed resistance of the spore population to the lethal treatment (Peleg 2000; van Boekel 2002), in which the most sensitive spore population is inactivated at a faster rate, followed by the slower and steady decline of a more resistant population (Tola and Ramaswamy, 2014). Although at 38°C $n$ was slightly higher ($=0.46$), the $n$ did not change within 50 to 70°C HPP temperature ($n=0.3$, Table 1). Cunha et al. (1998) has mentioned that $n$ should not change with temperature as it is related with the kinetic order, and others also reported Weibull $n$ not dependent of temperature and/or pressure (Bermúdez-Aguirre and Corradini, 2012; Evelyn and Silva, 2016; van Boekel, 2002).

In Weibull distribution, the $b$ parameter is the scale factor that relates with the spore inactivation rate. As can be seen from the Table 1, at 600 MPa, the higher the temperature, the higher was the value of $b$. The $b$ increased from 0.56 to 2.13 as the temperature increased from 38 to 70°C. Similarly, our past results with same strain in milk showed the increase of $b$ from 0.10 to 0.67 under the same conditions (Evelyn and Silva, 2015b). Daryaei et al. (2013) also obtained the increase of $b$ values from 0.81 to 1.66 as the 600 MPa HPP temperature was increased from 60 to 85°C. Fig. 4 shows a plot of $b$ as a function of HPP temperature, which increased linearly with temperature ($R^2 = 0.99$).
3.3. Modeling the thermal inactivation of psychrotrophic B. cereus spores in beef slurry

The thermal log survivors of the psychrotrophic B. cereus spores in beef slurry are presented in Fig. 5. Similar spore inactivation (≈ 3.0 log) was obtained after 20 min for the 80°C thermal process (Fig. 5) and 50°C HPP process (Fig. 2), thus demonstrating that HPP-thermal required a 30°C lower temperature than a thermal process alone to inactivate the B. cereus spores.

The survivors of B. cereus spores in beef slurry were linear with thermal processing time (Fig. 5). Therefore the first order kinetics fitted well, presenting good performance indices at 70, 80 and 90°C (MSE≤0.002, $R^2$≥0.998, $A^2$ between 1.01 and 1.03) and temperature dependence of the $D$-values ($R^2$=0.998) (Table 2). The $D$-values obtained were 1.0 min at 90°C, 6.9 min at 80°C and 46.0 min at 70°C. Similar to HPP-thermal resistance, our previous results with this strain also showed a slightly higher thermal resistance in milk with $D_{90^\circ C}$=2.0 min, $D_{80^\circ C}$=8.5 min and $D_{70^\circ C}$=78.5 min (Evelyn and Silva, 2015b), probably due to protective effect of milk components mentioned previously. Our previous studies with NZRM 984 spores in beef slurry (Evelyn and Silva, 2015b) demonstrated a similar $D_{90^\circ C}$-value (1 min). Byrne et al. (2006) determined $D_{90^\circ C}$-value of 10 min with B. cereus spores cocktail in pork roll as opposed to 1 min in beef slurry obtained in our study. A wide range of B. cereus spore heat resistance was reported in the literature, highly dependent on the strain, type of medium/food and sporulation conditions (Mazas et al., 1995; Montville et al., 2005). The z-value obtained in our work (11.9°C) with ICMP 12442 spores was in the range of B. cereus z-values obtained with strain ATCC 9818, F4165/75, CRA 1787 and F4165/75 in nutrient broth or distilled water modified and buffer (Ababouch and Busta,
1987; Casadei et al., 2001; Johnson et al., 1982), and in foods such as milk and orange juice with *B. cereus* ATCC 7004, ATCC 4342, ATCC 9818 spores (Montville et al., 2005).

The Weibull model was also fitted to the thermal log survivors in beef slurry in order to compare HPP-thermal with thermal results. As expected n=1.0, confirming the linearity and the estimated Weibull b values increased from 0.02 at 70°C to 1.03 at 90°C (Table 2). The HPP-thermal b values were higher than thermal b values. Similar b (≈1) were obtained for the 90°C thermal and 50°C HPP-thermal processes. The need of lower temperatures in the case of a pressure–thermal process was also observed in past studies with *B. cereus* and *C. perfringens* spores (Daryaei et al., 2013; Evelyn and Silva, 2015c, 2016; Luu Thi et al., 2014).

3.4. Specific energy requirements for equivalent pasteurization processes and process recommendations

The following processes resulting in 5-log reduction in *B. cereus* spores were selected for energy comparison: thermal processing at 90°C for 5 min and 600 MPa HPP-thermal process at 70°C for 20 min. For the calculation of specific energy for HPTP process, first the sensible heat (= 217 kJ/kg) to warm up the beef slurry from 2 to 65°C (beef slurry initial temperature before HPP) was estimated using Equation 4. Then, for the calculation of compression work (=151 kJ/kg), the initial and final temperatures were 65°C and 83°C, respectively. The volume expansivity (α) and isothermal compressibility (β) of water at 74°C (the average temperature during compression phase) were used: α=6.07×10^-4 K^-1; β=4.55×10^-10 Pa^-1 (Kell, 1975). The estimated specific energy for the HPP-70°C process was 367 kJ/kg (Eq. 4 and 5). The equivalent 90°C thermal process required 303 kJ/kg (Eq. 4). Rodriguez-Gonzalez et al.
(2015) also estimated higher energy consumption for HPP (338-483 kJ/kg) than thermal process (HTST, 167-228 kJ/kg) for 5D apple juice pasteurization.

