

# EFFECT OF HIGH HYDROSTATIC PRESSURE ON SPORES OF *GEOBACILLUS STEAROTHERMOPHILUS* SUSPENDED IN SOYMILK

YOKIUSHIRDHILGILMARA ESTRADA-GIRÓN<sup>1</sup>,  
JOSÉ A. GUERRERO-BELTRÁN<sup>3</sup>, BARRY G. SWANSON<sup>2</sup>  
and GUSTAVO V. BARBOSA-CÁNOVAS<sup>1,4</sup>

<sup>1</sup>*Biological Systems Engineering Department  
L.J. Smith Hall Room 213  
Washington State University  
Pullman, WA 99164-6120*

<sup>2</sup>*Food Science and Human Nutrition Department  
Washington State University  
Pullman, WA*

<sup>3</sup>*Depto. Ing. Química y Alimentos  
Universidad de las Américas, Puebla  
Sta. Catarina, Cholula, Puebla, México*

Accepted for Publication April 9, 2007

## ABSTRACT

*The inactivation of Geobacillus stearothermophilus spores (ATCC 7953) inoculated in soymilk was investigated using high hydrostatic pressure (550, 585 and 620 MPa) in combination with temperature (70, 80 and 90C) for selected times (2 s to 15 min). Inactivation of spores occurred at all selected treatments. Less than 10 CFU/mL of G. stearothermophilus were observed after 7 min of treatment at 620 MPa and 90C. An increase in the inactivation rate constant, at the highest pressure, was observed, resulting in a decrease in D values at all temperatures. D values were calculated as 10.6, 6.2 and 3.5 min for 70, 80 and 90C, respectively after pressurization at 620 MPa. z<sub>p</sub> values decreased as temperature increased with values ranging from 142 to 238 MPa. The activation energy required for inactivation of G. stearothermophilus spores in soymilk, at the selected treatments, was in the range of 37.9–57.4 kJ/mol.*

## PRACTICAL APPLICATIONS

In addition of being a healthy source of proteins, soymilk can be treated at high pressure as a pasteurization process instead of thermal treatment.

<sup>4</sup> Corresponding author. TEL: 509-335-6188; FAX: 509-335-2772; EMAIL: barbosa@mail.wsu.edu

Thermal treatment can change flavor and destroy nutrients of liquid food products. High pressurize processing machines, static and dynamics, are being used to study enzymic and microbial inactivation. Therefore, the use of these machines to pasteurize liquid food products can improve the nutritional content of soymilk. The combination of both pressure and temperature can reduce costs of pasteurization to deliver soymilk with good sensory and nutritional characteristics.

## INTRODUCTION

Thermal treatment is commonly used by the food industry for inactivation of pathogenic and foodborne microorganisms in food products. Using thermal treatment alone, high temperatures are necessary to reduce or completely destroy microbial populations in foods. However, processing of foods at high temperatures, or sterilization conditions, can result in products that are overheated and reduced in nutritional and sensorial quality.

The present tendency toward the application of emerging technologies has generated a number of alternatives other than thermal processing to obtain high-quality foods. In the last decade, much research has been done evolving the use of high hydrostatic pressure (HHP) as an alternative process alone or in combination with thermal treatment. Both alternatives can reduce the processing time of foods while minimizing damage of nutritional components and, at the same time, reducing microbial populations.

In designing a process capable of inactivating microorganisms, it is important to avoid product degradation in both nutritional and sensorial quality by selecting the best possible conditions. In the case of thermophilic spore-forming bacteria, which are extremely heat resistant, heat treatment is usually applied at a high temperature for long times to obtain a complete inactivation of microorganisms. However, HHP combined with heat can significantly reduce temperature intensity or treatment time to totally inactivate or significantly reduce spore counts in foods. Selecting the appropriate intensity of HHP is important to achieve the objectives regarding the production of safe and high-quality foods. Pressures below 100 MPa can induce growing of vegetative cells, while HHP ranging from 300 to 400 MPa can ensure the inactivation of spores (Russell 1982).

