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Combined Pressure-Thermal Inactivation Kinetics of *Bacillus amyloliquefaciens* Spores in Egg Patty Mince†

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ABSTRACT

Bacillus amyloliquefaciens is a potential surrogate for *Clostridium botulinum* in validation studies involving bacterial spore inactivation by pressure-assisted thermal processing. Spores of *B. amyloliquefaciens* Fad 82 were inoculated into egg patty mince (~1.4×10^8 spores per g), and the product was treated with combinations of pressure (0.1 to 700 MPa) and heat (95 to 121°C) in a custom-made high-pressure kinetic tester. The values for the inactivation kinetic parameter (*D*), temperature coefficient (z_T), and pressure coefficient (z_P) were determined with a linear model. Inactivation parameters from the nonlinear Weibull model also were estimated. An increase in process pressure decreased the *D*-value at 95, 105, and 110°C; however, at 121°C the contribution of pressure to spore lethality was less pronounced. The z_P-value increased from 170 MPa at 95°C to 332 MPa at 121°C, suggesting that *B. amyloliquefaciens* spores became less sensitive to pressure changes at higher temperatures. Similarly, the z_T-value increased from 8.2°C at 0.1 MPa to 26.8°C at 700 MPa, indicating that at elevated pressures, the spores were less sensitive to changes in temperature. The nonlinear Weibull model parameter *b* increased with increasing pressure or temperature and was inversely related to the *D*-value. Pressure-assisted thermal processing is a potential alternative to thermal processing for producing shelf-stable egg products.

Pressure-assisted thermal processing (PATP) offers new opportunities for the food industry to respond to the growing consumer demand for high-quality low-acid, shelf-stable products. During typical PATP, the food is subjected to a combination of elevated pressures (600 to 900 MPa) and moderate heat (90 to 121°C) for 1 to 3 min. One of the unique advantages of PATP is its ability to provide a rapid and uniform increase in the temperature of treated food samples. Uniform compression heating and expansion cooling on decompression help to reduce the severity of thermal effects encountered with conventional processing techniques. Although shelf-stable low-acid foods processed with this technology are not yet commercially available, the technology can be used for processing heat-sensitive products such as soups, egg products, coffee, tea, and mashed potatoes *(14)*.

Thermal sterilization of egg products has severe limitations. Such treatment results in the development of thermally induced off flavors, syneresis, and a green-gray discoloration of the egg products due the formation of iron-sulfur compounds *(5)*. When conventional thermal retorting and PATP were compared, PATP produced a more acceptable egg product because of a shorter period of exposure to heat *(3)*. Establishing a safe and efficient PATP method for sterilization requires proper definition of inactivation kinetic parameters for various target pathogenic and spoilage microbes under pressure, heat, and a combination of the two treatments. Few studies have addressed PATP spore inactivation kinetics over a range of pressure and temperature conditions *(15, 23, 25)*. Hence, the pressure and temperature coefficients for inactivation of bacterial spores during PATP are yet to be characterized *(9)*. These data would be extremely useful in developing a safe PATP method for a specific application. In contrast to the typical log-linear spore inactivation behavior during thermal processing, many researchers observed that PATP survivor curves were concave upward, with a linear inactivation period followed by a characteristic tailing *(1, 18, 20, 22)*. Therefore, the use of nonlinear rather than linear kinetic models to describe PATP spore inactivation may be more appropriate *(19)*. However, the influence of combined pressure-thermal treatment on the nonlinear kinetic model parameters is largely unknown *(20)*. The objectives of the study were (i) to determine the linear and nonlinear PATP kinetic parameters for inactivation of *Bacillus amyloliquefaciens* spores in egg patty mince at different pressures and temperatures and (ii) to evaluate the pressure and temperature coefficients (z_P and z_T, respectively) of *B. amyloliquefaciens* spores. Because *B. amyloliquefaciens* Fad 82 produces PATP-resistant spores, this bacterium has been proposed as a surrogate for *Clostridium botulinum (12)*, and its spores were used for this investigation.

MATERIALS AND METHODS

***B. amyloliquefaciens* spore preparation.** *B. amyloliquefaciens* Fad 82, a strain initially isolated from ropy bread, was kindly provided by M. Gänzle (Lehrstuhl für Technische Mikrobio-