Based on the models generated for HPTP predictions of pasteurization conditions at higher temperatures which deliver 6 log reductions (6D) in the psychrotrophic B. cereus spores in a shorter time can be made. For HPTP process, b values for higher temperatures (Figure 4) are 2.6 at 80°C and 3.0 at 90°C. Then, the HPTP times required for those temperatures (at a fixed n value of 0.3) are 17.1 min at 80°C and 9.7 min at 90°C. With respect to thermal Table 2 shows the D-values determined at 80 and 90°C, and the 6D thermal pasteurizations are 41.6 min at 80°C and 5.9 min at 90°C. These results suggest that the HPTP is a better process than thermal alone at temperatures ≤80°C, but from 90°C there is no advantage of using the HPTP for 6D reduction of psychrotrophic B. cereus spores in beef slurry.

4. Conclusion

Current study demonstrated that psychrotrophic B. cereus spore inactivation in beef slurry is temperature dependent for 600 MPa HPP-thermal processes, being higher for higher temperature. The 600 MPa HPP-70°C process enhanced B. cereus spore inactivation compared to thermal process alone at the same temperature. Weibull distribution was the best mathematical model to describe the non-linear inactivation of psychrotrophic B. cereus spores in beef slurry by HPP-thermal process, whereas first order kinetics was appropriate for thermal processing alone. The results demonstrated the benefit of the HPP technology and allowed a better understanding of the kinetics of pressure-thermal induced inactivation of psychrotrophic B. cereus spores in beef slurry.
Acknowledgments

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References


Cullen, P.J., Tiwari, B.K., Valdramidis, V.P., 2012. Status and trends of novel thermal and non-thermal technologies for fluid foods. In: Cullen, P.J., Tiwari, B. K., Valdramidis,


Robertson, R.E., Carroll, T., Pearce, L.E., 2008. Bacillus spore inactivation differences after combined mild temperature and high pressure processing using two pressurizing fluids. J. Food Protect. 71, 1186–1192.


**Table 1** Weibull model parameters for 600 MPa HPP-thermal inactivation of psychrotrophic *Bacillus cereus* ICMP 12442 spores in beef slurry*

*The Weibull model exhibited low MSE values (0.020–0.036), high $R^2$ (0.983–0.990), and close to 1.00 (1.04–1.09); The parameters’ values were expressed as means±standard deviation (SD) and obtained from three experiments.*

| T (°C) | Weibull
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>$b$ ± SD</td>
</tr>
<tr>
<td>70</td>
<td>2.13±0.15</td>
</tr>
<tr>
<td>60</td>
<td>1.52±0.13</td>
</tr>
<tr>
<td>50</td>
<td>1.14±0.14</td>
</tr>
<tr>
<td>38</td>
<td>0.56±0.09</td>
</tr>
</tbody>
</table>

*b and $n$ are the Weibull scale and shape factors, respectively (Eq. 1); The Weibull model exhibited low MSE values (0.020–0.036), high $R^2$ (0.983–0.990), and close to 1.00 (1.04–1.09); The parameters’ values were expressed as means±standard deviation (SD) and obtained from three experiments.*
Table 2 First order kinetic and Weibull model parameters for thermal inactivation of psychrotrophic *Bacillus cereus* ICMP 12442 spores in beef slurry*

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>First order</th>
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<th>Weibull</th>
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<tbody>
<tr>
<td></td>
<td>$D_T$-value ± SD (min)</td>
<td>$z$-value ± SE (°C)</td>
<td>$b$ ± SD</td>
</tr>
<tr>
<td>90</td>
<td>0.98±0.02</td>
<td>11.9±0.01</td>
<td>1.03±0.04</td>
</tr>
<tr>
<td>80</td>
<td>6.93±0.06</td>
<td>$R^2 = 0.98$</td>
<td>0.14±0.003</td>
</tr>
<tr>
<td>70</td>
<td>46.03±0.80</td>
<td>0.02±0.001</td>
<td>1.00±0.01</td>
</tr>
</tbody>
</table>

*D$_T$- and $z$-values are the first order kinetic parameters (Eq. 2 and 3); $b$ and $n$ are the Weibull scale and shape factors, respectively (Eq. 1); The parameters’ values were expressed as means±standard deviation (SD) and obtained from two experiments. Both models worked well presenting low MSE values (0.0001–0.002), high $R^2$ (0.998–0.999), and close to 1.00 (1.01–1.03).*
Fig. 1 HPP of beef slurry: example of temperature and pressure history for a high pressure thermal process (600 MPa, 60°C).
Fig. 2 Comparison of 600 MPa HPP-thermal and thermal inactivation of psychrotrophic *B. cereus* ICMP 12442 spores in beef slurry at 70°C (data points are average ± standard deviation).
Fig. 3 Weibull model fitted to psychrotrophic *B. cereus* ICMP 12442 (=ATCC 9139, ATCC 21) log survivors after 600 MPa HPP alone (38°C) and HPP-thermal processing (50, 60, 70°C) (data points are average ± standard deviation).
**Fig. 4** HPP combined with thermal processing for the inactivation of *B. cereus* ICMP 12442 spores: The effect of temperature on the Weibull $b$ parameter.
Fig. 5 First order kinetics fitted to psychrotrophic *B. cereus* ICMP 12442 (=ATCC 9139, ATCC 21) log survivors after thermal processing (70, 80, 90°C) (data points are average ± standard deviation).