Combination of HHP and temperature to inactivate *Geobacillus stearothermophilus* spores suspended in buffer solution or food systems has been reported elsewhere. Hayakawa *et al.* (1994) reported a reduction of the initial number of spores, after four to six compression–decompression cycles, at 600 MPa for 5 min in buffer solutions. Seyderhelm and Knorr (1992) pointed out that the initial counts ( $3 \times 10^6$  spores/mL) of *G. stearothermophilus* were reduced to less than 10 CFU/mL after treatment at 200 MPa and 90C,

350 MPa and 80C, or 400 MPa and 70C. The authors reported that the effects on spores inactivation were enhanced at pressures between 200 and 400 MPa in combination with temperatures between 60 and 90C. Kakugawa *et al.* (1996) reported that viable spore counts can be reduced from  $10^6$  to less than 100 CFU/mL using combinations of 200 MPa/110C for 30 min or 400 MPa/100C for 10 min. Furthermore, Ananta *et al.* (2001) inactivated *G. stearothermophilus* spores suspended in mashed broccoli and cocoa mass treated at HHP in the range of 50–600 MPa in combination with temperatures ranging from 60 to 120C. The authors also reported that spores inactivation was improved when increasing treatment intensity.

Recently, the food industry has focused more on the use of high pressure for food sterilization. Therefore, to determine the mechanism of inactivation of *G. stearothermophilus* spores under pressure, research is being conducted using rapid decompression methods after pressurization. The theory behind these methods is that rapid decompression increases the impact force on the spore coat. Also, the impact force upon rapid decompression is stronger than the force caused by pressurization alone. Therefore, the stress generated at some points will exceed the breaking threshold limit of the spore coat, and this will cause inactivation. Under decompression methods such as the link motion system, Hayakawa *et al.* (1998) reported 4-log reduction of spores (CFU/mL) suspended in buffer after 60 min of treatment at 200 MPa and 75C, whereas using the nonrotational rod valve and the E.G. seal methods at same levels of pressure and temperature, authors found 3-log reduction in spore counts.

The effects of the HHP-temperature treatment on microbial reduction are influenced by the medium composition in which spores are suspended. Consequently, some food constituents may protect microorganisms during exposure to high pressure and/or temperature treatments (Ogawa *et al.* 1992; Oxen and Knorr 1993). As suggested by Palou *et al.* (1998), it is important to take into account the composition of each particular food system to evaluate the effects of pressure treatment on microbial inactivation. The objective of this research is to assess the inactivation of *G. stearothermophilus* spores inoculated in soymilk using high pressure combined with temperature for different holding times (0–15 min) to determine the microbial inactivation rates.

## MATERIALS AND METHODS

### Spores Activation

Lyophilized pellets of *G. stearothermophilus* (ATCC 7953) were rehydrated adding 0.5 mL of nutrient broth (Difco, Becton Dickinson, Sparks, MD) and mixed carefully. The slurry was added with 5.5 mL of nutrient broth and mixed thoroughly. One hundred microliters of the spores suspension were

transferred to a second tube containing 6 mL of nutrient broth and then homogenized. Tubes were incubated at 55C for 24 h.

### **Spores Propagation**

After incubation, 0.1 mL of the spores suspension was spread on the surface of nutrient agar (Difco, Becton Dickinson) contained in petri dishes. All petri dishes were incubated at 55C for 5 days. After the germination and sporulation process, spores were harvested as follows. The surfaces of petri dishes were flooded with sterile distilled water and were washed twice. The spore suspension was placed in centrifuge tubes and centrifuged at  $5,000 \times g$  for 20 min. The supernatant was discarded and spores were suspended in NaCl solution (0.85%) to be centrifuged again. Spores were suspended in sterile peptone water, placed into vials and stored at a low temperature (4C) until use.