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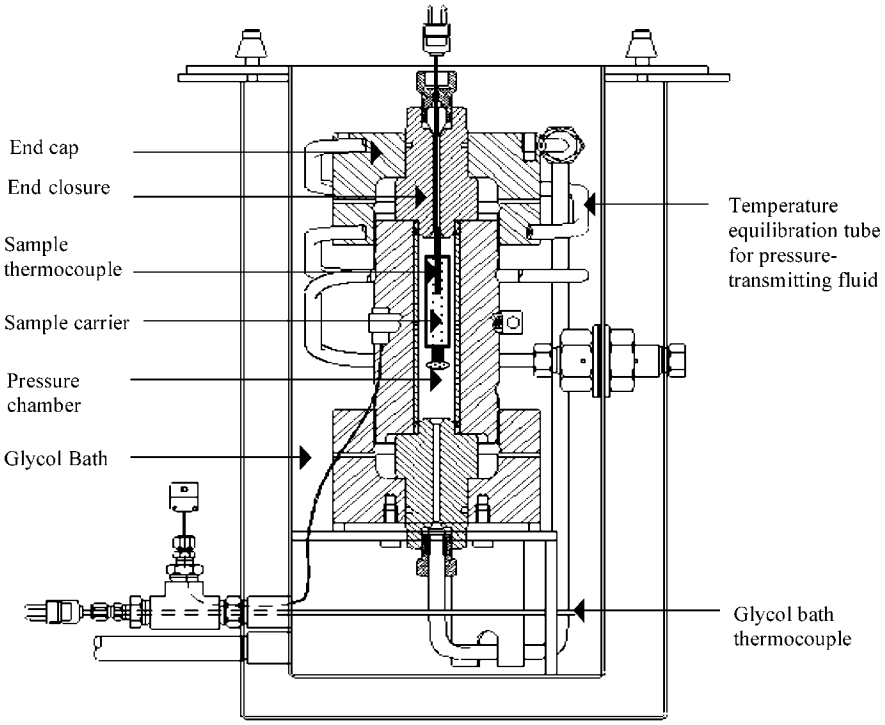

FIGURE 1. *Cross section of high-pressure kinetic tester (adapted from the pressure test unit PT-1 operation manual, Avure Technologies).*

logie, Technische Universität München, Freising, Germany). The bacterium was grown aerobically at 32°C for 24 h in Trypticase soy broth supplemented with 0.1% yeast extract (Difco, Becton Dickinson, Sparks, Md.). Preparation of spore suspension was adapted from the method of Margosch et al. *(12).* Freshly prepared cultures (100 μl) of *B. amyloliquefaciens* were spread plated onto Trypticase soy agar (TSA; Difco, Becton Dickinson) that was supplemented with 10 ppm $MnSO_4$ (Fisher Scientific, Pittsburgh, Pa.). The inoculated plates were incubated at 32°C until more than 95% sporulation was observed (at least 10 days) by microscopic examination. Spores were then collected by flooding the surface of the plates with 10 ml of sterile distilled water and scraping the colonies with a sterile glass spreader. The collected spore suspension was washed five times by differential centrifugation (from 2,000 to 8,000 × *g* for 20 min each at 4°C), sonicated for 10 min with a sonicator (SM275HT, Crest, ETL Testing Laboratory, Cortland, N.Y.) that has a peak power of 270 W, and then heated at 80°C for 10 min to destroy any remaining vegetative cells. The spore pellet was resuspended in deionized water to obtain approximately 10^9 spores per ml and stored at 4°C until used. The spore concentration was determined by pour plating 1 ml of the spore suspension on TSA and counting the number of colonies after incubation at 32°C for 48 h.

Preparation of egg samples. Frozen egg patties were obtained from a commercial egg processor (03-1426-9, Michael Foods, Minnetonka, Minn.). The egg patties are used in the fast food industry and are also available in the retail food market. The key ingredients of these patties were whole eggs, water, soybean oil, modified food starch, whey solids, salt, natural and artificial flavors, nonfat dry milk, xanthan gum, citric acid, and EDTA. According to the manufacturer, the egg patties were made by mixing whole eggs with dry and liquid ingredients, and then the mix was pumped into a mold within a flat cooking belt. Egg mix portions were cooked and preformed in a convection oven at 180 to 250°C for a predetermined time and then frozen and packaged *(10).* The round patties (8.9 ± 0.6 cm in diameter) weighed 42.5 ± 7.0 g, and their pH and water activity values were 7.25 and 0.99, respectively. One day before use in the experiments, the patties were thawed in a refrigerator at 4°C.

Thermal inactivation of spores. The thermal inactivation of *B. amyloliquefaciens* spores in the egg patty mince was determined at 95, 105, 110, and 121°C in custom-fabricated aluminum tubes (12 mm inside diameter, 42 mm high, and 3 mm wall thickness) *(11).* A sample of egg patty mince (0.9 g) was transferred into each aluminum tube, and 0.1 ml of the spore suspension (~10^9 spores per ml) was added to obtain a final spore concentration of approximately 1.4 × 10^8 spores per g of egg mince. Six tubes were then submerged simultaneously into a 28-liter circulating oil bath (Fisher Scientific), which was maintained at the desired target temperature. The sample temperature was monitored and recorded by inserting a K-type thermocouple (Omega Engineering, Stamford, Conn.) attached to a data logger (IOtech, Cleveland, Ohio) into a representative dummy aluminum cell containing the egg patty mince without spores. The heating time (come-up time) was recorded when the target temperature of the sample was reached. The process come-up times were approximately 3.58 min for 95°C, 3.33 min for 105°C, 3.25 min for 110°C, and 3.30 min for 121°C. The first aluminum tube was removed from the oil bath at the end of the come-up time. The other aluminum tubes were subjected to five different hold times (up to 180 min), and the hold time intervals were different for each target temperature. After the thermal treatments, the tubes containing samples were immersed promptly into an ice-water bath to avoid further inactivation. Spores surviving the thermal treatment were then counted.

