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Kinetics of *Bacillus cereus* Spore Inactivation in Cooked Rice by Combined Pressure–Heat Treatment

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ABSTRACT

The efficacy of pressure–heat treatment was evaluated for the inactivation of *Bacillus cereus* spores in cooked rice. The spores of *B. cereus* ATCC 9818 were inoculated (1.1×10^8 CFU/g) in a parboiled rice product (pH 6.0, water activity of 0.95) and inactivated to an undetectable level (<10 CFU/g) by treatment of 600 MPa and process temperatures of 60 to 85°C or 0.1 MPa and 85°C. Kinetic inactivation parameters were estimated with linear and nonlinear models. The potential recovery of injured bacteria was also evaluated during storage of the treated product for 4 weeks at 4 and 25°C. Depending on the process temperature, a 600-MPa treatment inactivated spores by 2.2 to 3.4 log during the 30-s pressure come-up time, and to below the detection limit after 4- to 8-min pressure-holding times. In contrast, a 180-min treatment time was required to inactivate the spores to an undetectable level at 0.1 MPa and 85°C. The decimal reduction time of spores inactivated by combined pressure–heat treatment ranged from 1.08 to 2.36 min, while it was 34.6 min at 85°C under atmospheric conditions. The nonlinear Weibull model scale factor increased, and was inversely related to the decimal reduction time, and the shape factor decreased with increasing pressure or temperature. The recovery of injured spores was influenced by the extent of pressure-holding time and process temperature. This study suggests that combined pressure–heat treatment could be used as a viable alternative to inactivate *B. cereus* spores in cooked rice and extend the shelf life of the product.

Bacillus cereus is a large, rod-shaped, gram-positive, facultative aerobic, endospore-forming bacterium with an optimum growth temperature of 28 to 35°C, a minimum growth temperature of 4°C, and a maximum of 48°C *(31)*. The ability of heat-resistant pathogenic *B. cereus* spores to survive pasteurization is a major concern in a variety of pasteurized low-acid foods with a limited refrigerated shelf life. *B. cereus* can cause two types of illness (poisoning) by two distinct types of toxin. Enterotoxigenic *B. cereus* can cause diarrheal illness through a wide variety of foods including meats, milk, vegetables, and fish, the signs and symptoms of which are watery diarrhea, abdominal cramps, and pain. Consumption of starchy foods (such as rice, potato, and pasta) and cheese products contaminated with *B. cereus* emetic toxin (cereulide) usually results in an emetic type of illness, characterized by nausea and vomiting *(31)*. However, enterotoxigenic (diarrheal) *B. cereus* was also detected in such products *(3, 27)*. In addition, foods such as sauces, puddings, soups, casseroles, pastries, and salads were also linked with *B. cereus* food poisoning outbreaks *(31)*. Storage of cooked rice at improper temperatures (e.g., room temperature) after cooking allows the heat-resistant spores of *B. cereus* that survived cooking to germinate, grow, and produce toxin in the product. Symptomatic similarities of *B. cereus* illness to *Staphylococcus aureus* intoxication (vomiting type) or *Clostridium perfringens* food poisoning (diarrheal type) could cause some of the *B. cereus* outbreaks to go unreported or to be misdiagnosed *(31)*. The decimal reduction time (*D*-value) of *B. cereus* spores can range from 2.2 to 5.5 min at 100°C to above 80 min at 80°C (strain dependent) *(6, 17)*. Combined pressure–heat treatment provides a possible alternative to conventional heat treatment to inactivate bacterial spores while minimizing the adverse thermal effects on the product quality attributes. Very limited studies evaluated combined pressure–heat resistance of *B. cereus* in rice products.

The current study was designed to determine the kinetic parameters of *B. cereus* spore inactivation in cooked rice as a selected low-acid, extended-shelf-life product by combinations of pressure and heat treatments, and to evaluate the storage stability of the pressure-heat–treated product by studying the potential injury and subsequent recovery of *B. cereus*.

MATERIALS AND METHODS

***B. cereus* strain.** *B. cereus* ATCC 9818 was selected for this study due to its high heat resistance *(10, 14, 17, 19)*. There is limited information on the type of toxin produced by *B. cereus* ATCC 9818. Miller *(16)* detected genes encoding nonhemolytic

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enterotoxins (*nheA, nheB,* and *nheC*) and enterotoxin FM (*entFM*) in this strain by PCR. The culture was obtained from the American Type Culture Collection (Manassas, VA).