### **Soymilk Preparation**

Raw soybeans were weighed and soaked in distilled water for 18 h at room temperature according to the Rouhana *et al.* (1996) procedure. After the soaking period, soybeans were washed and grounded (4 min) using a commercial laboratory blender model 31 BL91 (Waring, Torrington, CT). The slurry was filtered through a double layer of cheesecloth. The raw soymilk obtained in this manner had 7.8% of solids and pH 6.5. Soymilk was sterilized at 121C for 15 min and brought to room temperature before spores inoculation.

### **Pressure Treatments**

Fifty milliliters of inoculated soymilk ( $10^6$  CFU/mL) was placed in sterile  $7.6 \times 15.2$  cm plastic pouches (Bags Whirl Pak ~110 mL), sealed and held in ice water (1–2C) until HHP treatment. Plastic pouches containing soymilk were inserted into an outer polyethylene bag ( $16.3 \times 17.8$  cm), filled with water and heat-sealed with a TISH-300 type sealer (E-Z Audit Bankpak Inc., Baltimore, MD) prior to HHP treatment. Soymilk was treated at three pressures (550, 585 and 620 MPa) combined with three temperatures (70, 80 and 90C) at selected holding times (2 s to 15 min) using the isostatic pressing system (Engineering Pressure System Inc., Haverhill, MA) machine. A 5% Houghton Hydrolubic 123B soluble oil/water solution (Houghton International, Valley Forge, PA) was used as the pressure-transmitting medium. The heating system to heat the HHP-chamber up was turned on the night before pressurization. After pressurization, samples were held in ice water (1–2C) before estimating of survivors. Each test was performed in triplicate. The come-up time (CUT: time necessary to reach the working pressure) was measured using a chronometer.

## Microbial Count

The number of survivors was estimated using the plate count method with Tryptic soy agar (Difco, Becton Dickinson). Petri dishes were incubated at 55C for 48 h before counting. Petri dishes were cultured in duplicate for each serial dilution to estimate the number of survivors.

## First Order Kinetic Model

The inactivation of *G. stearothermophilus* spores was evaluated as a first order kinetic model:

$$\ln\left(\frac{N_t}{N_o}\right) = -kt$$

where  $N_t$  is the number of survivors at any time (CFU/mL),  $N_o$  is the initial microbial count (CFU/mL),  $k$  is the inactivation rate constant (1/min), and  $t$  is time (min). The decimal reduction time,  $D$  (min) indicates the time required to reduce the number of spores by 90%. The decimal reduction time ( $D$ ) can be obtained from the slope ( $D = -2.303/k$ ). The  $z_p$  (MPa) value is defined as the pressure needed to reduce or increase the  $D$  value by a factor of 10, and it is the reciprocal of the slope when plotting  $\log D$  versus pressure. The activation energy ( $E_a$ ) can be obtained using the Arrhenius model:

$$\ln k = \frac{E_a}{R} \frac{1}{T}$$

where  $T$  is absolute temperature (K) and  $R$  is the gas universal constant (8.314 J/mol K). The  $E_a$  (kJ/mol) can be computed from the slope when plotting  $\ln k$  versus  $1/T$ .

## Statistical Analysis

Data analysis was carried out using a Microsoft Excel program, and analysis of variance and least significant difference was performed with the SAS System (SAS Institute Inc. 1999).  $P < 0.05$  was selected as the decision for significant differences.