High-pressure microbial kinetic tester. A custom-made high-pressure kinetic tester (pressure test unit PT-1, Avure Technologies Inc., Kent, Wash.) was used in the study (Fig. 1). The unit is rated to 700 MPa pressure and 130°C process temperature. It has a 54-ml stainless steel pressure chamber immersed in a temperature-controlled bath, and the system is pressurized by an intensifier (M-340 A, Flow International, Kent, Wash.). The bath surrounding the pressure chamber was maintained at a suitable

TABLE 1. *Typical pressure-assisted thermal processing settings used in this study*

Process pressure (MPa)	Pressure chamber glycol bath temp (°C)	Come-up time (min)	Sample temp (°C) at different stages of processing[a]		
			Preheating (T_1)	Immediately before pressurization (T_2)	During pressure holding (T_3–T_4)
500	95	0.5 ± 0.1	60 ± 1.0	67.7 ± 0.6	95 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	75.7 ± 1.0	105 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	81.9 ± 0.3	110 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	92.6 ± 0.5	121 ± 1.0
600	95	0.6 ± 0.1	55 ± 1.0	61.0 ± 0.7	95 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	70.4 ± 1.2	105 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	75.9 ± 0.6	110 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	87.4 ± 0.8	121 ± 1.0
700	95	0.7 ± 0.1	50 ± 1.0	57.7 ± 1.1	95 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	67.1 ± 1.1	105 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	72.9 ± 0.4	110 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	84.1 ± 0.1	121 ± 1.0

[a] Figure 2 illustrates processing stages and the points at which T_1 through T_4 were measured. Data presented are mean ± standard deviation of two independent trials of various combinations of pressure, temperature, and holding time.

temperature (Table 1) so that isothermal process conditions could be maintained throughout the pressure-holding time. Propylene glycol (57-55-6, Avatar Corporation, University Park, Ill.) was used as the heating medium in the bath. Figure 2 shows sample pressure and temperature profiles at a combination of 700 MPa and 105°C. Whereas the pressure come-up time depended upon the target pressure (Table 1), the depressurization occurred in less than 4 s, regardless of the pressurization level. The sample temperature and chamber pressure were recorded every 2 s during the entire treatment cycle with K-type thermocouple sensors (model KMQSS-04OU-7, Omega Engineering, Stamford, Conn.) and pressure transducers (model 3399 093 006, Tecsis, Frankfurt, Germany), respectively. A data acquisition computer equipped with relevant hardware (Daq-Board/2000 16-bit, 200-kHz PCI card, DBK 81 seven-channel thermocouple expansion card, and DBK 203 expansion card; IOtech) and software (DasyLab 7.00.04; National Instruments Corp., Austin, Tex.) was used to record the data.

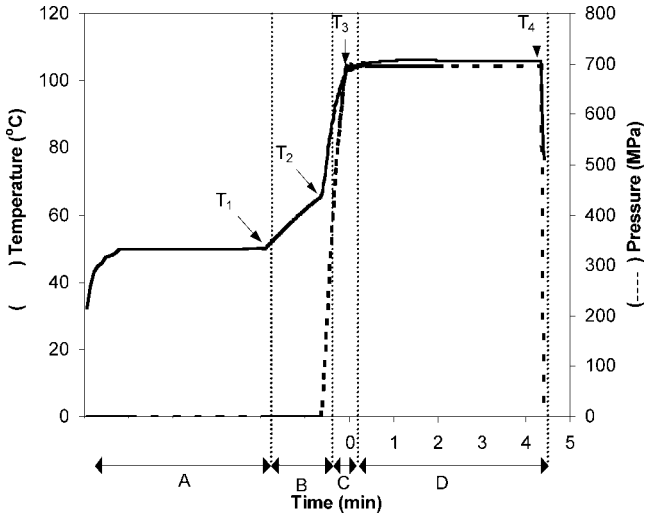

FIGURE 2. *Pressure and temperature history of a spore sample during preheating and pressure-assisted thermal treatment at 700 MPa and 105°C. Data are mean values for two independent trials. Time scale includes spore sample preheating time (min) in water bath prior to loading into pressure machine (A), time (min) for the sample in the pressure chamber to reach desired initial temperature before the commencement of pressurization (B), pressure come-up time (min) (C), and pressure hold time (min) (D). Depressurization time (<2 s) is not shown. Glycol bath was maintained at 105°C.*

Preparing microbial test samples for PATP. Pouches (5 by 2.5 cm) made from sterile filter bags (01-002-57, Fisher Scientific) were used as sample holders. A sample of egg patty mince (0.9 g) and 0.1 ml of the spore suspension (~10^9 spores per ml) were placed inside these pouches to obtain a final spore concentration of approximately 10^8 spores per g of egg mince. The pouches were then heat sealed (Impulse Food Sealer, American International Electric, Whittier, Calif.), and the contents of the pouch were mixed thoroughly. The packaged samples were then placed in a sample carrier consisting of a 10-ml polypropylene syringe (model 309604; Becton Dickinson) covered with two layers of insulating material (model 09-356; Fisher Scientific). Water was used as the pressure-transmitting fluid within the syringe. Prior to pressurization experiments, the sample carrier containing the spore-inoculated egg patty mince was preheated in a water bath (Isotemp 928, Fisher Scientific) to a suitable preprocessing temperature, T_1 (Table 1 and Fig. 2).

