The Effect of Growth Stage and Growth Temperature on High Hydrostatic Pressure Inactivation of Some Psychrotrophic Bacteria in Milk

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ABSTRACT

The effect of high hydrostatic pressure on the survival of the psychrotrophic organisms Listeria monocytogenes, Bacillus cereus, and Pseudomonas fluorescens was investigated in ultrahigh-temperature milk. Variation in pressure resistance between two strains of each organism were studied. The effect of growth stage (exponential and stationary phase), growth temperature (8 and 30°C) on pressure resistance, and sublethal pressure injury were investigated. Exponential-phase cells were significantly less resistant to pressure than stationary-phase cells for all of the three species studied (P < 0.05). Growth temperature was found to have a significant effect at the two growth stages studied. Exponential cells grown at 8°C were more resistant than those grown at 30°C, but for stationary-phase cells the reverse was true. B. cereus stationary-phase cells grown at 30°C were the most pressure resistant studied. L. monocytogenes showed the most sublethal damage compared to B. cereus and P. fluorescens. B. cereus spores were more resistant to pressure than vegetative cells. Pressure treatment at 400 MPa for 25 min at 30°C gave a 0.45-log inactivation. Pressure treatment at 8°C induced significantly less spore germination than at 30°C. This study indicates the importance of the history of a bacterial culture prior to pressure treatment and that bacterial spores require more severe pressure treatments, probably in combination with other preservation techniques, to ensure inactivation.

The potential for high hydrostatic pressure to preserve foods was recognized by Hite in 1899 (11) when he demonstrated that high pressure treatment at ambient temperature could be used to preserve milk. Now, more than a century later, the use of high hydrostatic pressure as a food preservation technique has started to gather momentum throughout the world, as an alternative to traditional heat-based methods that are more detrimental to the quality of the food. Treatment with high pressure, unlike high temperature processing, has little effect on the fresh-like, natural attributes associated with sensory quality including texture, color, and flavor (14) and does not significantly reduce the vitamin content in foods (3).

The effectiveness of any food preservation technique may be evaluated by its ability to eradicate any pathogenic microorganisms present and so to enhance product safety and to inactivate spoilage microorganisms to improve the shelf life of the food. Research to date on high pressure preservation is somewhat sporadic, but it does appear that high pressure is effective in the inactivation of a number of pathogens (27). Variation in pressure resistance between bacterial strains has been reported widely (1, 27, 33), and bacterial spores, well known for their resistance to physical and chemical treatments, are also resistant to pressure (18, 30).

Other intrinsic and extrinsic factors have been reported to influence the pressure sensitivity of microorganisms. In general, exponential-phase bacterial cells are more pressure sensitive than those in stationary, dormant, or death phase (16). Information is very limited on the effect of growth temperature on pressure sensitivity, but Lanciotti et al. (17) reported that cells grown at lower temperatures tend to be more pressure resistant. The effect of the pressure processing temperature on resistance has been reported widely. Tashashi (38) reported that increased lethality to microbial cells was observed for pressure treatments carried out at −20°C when compared to +20°C. Mild heating has also been shown to be effective particularly when combined with high pressure for the inactivation of pressure-resistant pathogens such as Escherichia coli O157 (19) and Staphylococcus aureus (26). The nature of the substrate can also affect the response of microorganisms to pressure. Patterson et al. (27) reported that ultrahigh-temperature (UHT) skimmed milk offered Listeria monocytogenes and E. coli O157:H7 more protection than poultry meat. As a result of these interacting factors a lower than expected or unsatisfactory level of microbial inactivation may be observed when pressure processing certain foods.

Milk is a nutritious medium that presents a favorable environment for the multiplication of microorganisms (10) and supports a wide range of spoilage and pathogenic bacteria. Recently the use of high pressure as a preservation technique for raw milk has been investigated (28, 41). However, a significant range and number of microbial stud-
ies are still required before high pressure processing could be considered as a suitable alternative to pasteurization.

Psychrotrophic bacteria are problematic because they can survive and grow at refrigeration temperatures. This is of particular concern in the case of pathogens including \textit{L. monocytogenes} and psychrotrophic strains of \textit{Bacillus cereus}, whereby large numbers can accumulate during storage, ultimately resulting in foodborne illness. \textit{Pseudomonas} spp. are considered to be the most important spoilage organisms in milk \cite{5} and are responsible for the spoilage of various dairy products due to the production of heat-stable enzymes, mainly lipases and proteases.