# RESULTS AND DISCUSSION

## Kinetics

Survival curves of *G. stearothermophilus* spores, suspended in soymilk, subjected at selected pressures, temperature and holding time are illustrated in

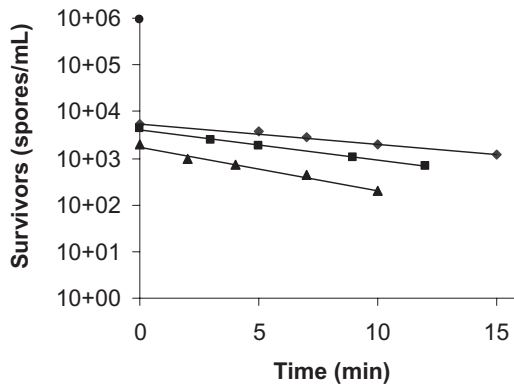


FIG. 1. INACTIVATION OF *G. STEAROTHERMOPHILUS* IN SOYMILK AT 70°C AND 550 (◆), 585 (■), AND 620 (▲) MPa. (●) INITIAL SPORES LOAD

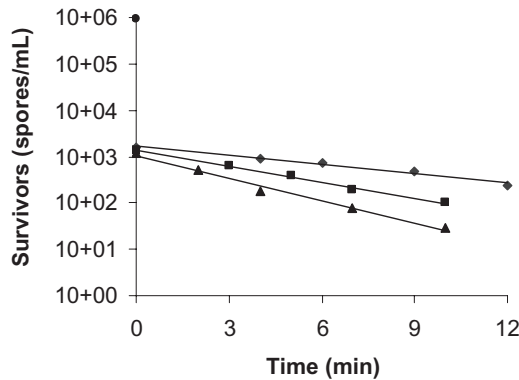


FIG. 2. INACTIVATION OF *G. STEAROTHERMOPHILUS* IN SOYMILK AT 80°C AND 550 (◆), 585 (■), AND 620 (▲) MPa. (●) INITIAL SPORES LOAD

Figs. 1–3 for 70, 80 and 90°C, respectively. The CUT to reach the working pressure were  $3.28 \pm 0.03$ ,  $3.45 \pm 0.05$  and  $3.80 \pm 0.31$  min for 550, 585 and 620 MPa, respectively. It was observed at three temperatures that survival fraction decreased linearly with time. Therefore, modeling was done using the first order kinetic model ( $R^2 > 0.970$ ) (Table 1) at the working pressures and temperatures. Spores were more rapidly inactivated as pressure, temperature and holding time increased. Initial spore loads were taken as the spore counts for 0 min of holding time (2 s) for the pressure–temperature combination. The CUT to achieve the desired pressure had a significant effect ( $P \leq 0.05$ ) on the

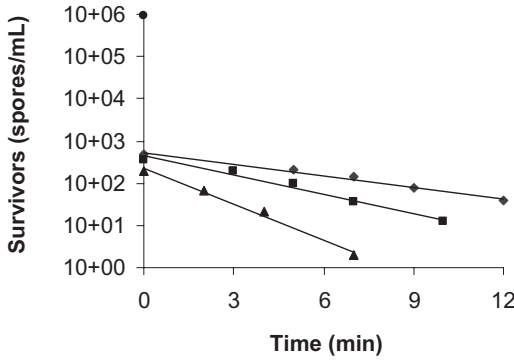


FIG. 3. INACTIVATION OF *G. STEAROTHERMOPHILUS* IN SOYMILK AT 90C AND 550 (◆), 585 (■), AND 620 (▲) MPa. (●) INITIAL SPORES LOAD

TABLE 1.  
D AND  $z_p$  VALUES FOR PRESSURE INACTIVATION OF *G. STEAROTHERMOPHILUS* AT SELECTED TEMPERATURES

Temperature (C)	D <sub>620 MPa</sub> (min)	R <sup>2</sup>	$z_p$ (MPa)	R <sup>2</sup>
70	10.6	0.98	238.0	0.99
80	6.2	0.98	178.0	0.97
90	3.5	0.97	142.0	0.98