PATP of egg patty mince. Each preheated sample carrier was immediately loaded into the chamber of the pressure kinetic tester, and pressurization began when the sample temperature reached a predetermined value, T_2 (Table 1 and Fig. 2). This temperature was estimated based on the following equation:

$$T_2 = T_3 - \{[(\mathrm{CH}) \times \Delta P] + \Delta T_H\} \quad (1)$$

where T_3 is the desired process temperature (°C), CH is the compression heating value of the test sample, ΔP is the pressure applied (MPa), and ΔT_H is the heat absorbed from the surrounding glycol bath during pressurization. Depending upon the target process temperature (T_3) and process pressure, ΔT_H was estimated on a trial-and-error basis. Compression heating values for egg samples were experimentally determined (data not shown) and were similar to the published value for water *(2)*. The test samples were subjected to a combination of process temperatures (95, 105, 110, and 121°C) and pressures (500, 600, and 700 MPa) for a maxi-

mum of 15 min. The process hold times were adjusted for each combination of process pressure and temperature so that enough data were collected for subsequent analysis. The process hold times did not include the pressure come-up time or the depressurization time. After depressurization, the samples were cooled immediately in an ice-water bath. The untreated control samples (non–pressure-treated inoculated egg patty mince) were heated at 80°C for 30 min to activate the spores for enumeration. Pouches containing the inoculated egg product (control and pressure treated) were opened aseptically, and their contents were used for determining the total viable spore count.

Enumeration of survivors. Heat- or PATP-treated samples (1 g) were mixed with 9 ml of 0.1% peptone water and homogenized for 2 min in a stomacher (Seward Lab Stomacher, Norfolk, UK). The homogenized sample was further serially diluted in peptone water, and the dilutions (1 ml) were pour plated on duplicate TSA plates. The plates were then incubated at 32°C for 48 h before enumeration. Colonies were counted with a dark-field Quebec colony counter (Leica Microsystems, Richmond Hill, Ontario, Canada). The minimum detection limit for the enumeration procedure was 10 spores per g of egg product.

Determination of kinetic inactivation parameters. The decimal reduction time (*D*-value) for different pressure and temperature combinations was calculated from the linear portion of the survivor curve, which occurred immediately after the come-up time, using the equation

$$\log\frac{N}{N_0''} = \frac{-t}{D} \tag{2}$$

where N_0'' is the initial spore count measured immediately after the process (thermal or pressure) come-up time and N is the spore count after exposure to the lethal treatment for a specific time (t). The pressure coefficient, z_P (MPa), at constant temperature (i.e., the pressure required at constant temperature to achieve a 10-fold change in the *D*-value) was estimated as a negative reciprocal of the slope resulting from plotting log *D* against pressure. Similarly, the temperature coefficient, z_T (°C), at constant pressure (i.e., the temperature change required at constant pressure to achieve a 10-fold change in the *D*-value) was estimated as a negative reciprocal of the slope resulting from plotting log *D* against temperature. The reaction rate constant, k (min^{-1}), is inversely related to the *D*-value and was determined using the relationship

$$k = \frac{2.303}{D} \tag{3}$$

The volume change of activation ΔV (m^3 $mole^{-1}$), which is a measure of the net effect of pressure reactions causing physiological changes at constant temperature, was estimated using the following equation *(4, 16)*:

$$\Delta V = -RT\left(\frac{\Delta \ln k}{\Delta P}\right)_T \tag{4}$$

where P is the pressure (MPa), T is the absolute temperature (°K), and R is the universal gas constant (8.314×10^{-6} m^3 $mole^{-1}$ MPa K^{-1}). The energy of activation E_a (J $mole^{-1}$), which describes the effect of temperature changes on the reaction rate (at constant pressure), was obtained from the following equation:

$$E_a = -R\left(\frac{\Delta \ln k}{\frac{1}{\Delta T}}\right)_P \tag{5}$$

where k is the rate constant (min^{-1}), T is the absolute temperature (°K), and R is the universal gas constant (8.314 J $mole^{-1}$ K^{-1}).

Weibull model parameter estimation and curve fitting. The Weibull model was initially proposed by Peleg and Cole *(19)* to describe microbial inactivation curves. The Weibull model is described by the following equation:

$$\log\frac{N}{N_0''} = -bt^n \tag{6}$$

where b and n are the scale and shape factors, respectively *(19)*.

The curve fitting and model parameter estimation were achieved with the nonlinear (PROG NLIN) procedure of the Statistical Analysis System software (SAS, release 9.2.1, SAS Institute Inc., Cary, N.C.). Mean square error (MSE), regression coefficient (R^2), and accuracy factor (A_f) were used to evaluate the goodness of fit. A relatively small MSE value and large R^2 value is indicative of a good fit to the model *(17)*, and an A_f value of 1 indicates that the model produces a perfect fit to the data *(24)*. Although MSE and R^2 were obtained from the analysis, the A_f values were calculated with the following equation:

$$A_f = 10^{[\Sigma\,|\log(\text{predicted/observed})|]/n} \tag{7}$$

Data analysis. All data were analyzed with the SAS software. The general linear model and least significant difference procedures were used to compare means. Mean differences among PATP treatments and holding times were calculated with Fisher's least significant difference method, with significance at the 5% level ($P < 0.05$). Two independent trials of thermal and PATP experiments were carried out.