This study investigated three psychrotrophic organisms \textit{L. monocytogenes}, \textit{B. cereus}, and \textit{P. fluorescens} that can be of concern in milk. The aims of this study were to determine: (i) the effect of growth temperature on pressure resistance; (ii) the effect of stage of growth on pressure resistance; (iii) variation in pressure resistance between different strains of the same organism; and (iv) survival and recovery (sublethal injury). The substrate used throughout this experiment was UHT skimmed milk.

\textbf{MATERIALS AND METHODS}

\textbf{Microorganisms.} Two strains of each microorganism, \textit{L. monocytogenes}, \textit{B. cereus}, and \textit{P. fluorescens}, were studied. The \textit{L. monocytogenes} strains used were NCTC 11994 (LM1) and the Scott A strain (LM2). The \textit{B. cereus} strains were NCFB 578 (BC1) and 1031 (BC2), obtained from the culture collection held in the Food Microbiology Unit of the Food Science Division. The \textit{P. fluorescens} strains studied were ANA11 (PF1) isolated from pressure-treated chicken in our own laboratory and NCDO 1524 (PF2). The plates were incubated at 30°C that served as stock cultures for subsequent experiments. The cultures were grown in tryptone soy broth (Oxoid CM129) supplemented with 0.6% yeast extract (Oxoid L21) (TSBYE) prior to inoculation into milk.

\textbf{Growth curves.} Growth curves were obtained for each organism to ascertain incubation times at 8 and 30°C. The methods used were as follows: for 8°C curves, TSBYE (100 ml) was inoculated using a 10^{-4} dilution of an overnight culture for LM1, LM2, PF1, and PF2 and using a 10^{-2} dilution of an overnight culture for BC1 and BC2. Each broth was incubated at 8°C and cells were enumerated every 4 h until stationary phase was reached. When stationary phase was achieved, the broth was sampled every 8 h until the beginning of death phase was observed. Aliquots (1 ml) were removed from the broth and enumerated by serial dilution using sterile maximum recovery diluent (Oxoid CM733) (MRD) and plating 0.1 ml (in duplicate) onto TSBYE in duplicate using the spread plate technique \cite{4}. The plates were incubated at 30°C for 24 h. For 30°C curves, TSBYE (100 ml) was inoculated using a 10^{-4} dilution of an overnight culture for all of the strains studied. The broth cultures were incubated at 30°C and sampled hourly for enumeration as described previously until stationary phase was achieved. The broths were then sampled for enumeration every 4 h. Sampling of the broth cultures continued until the beginning of the death phase. Each growth curve was prepared in duplicate.

\textbf{Preparation of inocula.} For vegetative cells, mid-exponential- and mid-stationary-phase cultures of LM1, LM2, BC1, BC2, PF1, and PF2 were prepared as described above for the growth curves. Results from the growth curves were used to determine each growth temperature/growth stage combination. These are given in Table 1.

The cells were harvested by centrifugation (Centaur 2 MSE, Fisons, England) of 20 ml of stationary-phase cells and 50 ml of exponential-phase cells at 2,400 × g for 20 min. Pellets were washed once in phosphate-buffered saline.

For spores, the method used was modified from Jacquette and Beuchat \cite{14}. Nutrient agar (Oxoid CM3) supplemented with 50 mg liter^{-1} manganese sulfate was used as the sporulation medium. Vegetative cell cultures of BC1 and BC2 were grown in TSBYE at 30°C overnight and 0.1 ml was surface spread on each plate of nutrient agar + 50 mg liter^{-1} MnSO_{4}. The plates were incubated at 30°C for 72 to 76 h, resulting in >85% cell sporulation. Sporulation was observed microscopically following the Schaeffer Fulton modification of the Wirtz method \cite{31}.

To harvest the spores, 5 ml of sterile distilled water was placed on each agar plate and rubbed gently with a glass spreader. Suspensions of cells and spores were filtered through sterile glass wool and collected in 50 ml centrifuge tubes (Corning International, Corning, N.Y.). This process was repeated, pooled suspensions centrifuged at 2,600 × g at 5°C for 20 min and the supernatant discarded. The resulting pellet was resuspended in 50 ml of sterile distilled water and centrifuged at 2,600 × g at 5°C for 10 min. This procedure was repeated a further two times. The final pellet was resuspended in sterile distilled water and stored at 3°C until used as inocula in subsequent experiments.