TABLE 2.  
EFFECT OF THE COME UP TIME FOR THE INACTIVATION OF *G. STEAROTHERMOPHILUS* AT SELECTED PRESSURES AND TIMES

Pressure (MPa)	Log (CFU/mL)* Temperature (C)		
	70	80	90
550	3.72 <sup>a</sup>	3.19 <sup>a</sup>	2.69 <sup>b</sup>
585	3.63 <sup>a</sup>	3.14 <sup>a</sup>	2.55 <sup>b</sup>
620	3.30 <sup>a</sup>	3.08 <sup>a</sup>	2.29 <sup>b</sup>

\* Initial microbial load: 6<sub>a</sub> log cycles (CFU/mL).  
a,b Different sub index letters indicate differences among treatments (temperatures).

inactivation of *G. stearothermophilus* (Table 2) as well as combination of pressures and 90C. The highest spores reduction from the initial microbial counts (10<sup>6</sup> CFU/mL) was observed at 620 MPa and 90C, decreasing 3.71 log cycles.

*G. stearothermophilus* spores have been evaluated by several authors as one of the extremely pressure-resistant microorganisms (Gould and Sale 1970; Sale *et al.* 1970). Comparing the pressure resistance of *G. stearothermophilus* with other bacilli, the pressure-resistance is as follows: *G. stearothermophilus* > *B. coagulans* > *B. subtilis* > *B. cereus* > *B. polymyxa* > *B. megaterium* (Lechowich 1993; Ananta *et al.* 2001).

According to Sale *et al.* (1970), the resistance of spores to HHP suggests that spore proteins are protected against solvation and ionization. In addition, the structure and thickness of the bacterial spore coats also contribute to this high resistance to pressure. Therefore, spores of *G. stearothermophilus* can be injured with pressure and temperature as well as the medium in which the spores are suspended.

On the other hand, little reduction in spore counts was observed at 70C at the working pressures (Fig. 1). Based on the initial number of spores after treatment for 2 s (3.72 log cycles), less than 1 log cycle reduction was observed at 550 MPa after 15 min of treatment. One log cycle reduction of spores was observed after 10 min of treatment at 620 MPa. Therefore, increasing pressure from 550 to 620 MPa did not result in significant spores reduction. This may suggest that the combination of pressure and heat for longer times (above 60 min) is sometimes necessary to increase spore inactivation. Therefore, high costs in food processing could be worthless unless the food product be valuable to be processed by this approach and improve its shelf-life characteristics.

A reduction of 1.13 log cycles was observed at 585 MPa and 80C after 10 min of treatment (Fig. 2). Increasing pressure to 620 MPa at the same temperature and holding time resulted in an inactivation of about 1.7 log cycles. The inactivation of *G. stearothermophilus* spores was improved at 80C at selected combinations of pressure–temperature. However, these results indicate that higher levels of pressure and heat are required to increase spores inactivation. Nevertheless, substantial reduction of spores was accomplished using this pressure–temperature combination.

The inactivation of *G. stearothermophilus* spores suspended in soymilk treated at 90C and selected pressures was more effective (Fig. 3) in comparison with treatments at lower temperatures (Figs. 1 and 2). Spore counts of the inoculated soymilk treated at 90C and 620 MPa during 4 min were reduced to less than 10 CFU/mL. After 7 min of treatment, less than 10 CFU/mL was observed. Therefore, this pressure–temperature combination significantly improved spores inactivation, as the inactivation rate values obtained at 70 and 80C were smaller than those obtained at 90C when working at 620 MPa. As mentioned by some authors (Moerman *et al.* 2001), working at high pressure to inactivate spores is an opportunity to decrease thermal treatment. Therefore, an equal or similar reduction of spores in foods could be accomplished using