RESULTS AND DISCUSSION

Figure 3 is a comparison of survivor curves for *B. amyloliquefaciens* during treatment of spores in egg patty mince by combinations of pressure (0.1 to 700 MPa) and heat (95 to 121°C). In general, PATP inactivation of *B. amyloliquefaciens* spores was biphasic, with rapid initial inactivation immediately after the pressure come-up time followed by a characteristic tailing during the extended pressure-holding times. Similar biphasic PATP inactivation has been observed for other spores, such as those of *Bacillus stearothermophilus (1, 18, 20)*, *C. botulinum (21, 22)*, and *Clostridium thermosaccharolyticum (12)*.

The reduction in number of spores during the pressure come-up time varied between 0.1 and 1.2 log spores per g of egg product, depending on the treatment. Within the range of conditions tested in the present study, lower pressure-heat combinations (e.g., 500 MPa at 95 to 105°C) resulted in no or limited reduction in the spore count during the pressure come-up time, but application of higher pressure (700 MPa) and temperature (121°C) inactivated up to 1.2 log spores per g during that period (Fig. 3). Margosch et al. *(12)* also reported a reduction in the population (<0.5-log reduction) of *B. amyloliquefaciens* spores during a 5-min pressure come-up time for PATP at 600 MPa and 80°C. These authors also reported reduction in numbers of other spores tested, such as *C. botulinum* TMW 2.357 (1.5-log reduction), *C. thermosaccharolyticum* (3-log reduction), and *Bacillus subtilis* (>5-log reduction) during similar come-up times. These observations suggest that different spores are likely to have different resistances during the pressure come-up time and highlight the importance of documenting the PATP come-up time and the corresponding spore inactivation.

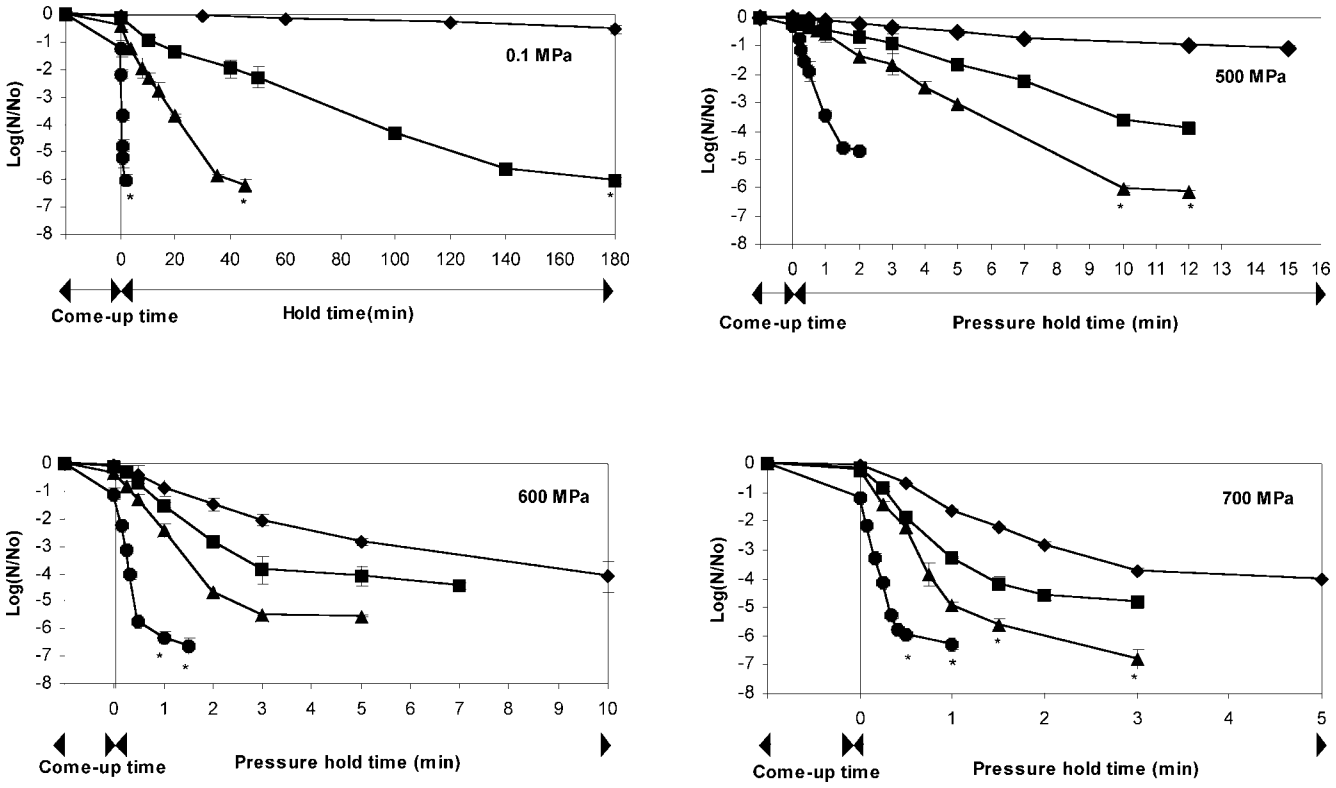

FIGURE 3. *Log survivor fraction of* Bacillus amyloliquefaciens *spores in egg patty mince subjected to pressures of 0.1 to 700 MPa at temperatures of 95 to 121°C: 95°C (♦), 105°C (■), 110°C (▲), and 121°C (●). * Number of survivors was an estimate (<20 spores per g).*