The numbers of spores present in the stock solutions of BC1 and BC2 were determined by serial dilution (in MRD) of the heat-shocked spores. Heat shocking was carried out by placing 3 ml of spores in plastic 13.5-ml tubes (Bibby Sterilin Ltd., Stone, Staffordshire, England) and heat shocking at 80°C for 10 min (allowing a 5 min come-up time). The appropriate dilutions were spread plated onto TSBYE and the plates were incubated at 30°C for 24 h. Plating of these spore suspensions before and after heat treatment did not result in significantly different counts, thus indicating that the spore suspensions consisted exclusively of spores.

The spore mixture was held on ice throughout all inoculation procedures.

\textbf{Inoculation of milk.} UHT-treated skimmed milk was purchased from a local supermarket and stored at 4°C until required.

For vegetative cells, UHT skimmed milk was inoculated with washed cell pellets for each strain of organism and for each growth temperature or stage of growth combination to give an approximate cell concentration as summarized in Table 2. The cell suspensions were thoroughly mixed to give a final volume of 20 ml for each sample. The sample was dispensed in 2.5-ml aliquots.
into sterile polyethylene/polyamide pouches (Somerville Packaging, Lisburn, Northern Ireland) measuring 7 by 7 cm and sealed using a heat sealer (R.S. Components, Corby, Northants, England), taking care to exclude as much air as possible. The remainder was retained for enumeration as a control sample. Each pouch was placed in a larger pouch (7 by 14 cm) and heat sealed. The pouches were grouped by treatment and placed in a second bag and heat sealed excluding as much air as possible.

For spores, UHT milk was inoculated to a final concentration of approximately 10^7 spores ml^-1 using the stock spore solutions of BC1 and BC2. Aliquots of 4.1 ml were dispensed and packaged as outlined for the vegetative cells. The remainder was retained for heat shock treatment (80°C for 10 min) as previously described, prior to enumeration.

**High pressure treatment.** Samples were pressure treated in a Stansted Foodlab 9000 high pressure isostat capable of operation at 900 MPa (Stansted Fluid Power Ltd., Stansted, UK). The Stansted Foodlab 9000 had a high pressure cylinder with internal bore diameter 70 mm and length 203 mm. Samples were submerged in 15% castor oil:85% ethanol. The temperature inside the cylinder was monitored by a thermocouple that was immersed in the pressurization fluid during treatment. The pressure come-up time was approximately 200 MPa/min, and the pressure release time was fixed at 2 min. The temperature increase due to the adiabatic heating effect during pressure application was approximately 3°C per 100 MPa.

For vegetative cells, UHT skimmed milk inoculated with *L. monocytogenes* strains LM1 and LM2 and *B. cereus* strains BC1 and BC2 were processed using 400 MPa and *P. fluorescens* strains PS1 and PS2 using 250 MPa because *Pseudomonas* spp. are intrinsically more pressure sensitive than either *L. monocytogenes* or *B. cereus*. BC1 and BC2 showed a 100-fold difference in sensitivity to pressure compared to PS1 and PS2. This difference was observed after 10 min of pressure processing at 400 MPa and 250 MPa, respectively.

For spores, BC1 and BC2 were exposed to 400 MPa at 8°C and cells grown at 30°C for processing times of 0 to 30 min in 5-min intervals. After treatment, pouches were immediately placed on ice to prevent further germination and analyzed within 15 min of treatment. In each case an untreated sample acted as a control and was used to provide an estimate of the initial numbers of viable microorganisms present in each treated sample prior to pressurization. The initial number of spores present in the control was estimated following heat shock treatment. For all samples, pressure treatment took place within 4 h of inoculation.