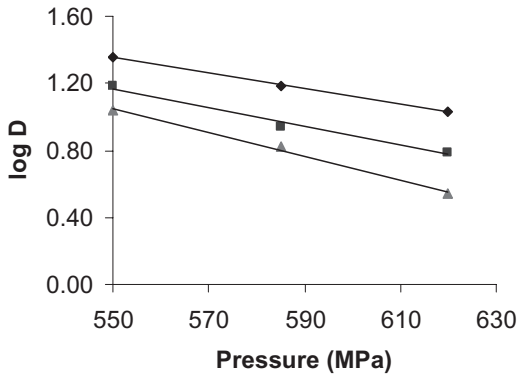


FIG. 4. PRESSURE DEPENDENCE OF  $D$  VALUES FOR INACTIVATION OF *G. STEAROTHERMOPHILUS* AT 70 (◆), 80 (■), AND 90C (▲)

temperatures below 100C and high pressure. Soymilk is a high-moisture content food; therefore, it cannot be discarded the possibility that spores inactivation could have been enhanced because of the high moisture content of soymilk as well.

The inactivation of *G. stearothermophilus* suspended in soymilk was described by a first order kinetics model ( $R^2 > 0.97$ ). Ardia (2004) observed a linear behavior of the inactivation rate constants of *G. stearothermophilus* spores suspended in buffer solution treated in the range from 100 to 600 MPa.

$D$  values obtained at the working conditions are illustrated in Fig. 4 and listed in Table 1 (at 620 MPa).  $D$  values for inactivation of vegetative cells and spores depend not only on the treatment conditions, but also on the type of medium in which the microorganism is suspended as well as type of species and strains.  $D$  values for *G. stearothermophilus* spores suspended in soymilk decreased as pressure increased at constant temperature and  $D$  values decreased as temperature increased at constant pressure (Fig. 4). Therefore, using high pressure and temperature could improve the inactivation of *G. stearothermophilus* in food systems for short treatment times. For instance, 3.51 min are required to inactivate 90% (1 log cycle reduction) of *G. stearothermophilus* spores, suspended in soymilk, and treated at 620 MPa and 90C (Table 1). Therefore, the reduction of physical (protein denaturation) and chemical (sensory changes because of burning effects at high temperature) changes can generate safe and high-quality food products.

Large  $D$  values were observed at 550, 585 and 620 MPa at both temperatures 70 and 80C. As an example,  $D$  value obtained at 620 MPa and 70C was estimated as 10.6 min, whereas  $D$  value obtained at 550 MPa at the same

temperature was 22.7 min (1.35 log cycles), indicating that *G. stearo-thermophilus* spores exhibited higher resistance at the lower pressure–temperature combinations.

Figure 4 illustrates  $D$  values as a function of pressure at 70, 80 and 90C. A linear trend was observed ( $R^2 > 0.97$ ). The  $z_p$  values computed from the slope (1/MPa) decreased as temperature increased (Table 1). For *G. stearo-thermophilus* spores suspended in soymilk, the lower the thermal conditions, the higher the pressure change required to reduce one  $D$  value (1 log cycle).

### Activation Energy

Figure 5 represents the effect of temperature on the inactivation rate constant for *G. stearo-thermophilus* spores suspended in soymilk. It was observed that the higher the temperature or pressure, the higher the inactivation constant rate at constant pressure or temperature, respectively. On the other hand, the temperature dependence of the inactivation rate constant was adequately described with the Arrhenius model in view of the fact that the correlation coefficients were higher than 0.960. Also, the  $E_a$  increased as pressure was increased (Table 3).

Labuza (1984) has reported  $E_a$  values in the range of 210–627 kJ/mol for thermal inactivation of spores and Lund (1977) has reported  $E_a$  values in the range of 221–347 kJ/mol intended for sterilization purposes. However,  $E_a$  values are specific for each system and may vary depending on factors such as pH, water activity and medium composition, among others factors. When combining pressure and temperature,  $E_a$  values are not only different because