The lethality of PATP against *B. amyloliquefaciens* spores increased with increases in process pressure at a given temperature (Fig. 3). For example, the *D*-value for PATP at 105°C decreased with a pressure increase from 500 to 700 MPa (Table 2). Similar changes in the *D*-value with pressure were reported for the PATP of *Clostridium sporogenes* spores in meat broth *(26)* and *B. stearothermophilus* spores in egg *(20).*

TABLE 2. *Linear and Weibull model kinetic parameters of* Bacillus amyloliquefaciens *spores during thermal or pressure-assisted thermal processing in egg patty mince*[a]

Pressure (MPa)	Temp (°C)	Linear, *D* (min)	Weibull[b]				
			b (95% CI)	*n* (95% CI)	MSE	R^2	A_f
500	95	11.58 ± 0.39	0.15 (0.12–0.18)	0.75 (0.66–0.83)	0.01	0.99	1.14
	105	2.9 ± 0.16	0.31 (0.23–0.38)	1.03 (0.85–1.02)	0.03	0.99	1.10
	110	1.66 ± 0.01	0.66 (0.43–0.71)	0.94 (0.86–1.21)	0.08	0.99	1.09
	121	0.32 ± 0.03	2.83 (2.60–3.07)	0.76 (0.63–0.89)	0.11	0.98	1.16
600	95	1.79 ± 0.10	0.91 (0.79–1.04)	0.66 (0.59–0.73)	0.03	0.99	1.12
	105	0.72 ± 0.01	1.60 (1.24–1.97)	0.56 (0.42–0.71)	0.23	0.97	1.40
	110	0.46 ± 0.01	2.30 (1.76–2.84)	0.59 (0.41–0.77)	0.44	0.97	1.36
	121	0.11 ± 0.02	4.92 (4.33–5.52)	0.51 (0.34–0.68)	0.52	0.97	1.23
700	95	0.80 ± 0.01	1.63 (1.35–1.92)	0.60 (0.46–0.74)	0.12	0.98	1.19
	105	0.31 ± 0.01	2.83 (2.49–3.17)	0.55 (0.41–0.69)	0.20	0.98	1.21
	110	0.21 ± 0.02	3.94 (3.51–4.36)	0.53 (0.40–0.67)	0.38	0.98	1.21
	121	0.08 ± 0.01	5.83 (5.01–6.65)	0.47 (0.32–0.61)	0.37	0.97	1.22
0.1	95	349 ± 49					
	105	24 ± 3.4					
	110	5.90 ± 0.87					
	121	0.25 ± 0.01					

[a] Kinetic parameters were estimated based two independent trial combinations of thermal and pressure-assisted thermal processing experiments. The Weibull model was not applied for thermal inactivation curves.

[b] The smaller the MSE (mean square error) values and the higher the R^2 values, the better the model fits the data. A_f values closer to 1 indicate that the model produces a closer fit to the data.

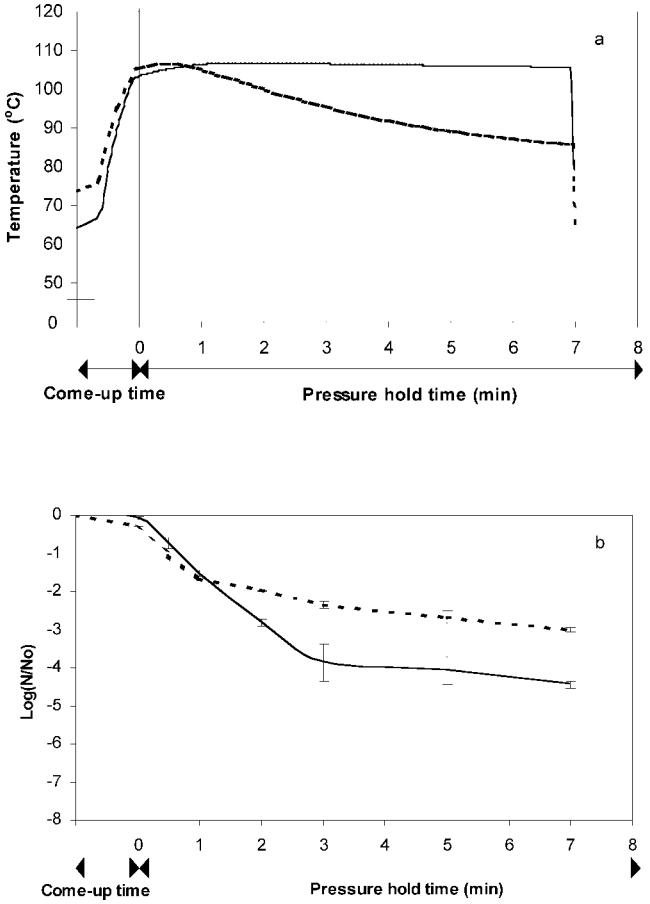

FIGURE 4. *Time-temperature profile and the corresponding inactivation of* Bacillus amyloliquefaciens *spores in egg patty mince subjected to a pressure-assisted thermal treatment at 600 MPa and 105°C. (a) Time-temperature profile; (b) survivor plot;* ———*, isothermal treatment at 105°C;* – – –*, nonisothermal treatment in the range of 105 to 85°C.*