**Enumeration.** For vegetative cells, after treatment, serial dilutions were prepared in sterile MRD, and surviving cells were enumerated in duplicate by plating 0.1-ml volumes on TSAYE and in the appropriate selective agar: *Listeria* selective agar Oxford formulation (OX) (*Listeria* selective agar base [Oxoid CM856] + Oxford selective supplement [Oxoid SR140]) for *L. monocytogenes*, *Bacillus cereus* selective agar (BCS) (*Bacillus cereus* agar base [Oxoid CM617] + *Bacillus cereus* selective supplement [Oxoid SR99] + egg yolk emulsion [Oxoid SR47]) for *B. cereus*; and *Pseudomonas* selective agar CFC formulation (PSA) (*Pseudomonas* selective agar base [Oxoid CM559] + *Pseudomonas* selective supplement CFC formulation [Oxoid SR103]) for *P. fluorescens*. The plates were incubated for 48 h at 30°C, and the number of surviving organisms enumerated as CFU. The use of this differential plating technique, consisting of TSAYE and the appropriate selective agar, enabled injury to be monitored. Both injured and noninjured cells are able to form colonies on TSAYE, whereas only noninjured cells form colonies on selective agars. The entire procedure was repeated on three separate occasions. Analysis of variance was carried out on the data obtained.

For spores, after pressure treatment serial dilutions were prepared in MRD, and the total counts (spores and germinated cells) were determined by direct plating on TSAYE. The number of spores surviving each treatment was determined by heating 3-ml aliquots at 80°C for 10 min to inactivate any germinated spores and then spread plating on TSAYE. All plates were incubated at 30°C for 24 h. Direct comparison of the two plate counts was used as an indicator of pressure-induced germination. The percentage germination rate was calculated using the formula (1 − residual spores/original spores) × 100 (40). Analysis of variance was carried out on the data obtained for pressure-induced germination.

**RESULTS**

**Pressure sensitivity of vegetative organisms.** The results for *L. monocytogenes* are shown in Figure 1a through 1d, *B. cereus* in Figure 2a through 2d, and *P. fluorescens* in Figure 3a through 3d. Time 0 treatments for all three organisms are displayed as time 0.1 min to enable the effect of pressure come-up to be seen clearly on the graphs. For all of the organisms and strains studied, exponential-phase cells were significantly more sensitive to pressure than stationary-phase cells (*P < 0.05*). BC1 and BC2 showed a

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**TABLE 2.** Approximate final cell concentration (log CFU ml^-1) in UHT milk for each growth temperature/stage of growth combination for strains of *L. monocytogenes* (LM), *B. cereus* (BC), and *P. fluorescens* (PF)

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Exponential</th>
<th>Stationary</th>
<th>Exponential</th>
<th>Stationary</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM1</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>LM2</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>BC1</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>BC2</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>PF1</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>PF2</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

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**TABLE 3.** Pressure processing times: growth temperature/stage of growth combinations for *L. monocytogenes*, *B. cereus*, and *P. fluorescens*

<table>
<thead>
<tr>
<th></th>
<th>Exponential phase (min)</th>
<th>Stationary phase (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8°C</td>
<td>30°C</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>0–10</td>
<td>0–10</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>0–5</td>
<td>0–5</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>0–5</td>
<td>0–10</td>
</tr>
</tbody>
</table>

a Time intervals used for sampling and enumeration.
FIGURE 1. Survivor curves for L. monocytogenes (LM1 and LM2) following pressure treatment (400 MPa) at 8 and 30°C. Enumeration on TSAYE and on OX: (a) LM1 exponential-phase cells; (b) LM2 exponential-phase cells enumerated; (c) LM1 stationary-phase cells; (d) LM2 stationary-phase cells enumerated on OX. TSAYE 8°C ( ), OX 8°C ( ); TSAYE 30°C ( ); OX 30°C ( ). N₀, initial number; N, number of survivors; LSD, least significant difference (5% level).

6.04-log and 5.25-log reduction when exposed to 400 MPa followed by immediate decompression (0 min pressurization). Exponential cells grown at 8°C were significantly (P < 0.05) more resistant to pressure than exponential cells grown at 30°C for both strains of L. monocytogenes and B. cereus, and for only strain 1 of P. fluorescens.

Stationary-phase cells grown at 30°C were found to be the most pressure-resistant cells in this study. In the case of B. cereus these cells were significantly more resistant to pressure than stationary-phase cells grown at 8°C (P < 0.05); however, this was not so for L. monocytogenes or P. fluorescens. Throughout the entire experiment, for all of the organisms studied, B. cereus stationary-phase cells grown at 30°C were found to be the most pressure-resistant vegetative cells with 3.44-log and 2.90-log reductions observed for BC1 and BC2, respectively, following a pressure treatment of 400 MPa at 30°C for 18 min.