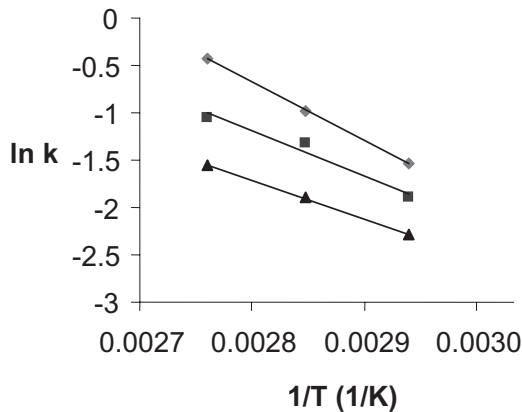


FIG. 5. TEMPERATURE DEPENDENCE OF THE INACTIVATION RATE CONSTANT ( $k$ ) FOR *G. STEAROTHERMOPHILUS* AT 550 (▲), 585 (■), AND 620 (◆) Mpa

TABLE 3.  
 $E_a$  VALUES FOR THERMAL INACTIVATION OF *G.*  
*STEAROTHERMOPHILUS* AT SELECTED PRESSURES

Pressure (MPa)	$E_a$ (kJ/mol)	$R^2$
550	37.9	0.99
585	43.7	0.98
620	57.4	0.98

of such factors, but also because of the selected conditions of pressure and temperature. Therefore, the comparison of  $E_a$  values from thermal treatment and/or from pressure–temperature combinations is not possible.

In general, during thermal treatment, the inactivation process of native proteins (unfolding) in food systems or microorganisms is usually accompanied by large activation energies. On the other hand, under high-pressure treatments, unfolding and denaturation of monomeric structures, protein aggregation, or gelation (if protein concentration and pressure are high enough) may occur. Therefore, it is expected to obtain large activation energies in the inactivation of spores, which are structured of several protein layers. However, under the working conditions in this research, most of the initial population was inactivated during the come-up time. Consequently, the  $E_a$  values explain only data of the inactivation of spores suspended in soymilk at the pressure–temperature conditions during holding time. The low  $E_a$  values obtained in this research could be because of the synergistic effect of pressure and temperature applied simultaneously. These  $E_a$  values can be defined as the energy required for inactivating the fraction of spores remaining after the come-up time; however, more studies are needed to make a more precise statement. No data regarding activation energies had been resorted for *G. stearothermophilus* in model or food systems treated at high pressure alone or in combination with temperature.

## CONCLUSIONS

Inactivation of *G. stearothermophilus* spores suspended in soymilk was achieved at the selected pressure–temperature–time conditions applied in this research. The highest levels of pressure and temperature resulted in faster spores inactivation and in greater inactivation rate constants. The come-up time had a significant effect on the inactivation of spores at the selected pressure–temperature combinations. Less than 10 CFU/mL of *G. stearothermophilus* was counted after 7 min of treatment at 620 MPa and 90C. On the

other hand, around 4.1, 5.3 and 5.8 log cycles for *G. stearothermophilus* inactivation were achieved after treatment at 620 MPa for 10, 10 and 7 min, respectively. The  $z_p$  values were estimated as 238, 178 and 142 MPa for temperatures of 70, 80 and 90°C. Low  $E_a$  values were observed when using combinations of pressure and temperature to inactivate *G. stearothermophilus* suspended in soymilk.

### ACKNOWLEDGMENTS

The authors are grateful for the financial support of Y. Estrada-Girón provided by CONACyT (National Council for Science and Technology, Mexico).