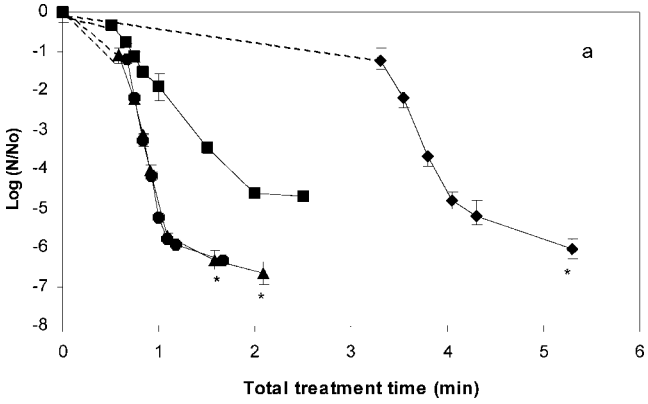

FIGURE 5. *Log survivor fraction of* Bacillus amyloliquefaciens *spores in egg patty mince subjected to a process temperature of 121°C at pressures of 0.1 to 700 MPa: 0.1 MPa (◆), 500 MPa (■), 600 MPa (▲), and 700 MPa (●). Total treatment time was come-up time (– – –) and process hold time (———). * Number of survivors was an estimate (<20 spores per g).*

Comparison of survivor curves (Fig. 3) illustrates as expected that increases in process temperature (at constant pressure) decreased the spore viability considerably. A temperature rise from 95 to 121°C at all the pressures tested decreased the *D*-value significantly ($P < 0.05$) (Table 2). The effect of rising process temperatures increasing the PATP inactivation rates also has been observed for spores of *B. stearothermophilus* in mashed broccoli *(1)* and *C. sporogenes* in meat broth *(15)*. Different combinations of pressure and temperature can bring about a similar level of *B. amyloliquefaciens* spore inactivation. For example, PATP at 600 MPa and 105°C produced lethality similar to that of PATP at 700 MPa and 95°C.

Researchers have hypothesized that the nonisothermal conditions inside the pressure vessel can influence spore inactivation *(6, 20, 27)*. Figure 4 shows the effect of temperature decline (nonisothermal conditions) during PATP on the survivor curves of *B. amyloliquefaciens* spores. The decline in temperature was simulated in the pressure kinetic tester by maintaining the temperature of the glycol bath below that achieved in the pressure chamber from compression heating. When spore survivor curves obtained under isothermal and nonisothermal conditions were compared, the nonisothermal conditions were less lethal overall (Fig. 4), which illustrates the importance of maintaining uniform conditions during PATP.

The interval between the beginning of the isobaric-thermal process (i.e., when the targeted pressure and temperature are achieved) and the tailing of the spore population appeared to be a function of processing conditions. Visual examination of Figure 3 suggests that at a high process pressure (700 MPa) and temperature (121°C), tailing occurs early, and these processing conditions were effective in decreasing spore populations by up to 4.6 to 5.8 log spores per g of egg product. Extended treatment appears to be ineffective for increasing lethality. More research is needed to understand bacterial tailing and to develop procedures to minimize it and enhance process lethality.

Spore resistance during thermal processing or PATP. Resistance of *B. amyloliquefaciens* spores to PATP (as measured by the kinetic parameter, *D*-value) was significantly less than that to thermal processing at an equivalent process temperature (Table 2). Similar results have been reported when other spores (*Clostridium tyrobutyricum*, *C. thermosaccharolyticum*, *C. sporogenes*, and *B. stearothermophilus*) were subjected to PATP *(12, 20, 26)*. However, synergy between heat and pressure diminishes at higher temperatures, and heat becomes the dominant contributor to lethality at 121°C. In Figure 5, thermal treatment at 121°C (6.04-log reduction after 3.3-min come-up time and 2-min process hold time) appears to be more lethal than PATP treatment at 500 MPa and 121°C (4.38-log reduction after 0.5-min come-up time and 2-min pressure hold time). However, a considerable part of heat treatment lethality (1.23-log reduction in spore numbers) occurred during the longer come-up time (3.3 min), whereas the shorter PATP come-up time (0.5 min) resulted in only a 0.32-log reduction. The come-up time in the pressure kinetic tester used in this study cannot be changed; therefore, no attempts were made to match the come-up times of PATP and thermal treatments. In the absence of such comparable come-up times, it may not be possible to draw meaningful conclu-

TABLE 3. *Pressure coefficients (z_P and ΔV) for* Bacillus amyloliquefaciens *spores suspended in egg patty mince at different temperatures during pressure-assisted thermal processing*

Process temp (°C)	z_P (MPa)[a]	ΔV ($\times 10^{-5}$ m^3/mole)[b]
95	170 ± 1	−4.4
105	206 ± 2	−3.7
110	220 ± 6	−3.3
121	332 ± 42	−2.2

[a] A higher z_P value implies less sensitivity to pressure change.
[b] A less negative ΔV value implies less sensitivity to pressure change.