Spore preparation. *B. cereus* ATCC 9818 culture was sporulated on nutrient agar supplemented with 6 g/liter yeast extract (BD, Sparks, MD) and 10 mg/liter $MnSO_4 \cdot H_2O$ (Fisher Scientific, Pittsburgh, PA). The culture was initially grown aerobically in Trypticase soy broth supplemented with 0.6% yeast extract (TSBYE; BD) at 30°C for 2 days, and was then inoculated (0.5 ml) on nutrient agar (NA) that was incubated aerobically at 30°C for 7 days. Once >90% sporulation was achieved (verified with phase-contrast microscopy), the spore crop was harvested by flooding the plates with cold, sterile deionized water and washed five times (14,000 × *g,* 10 min, 4°C). The final spore suspension in sterile deionized water (10^9 CFU/ml) was heat shocked (80°C, 15 min) to kill any remaining vegetative cells, and then stored at 4°C until used.

Cooked rice. A commercial shelf-stable cooked-rice product was used for this study to minimize product variability between experiments. The product had a total fat of 2.4%, total carbohydrates of 32%, protein content of 3.2%, sodium of 0.12%, and potassium of 0.044%. The pH of the rice was 6.0, and the water activity (a_w) 0.95.

Sample preparation. To prepare the samples for combined pressure–heat treatment and thermal processing, aliquots (0.2 ml) of *B. cereus* spore suspension with a concentration of ~10^9 CFU/ml and 1.8 g of rice were aseptically transferred into sterile polyethylene pouches (5 by 2.5 cm; 01-002-57, Fisher Scientific) and mixed to obtain ~10^8 CFU/g before processing. The pouches were then heat sealed and kept at 4°C for 1 h for preconditioning purpose.

Combined pressure–heat treatment. A high pressure microbial kinetic tester (PT-1, Avure Technologies, Inc., Kent, WA) was used for pressure–heat treatment of rice in this study. The unit was equipped with an intensifier (M-340 A, Flow International) capable of generating pressures up to 700 MPa. A 54-ml stainless steel pressure chamber was immersed in a temperature-controlled bath. Propylene glycol (Houghton Safe-620TY, Houghton International, Inc., Valley Forge, PA) was used as the heat- and pressure-transmitting medium in the temperature-controlled bath and pressure chamber. For combined pressure–heat treatment applications, the temperature of the external glycol bath was set at the desired target process temperature (60 to 85°C) to minimize any heat loss during the test. The high pressure processor had a pressurization rate of 14.3 MPa/s, while depressurization occurred within 2 s for all treatments. A K-type thermocouple sensor (model KMQSS-04OU-7, Omega Engineering, Stamford, CT) inserted into the sample holder and mounted to the top end of the pressure chamber was used to monitor the sample temperature during process. Another K-type thermocouple sensor measured the glycol bath temperature. The chamber pressure was recorded every second with a pressure transducer (model 3399 093 006, tecsis GmbH, Frankfurt, Germany) by using a computerized data acquisition system. The samples holder was made of a 10-ml polypropylene syringe (model 309604, BD) wrapped with two layers of sport tape (CVS Pharmacy, Inc., Woonsocket, RI), which served as insulating material. After placing duplicate pouches containing rice samples inside the syringe, the void volume of the syringe was filled with water. Insulating the syringe further helped minimize the heat exchange between the syringe and the surrounding glycol bath, which had higher heat of compression than that of water. The sample temperature was brought to an empirically determined *(18)* preprocessing temperature (T_1) within 2 min by placing the syringe containing pouches in a water bath (Isotemp 928, Fisher Scientific). The syringe was then immediately loaded into the pressure chamber.

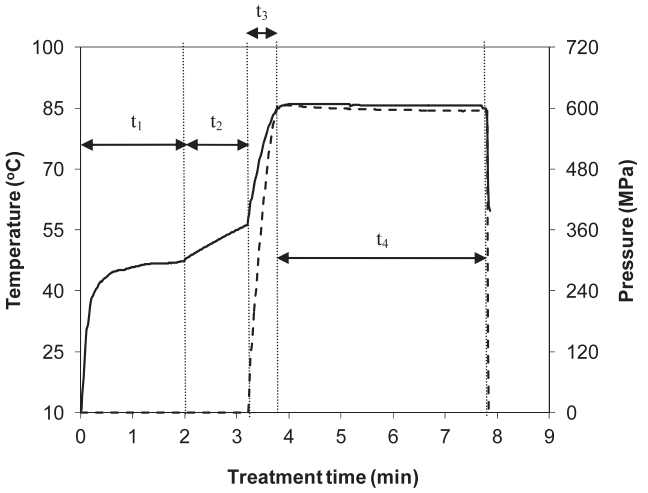

FIGURE 1. *Representative pressure and temperature histories of rice samples during pressure–heat treatment (600 MPa and 85°C) for 4 min.* t_1*, preheating time in a water bath (47°C);* t_2*, preheating time inside the pressure chamber;* t_3*, pressure come-up time;* t_4*, pressure holding time. The dashed and solid lines represent pressure and temperature changes, respectively.*