Pressure resistance was variable between the strains studied. Significant differences (P < 0.05) were found between the two strains for each of the three organisms studied.

The extent of pressure injury, indicated by the difference in the counts obtained on nonselective (TSAYE) and the appropriate selective agar (OX, BCS, or PSA) varied between the different organisms studied and the stage of growth. Sublethal injury was most marked in L. monocytogenes stationary-phase cells, where there was an 8-log inactivation for LM2 on OX after 24 min pressurization, compared to a 4-log inactivation on TSAYE. B. cereus and P. fluorescens strains showed a lower degree of pressure injury with a 0.5- to 1.0-log difference observed between the counts on the nonselective and selective agars.

Pressure inactivation of spores. The results for spore inactivation using a pressure treatment of 400 MPa at 8°C and 30°C for BC1 and BC2 are shown in Figure 4a and 4b. Pressure treatment at 8°C did not inactivate the spores of either BC1 or BC2, but instead appeared to increase...
counts by about 0.5 log after 30 min. Spore numbers for BC2 were reduced significantly ($P < 0.05$) by a 0.45-log reduction following a 25-min treatment time, which was the greatest reduction observed.

**Pressure germination of spores.** The results obtained for this experiment are shown in Figure 5. Pressure-induced germination of spores at 8°C was significantly less than that observed following pressurization at 30°C ($P < 0.001$). Pressure treatment of 400 MPa for 30 min at 8°C induced 13.4 and 8.3% germination for BC1 and BC2, respectively, compared to 75.8 and 18.7% observed at 30°C for 30 min. BC1 spores showed 38.2% germination when exposed to 400 MPa at 30°C followed by immediate decompression (0 min pressurization), whereas BC2 spores showed less germination (9.6%). When pressure treatment was carried out at 8°C (0 min pressurization), spore germination was reduced and values of 4.3 and 2.1% for BC1 and BC2 were observed.

**DISCUSSION**

Milk was one of the first foods to be treated with high pressure (11). Since then, several authors have reported the effects of high pressure on the natural microflora present in milk (24, 28, 39, 41). The effect of pressure on microorganisms inoculated into milk as investigated here has also been widely reported (7–9, 27, 33, 37).

It is clear from the results obtained in this study that the stage of growth of bacterial cells greatly affects inactivation by pressure treatment. Pressure inactivation of vegetative cells showed mainly resistant tails, whereas straight-line inactivation was observed for *B. cereus* exponential-phase cells grown at 30°C. The resistant tails observed for the organisms studied in this experiment were previously described by other authors (21), who reported that the resistant tail populations of *Salmonella Typhimurium* and *Salmonella Senftenberg* were not significantly different in pressure resistance from the original culture. It has been
suggested that this survival pattern reflects the heterogeneity of the cell population (6). Our results also show pressure variability between strains that is in agreement with the work reported by Patterson et al. (27) for a variety of pathogens, Benito et al. (1) for E. coli O157, and Simpson and Gilmour (33) in relation to L. monocytogenes.

Studies involving different stages of growth to date are very limited, but all agree that stationary-phase cells are more resistant to pressure than exponential-phase cells for both gram-negative and gram-positive organisms (2, 13, 16, 20, 35). This is in agreement with the findings of this study.

Pressure resistance of microorganisms has been shown to be affected by growth temperature. Lanciotti et al. (17) reported that both L. monocytogenes and E. coli were found to be more pressure resistant when grown at lower temperature. Smelt et al. (34) reported that Lactobacillus plantarum in exponential phase was more resistant to pressure when grown at suboptimal temperature. Casadei and Mackey (2) reported that cells in stationary phase became more resistant to pressure as growth temperature increased and that exponential cells became less resistant to pressure as growth temperature increased. The results obtained for L. monocytogenes, B. cereus, and P. fluorescens for both exponential- and stationary-phase cells are similar to previous observations (2, 38). Cell membrane fatty acid composition has been reported to affect heat resistance in E. coli (15, 42). This is also thought to be so for high pressure-treated bacterial cells; however, a recent study by Benito et al. (1) involving E. coli O157 reported that there was no obvious relationship between pressure resistance of some strains and their membrane composition.