TABLE 4. *Temperature coefficients (z_T and E_a) for* Bacillus amyloliquefaciens *spores suspended in egg patty mince at different pressures during pressure-assisted thermal processing*

Process pressure (MPa)	z_T (°C)[a]	E_a ($\times 10^5$ J/mole)[b]
0.1	8.2 ± 0.2	3.4
500	16.7 ± 0.4	1.7
600	21.5 ± 1.5	1.3
700	26.8 ± 1.2	1.1

[a] A higher z_T value implies less sensitivity to temperature change
[b] A lower E_a value implies less sensitivity to temperature change.

sions about the presumed protective effects of pressure at higher temperatures.

Pressure and thermal coefficients of *B. amyloliquefaciens* spores. The z_P values of *B. amyloliquefaciens* spores subjected to PATP increased from 170 MPa at 95°C to 332 MPa at 121°C (Table 3). At 121°C, a similar z_P value (370 MPa) was reported for *B. stearothermophilus* spores *(23).* Our results suggest that the spores became increasingly less sensitive to pressure changes as the processing temperature increased. Therefore, contribution of pressure to spore lethality was less pronounced at higher (121°C) than at lower (95, 105, or 110°C) process temperatures. This observation is consistent with that of Miglioli et al. *(15),* who estimated z_P values for *C. sporogenes* at different PATP temperatures. Values for activation volume (ΔV) at all temperatures (Table 3) were negative, indicating that pressure has a lethal effect on *B. amyloliquefaciens* spores; this result is consistent with the Le Chatelier's principle. In general, a less negative ΔV value signifies less sensitivity of spores to changes in pressure *(16).* In the current study, the ΔV value became less negative with an increase in process temperature (-4.4×10^{-5} m^3/mole at 95°C to -2.3×10^{-5} m^3/mole at 121°C); therefore, the activation volume concept further confirms that the spores became less sensitive to pressure changes when the processing temperature increased.

B. amyloliquefaciens spores were more sensitive to temperature changes at atmospheric pressure ($z_T = 8.2$°C) than at higher pressure ($z_T = 26.8$°C at 700 MPa) (Table 4). Similar observations were made by Miglioli et al. *(15),* who reported z_T values of 11.8 and 17.1°C for *C. sporogenes* at 0.1 and 700 MPa, respectively. The calculated activation energy for *B. amyloliquefaciens* spores at ambient pressures was similar to the published value of 2.5 to 3.4 $\times 10^5$ J/mole for spore destruction *(8).* Activation energy decreased with increasing pressure (from 3.4×10^5 J/mole at 0.1 MPa to 1.1×10^5 J/mole at 700 MPa), suggesting that pressure synergistically contributed to spore lethality within the range of conditions studied.

Influence of pressure and temperature on inactivation parameters of the Weibull model. For the model parameter estimation and curve fitting, spore lethality during the pressure come-up time was not considered. Preliminary model fitting was done using both the Weibull and log-logistic models; however, the Weibull model was chosen to describe the inactivation curves because it resulted in a better fit to the raw data (data not shown). Many researchers *(7, 13, 20, 28)* also have preferred the Weibull model to describe nonlinear log survivor curves because of its mathematical simplicity and ability to address microbial tailing.

The Weibull model parameter *b* increased with increases in pressure and temperature (Table 2). A higher *b* value corresponds to a steeper slope of the log survivor curve, which in turn implies that spore inactivation occurred at a faster rate. Consequently, there is an inverse relationship between *b* and *D*-values (Table 2), which can be described by the following equation:

$$b = \frac{t^{1-n}}{D} \tag{8}$$

Thus, when $n = 1$ (indicating a linear inactivation), *b* and *D* are inversely proportional to each other.

A value of *n* that is less than 1 indicates that the survivor curve is concave upward with the presence of tailing *(19).* In our study, most inactivation curves (fitted with Weibull models) produced *n* values less than 1 (Table 2). An increase in pressure at a constant process temperature resulted in curves with a distinct linear slope region followed by a near-horizontal region (i.e., a sharp tailing) (Fig. 3). Correspondingly, the value of the parameter *n* also decreased. Thus, curves in which tailing occurs suddenly have a lower *n* value than do curves in which the tailing is gradual.

Different combinations of pressure and temperature bring about similar lethality for *B. amyloliquefaciens* Fad 82 spores during PATP. *D*-values decreased with increases in pressure or temperature and were inversely related to the values of the Weibull parameter, *b*. The processing resistance parameter, the *D*-value for *B. amyloliquefaciens* spores in egg patty mince, was lower for PATP than for thermal processing at an equivalent temperature; however, *B. amyloliquefaciens* spores were less sensitive to pressure changes at 121°C (indicated by the higher z_P and less negative ΔV values) than at lower temperatures. The sensitivity of *B. amyloliquefaciens* spores to temperature changes was less at 700 MPa (indicated by the higher z_T and lower E_a values) than at lower pressures.

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