### REFERENCES

- ANANTA, E., HEINZ, V., SCHLÜTER, O. and KNORR, D. 2001. Kinetic studies on high-pressure inactivation of *Bacillus stearothermophilus* spores suspended in food matrices. *Innov. Food Sci. Emerg. Technol.* 2, 261–272.
- ARDIA, A. 2004. *Process considerations on the application of high pressure treatment at elevated temperature levels for food preservation*. Genehmigte Dissertation, Tag der wissenschaftlichen Aussprache, Berlin D 83.
- GOULD, G.W. and SALE, A.J.H. 1970. Initiation of germination of bacterial spores by hydrostatic pressure. *J. Gen. Microbiol.* 60, 335–346.
- HAYAKAWA, I., FURUKAWA, S., MIDZUNAGA, A., HORIUCHI, H., NAKASHIMA, T., FUJIO, Y., YANO, Y., ISHIKURA, T. and SASAKI, K. 1998. Mechanism of inactivation of heat-tolerant spores of *Bacillus stearothermophilus* IFO 122550 by rapid decompression. *J. Food Sci.* 63(3), 371–374.
- HAYAKAWA, I., KANNO, T., YOSHIYAMA, K. and FUJIO, Y. 1994. Oscillatory compared with continuous high pressure sterilization on *Bacillus stearothermophilus* spores. *J. Food Sci.* 59, 164–167.
- KAKUGAWA, K., OKAZAKI, T., YAMAUCHI, S., MORIMOTI, K., YONEDA, T. and SUSUKI, K. 1996. Thermal inactivation behavior of *Bacillus stearothermophilus* under pressure. In *High Pressure Bioscience and Biotechnology* (R. Hayashi and C. Balby, eds.) pp. 171–174, Elsevier Science BV, Amsterdam.
- LABUZA, T.P. 1984. Application of chemical kinetics deterioration of foods. *J. Chem. Educ.* 61(4), 348–358.

- LECHOWICH, R.V. 1993. Food safety implications of high hydrostatic pressure as a food processing method. *Food Technol.* 47(6), 170–172.
- LUND, D.B. 1977. Design of thermal processes for maximizing nutrient retention. *Food Technol.* 31(2), 71–78.
- MOERMAN, F., MERTENS, B., DEMEY, L. and HUYGHEBAERT, A. 2001. Reduction of *Bacillus subtilis*, *Bacillus stearothermophilus* and *Streptococcus faecalis* in meat batters by temperature-high hydrostatic pressure pasteurization. *Meat Sci.* 59, 115–125.
- OGAWA, H., FUKUHISA, K. and FUKUMOTO, H. 1992. Effect of hydrostatic pressure on sterilization and preservation of citrus juice. In *High Pressure and Biotechnology*, Colloque INSERM Vol 224 (C. Balny, R. Hayashi, K. Heremans and P. Masson, eds.) p. 269, John Libbey Eurotext, Montrouge, France.
- OXEN, P. and KNORR, D. 1993. Baroprotective effect of high solute concentrations against inactivation of *Rhodotorula rubra*. *Lebensm.-Wiss. Technol.* 26, 220–223.
- PALOU, E., LOPEZ-MALO, A., BARBOSA-CANOVAS, G.V. and SWANSON, B.G. 1998. High pressure treatment in food preservation. In *Handbook of Food Preservation* (M.S. Rahman, ed.) pp. 533–576, Marcel Dekker, New York, NY.
- ROUHANA, A., ADLER-NISSEN, J., COGAN, U. and FRØKLÆR, H. 1996. Heat inactivation kinetics of trypsin inhibitors during high temperature-short time processing of soymilk. *J. Food Sci.* 61(2), 256–269.
- RUSSELL, A.D. 1982. Inactivation of bacterial spores by hydrostatic pressure. In *The Destruction of Bacterial Spores* (A.D. Russell, ed.) p. 259, Academic Press, London and New York, NY.
- SALE, A.J.H., GOULD, G.W. and HAMILTON, W.A. 1970. Inactivation of bacterial spores by hydrostatic pressure. *J. Gen. Microbiol.* 60, 323–334.
- SAS Institute Inc. 1999. *Statistical Software and User's Guide*. Version 8 (TS MO). Cary, NC.
- SEYDERHELM, I. and KNORR, D. 1992. Reduction of *Bacillus stearothermophilus* spores by combined high pressure and temperature treatment. *Zeitschrift für Lebensmittel-technologie und Verfahrenstechnik* 43, EFS17.