Pressure treatment is known to cause microbial sublethal injury. The counts obtained for LM1 and LM2 stationary-phase cells on OX decreased much more rapidly and to a greater extent than counts on nonselective TSAYE. The observed divergence of the counts indicated that the surviving population of cells had suffered injury throughout the range of treatment times. This was also reported by

FIGURE 3. Survivor curves for P. fluorescens (PF1 and PF2) following pressure treatment (250 MPa) at 8 and 30°C. Enumeration on TSAYE and on PSA: (a) PF1 exponential-phase cells; (b) PF2 exponential-phase cells; (c) PF1 stationary-phase cells; (d) PF2 stationary-phase cells. TSAYE 8°C ( ); PSA 8°C ( ); TSAYE 30°C ( ); PSA 30°C ( ). N₀, initial number; N, number of survivors; LSD, least significant difference (5% level).

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Simpson and Gilmour in a variety of substrates including UHT milk and raw and cooked chicken mince (33). The differences between counts on selective and nonselective agar for *P. fluorescens* and *B. cereus* were much lower. The larger difference in counts for the *L. monocytogenes* strains may be due to the stringent, five different antibiotic cocktail used in the preparation of OX, when compared to the single antibiotic used for BCS and the three antibiotics present in PSA, or *L. monocytogenes* could be intrinsically more susceptible to sublethal injury. The reduced sublethal damage observed for *P. fluorescens* may be due to the use of a less severe pressurization process (250 MPa), although this species is more susceptible to lower pressures. Microorganisms are believed to be resistant to selective chemical inhibitors including antibiotics due to their ability to exclude such agents from the cell, mainly by the action of the cell membrane. However, if the membrane becomes damaged, this tolerance is lost (29). The cell membrane is considered to be a primary site for the inactivation or injury of bacterial cells by high pressure (12). The difference between the counts on selective and nonselective agars would appear to support this.

Sublethal injury is an important consideration for any preservation method. Given favorable conditions such as prolonged storage in a suitable substrate, sublethally injured cells may be able to repair. This recovery potential is problematic but has particular significance for psychrotrophic foodborne pathogens that can survive and grow at refrigeration temperatures.

The mode of bacterial spore inactivation by pressure is open to speculation. However, it is now generally accepted that pressures up to 300 MPa induce germination and cause death to the germinated spore. Pressurization temperature is known to be one of the most important factors affecting pressure sensitivity of bacterial spores and can enhance the lethal effect (22, 23, 30). Generally, initiation of germination increases with increased temperature as observed in this study. The observed spore germination in this study was variable, as BC1 and BC2 showed 75.8 and 18.7% germination, respectively, following a 30-min pressure treatment of 400 MPa at 30°C. This may be because BC2 is known to have slow-germinating spores. Spore germination of *Clostridium sporogenes* was reported by Mills et al. (22) who observed 55.9% germination following a pressure treatment of 400 MPa at 40°C for 30 min. However Nakayama et al. (25) compared the pressure and heat resistance of six *Bacillus* strains and reported that *Bacillus stearothermophilus*, a very heat-resistant strain, was the most pressure sensitive, whereas a heat-sensitive strain of *Bacillus megaterium* was not inactivated following a treatment of 1,000 MPa for 400 min. The temperatures applied in the current study were used to compare the inactivation of spores with vegetative cells. From the data obtained, a small increase in CFU ml⁻¹ was observed for both strains of *B. cereus* at 8°C. The observed increase in numbers following pressurization at 30°C resulted in a very slight inactivation of <0.5-log reduction after 30 min pressurization compared to a >2-log reduction of 30°C stationary-phase cells. Mills et al. (22) also reported a <0.5-log reduction of *Cl. sporogenes* spores following a
pressure treatment of 400 MPa at 40°C for 30 min. Therefore, from our results and reports from many other authors, pressure inactivation alone is highly inefficient for the inactivation of bacterial spores. Techniques such as oscillatory pressure cycling have been reported as significantly more efficient for the inactivation of bacterial spores. Sojka and Ludwig (36) reported a >8-log inactivation of B. subtilis spores using this method.

Before any food product can be produced commercially using high pressure processing, optimization of processing conditions is essential to ensure product safety. As demonstrated by this study, many intrinsic and extrinsic factors affect pressure resistance of microorganisms. This therefore presents an interesting but very real challenge to the food industry.

ACKNOWLEDGMENT

The authors thank Dr. Christine Best for excellent technical assistance.

REFERENCES