The pressure–temperature histories in the vicinity of the sample within the syringe were recorded. The pressurization started when the sample temperature reached a predetermined value, T_2, which was estimated with equation 1, where T_3, CH, and ΔP are the desired target temperature (in degrees Celsius), heat of compression value of the sample (in degrees Celsius at 100 MPa), and process pressure (in megapascals), respectively. ΔT_H is the temperature gained by the test sample during loading within the pressure chamber as well as pressurization *(18)*:

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

The combined pressure–heat treatment of rice was conducted at 600 MPa and various process temperatures (60 to 85°C) for ≤15 min. The target pressure (600 MPa) is a commonly used pressure level in food pasteurization. The time required to reach the target pressure of 600 MPa (pressure come-up time) was about 30 s. The pressure holding time did not include the pressure come-up time or the depressurization time. After depressurization, samples were immediately removed from the pressure chamber and cooled in an ice-water bath (4°C) to prevent further spore inactivation. Figure 1 shows the pressure and temperature histories of the samples processed at 600 MPa and 85°C for 4 min. Surviving *B. cereus* populations in rice were enumerated (as described below) within 3 h after the treatment as well as during storage of treated samples up to 4 weeks at 4 and 25°C. All experiments were conducted in two independent pressure–heat treatment trials by using duplicate rice samples.

Thermal processing. Thermal processing experiments were carried out at 85°C and 0.1 MPa with a 35-liter circulating oil bath (NESLAB EX-35 Digital One, Thermo Fisher Scientific, Inc., Waltham, MA). Custom-fabricated thermal death time disks (TDT disks; 18-mm diameter and 4.5-mm height) were used as sample

holders *(11)*. A 1-g portion of the inoculated rice sample (containing $\sim 10^8$ spores per g) was placed in each of the TDT disks. The sample temperature was monitored and recorded by inserting a K-type thermocouple (Omega Engineering) attached to a data logger (IOtech, Inc., Cleveland, OH) into a TDT disk containing rice without spores. Two water baths and two oil baths were used to match the temperature history of thermal processing to that of pressure–heat treatment *(9, 18)*. TDT disks containing rice samples were preheated in the first water bath for 2 min at 47°C and then in the second water bath until they reached 55°C. The samples were then transferred to the first oil bath, which was maintained at 93°C and finally to the second oil bath (85°C) once the sample temperature reached around the target process temperature (i.e., 85°C). At specific holding times, the disks were removed from the second oil bath and immediately immersed in an ice-water bath (4°C) to avoid further spore inactivation. It took about 2 min to bring the sample temperature to 4°C. Surviving *B. cereus* populations were enumerated within 3 h after the treatment as well as during storage of processed rice, up to 4 weeks at 4 and 25°C. All experiments were carried out in two independent trials by using duplicate rice samples.

Enumeration of survivors. The viable counts of *B. cereus* spores in rice subjected to pressure–heat treatment and thermal processing were enumerated after treatment and during storage of samples at 4 and 25°C for 4 weeks on NA by using the spread-plate method. Rice samples were homogenized with sterilized 0.1% peptone water in a Seward Laboratories Stomacher (Basingstoke, Hampshire, UK) at 230 rpm for 2 min for the preparation of serial dilutions. After preparing serial dilutions, 0.1-ml aliquots of the appropriate dilutions were spread plated on NA. The plates were incubated at 30°C for 2 days before enumeration of survivors. The population of spores inactivated to below the plate count detection limit (<10 CFU/g) was also estimated with a three-tube most-probable-number (MPN) method (detection limit of 0.48 log MPN/g) *(30)*. After preparation of serial dilutions, nine TSBYE tubes were inoculated at 3 × 0.1, 3 × 0.01, and 3 × 0.001 ml of inocula, and then incubated at 30°C up to 15 days to allow the recovery, germination, and growth of injured spores. The growth medium was then streaked on NA that was incubated at 30°C for 2 days to indicate any spore growth.

Statistical analysis of data. Statistical analysis was performed by applying analysis of variance and multiple comparisons of means of each treatment (process temperature and holding time) by using the least significant difference test at a confidence level of 95% (LSD_{95}). Analysis of data was carried out with Statistical Analysis System (SAS) software 9.2 (SAS Institute Inc., Cary, NC); the difference between mean values greater than the LSD_{95} was considered to be significant.

Kinetics of combined pressure–heat inactivation of spores in rice. The linear first-order kinetics and nonlinear Weibull and log-logistic models were used as follows to estimate the kinetic parameters of *B. cereus* spore inactivation in rice. The come-up time reductions were documented, and the spore count immediately after come-up time (N'_0) was considered the initial count. N in the following equations indicates the number of survivors after exposure to a lethal treatment for a specific time (t).

First-order kinetics. The D-value (the time required to reduce spore population by 1.0 log) for different process conditions were calculated from the linear portion of the survival curve after come-up time by using equation 2:

$$\log \frac{N}{N'_0} = -\frac{t}{D} \tag{2}$$

The temperature coefficient, z_T (in degrees Celsius), at constant pressure (i.e., the temperature change required at constant pressure to achieve a 10-fold change in the D-value) was also estimated as the negative reciprocal of the slope resulting from plotting log days against process temperature *(12)*.

Weibull model. The scale factor (b) and shape factor (n) of the cumulative form of the Weibull distribution (equation 3) as used by Peleg and Cole *(21)* were calculated:

$$\log \frac{N}{N'_0} = -bt^n \tag{3}$$

Log-logistic model. The parameters A (lower asymptote–upper asymptote), σ (maximum rate of inactivation), and τ (log time to the maximum rate of inactivation) of the log-logistic model as used by Cole et al. *(7)* and modified by Chen and Hoover *(5)* (equation 4) were calculated:

$$\log \frac{N}{N'_0} = \frac{A}{1+e^{\frac{4\sigma(\tau-\log\ t)}{A}}} - \frac{A}{1+e^{\frac{4\sigma(\tau+6)}{A}}} \tag{4}$$

Since log t at 0 min is not defined, a small value of t ($t = 10^{-6}$) was used to approximate $t = 0$ *(23)*.

The nonlinear curve fitting and model parameter estimation were achieved with the nonlinear PROG NLIN procedure of the SAS software. To validate the accuracy of the models and to make a comparison between the models, the mean square error (MSE; equation 5), regression coefficient (R^2), and accuracy factor (A_f; equation 6) values were calculated *(12, 22, 23)*. In the following equations, n stands for the number of observations. A relatively small MSE value and large R^2 value indicates a good fit to the model. An A_f value of close to 1 also indicates that the model produces a perfect fit to the data.

$$\text{MSE} = \frac{\sum(\text{predicted} - \text{observed})^2}{n} \tag{5}$$

$$A_f = 10^{[\sum|\log(\text{predicted}/\text{observed})|]/n} \tag{6}$$

In addition to the above, from the data on three-tube MPN recovery studies, a linear equation was also developed to estimate the process holding time required to achieve a 7.0-log reduction of *B. cereus* spores in rice at 600 MPa and each process temperature while preventing the spore recovery during storage of the treated product.

RESULTS AND DISCUSSION

Survival of *B. cereus* spores in rice after pressure–heat and thermal treatments. In the current study, enumeration of survivors on NA showed *B. cereus* spore inactivation to be below the detection limit (<10 CFU/g, i.e., ≥7.0-log reduction) after pressure holding times of 8, 6, and 4 min at 60, 75, and 85°C, respectively. The inactivation of *B. cereus* spores by combined pressure–heat treatment in buffer and food matrices was studied by few researchers. Raso et al. *(24)* reported a 5- to 6.5-log reduction of *B. cereus* ATCC 14579 in McIlvaine citrate phosphate buffer at pH 6.0 by high pressure treatment at 690 MPa and 40°C for 2 min when *B. cereus* was sporulated at 30 or 37°C. For those sporulated at 20°C, inactivation increased when pH was decreased. Oh and Moon *(20)* found

TABLE 1. *Recovery of* Bacillus cereus *ATCC 9818 spores in treated rice during storage of samples at 25°C for 4 weeks*[a]

Process conditions	Recovered *B. cereus*	
	1 wk of storage	4 wk of storage
60°C, 600 MPa, 8 min	1.2 ± 0.3 log MPN/g	4.8 ± 0.4 log CFU/g
60°C, 600 MPa, 15 min	<0.48 log MPN/g	<0.48 log MPN/g
75°C, 600 MPa, 6 min	0.96 ± 0.2 log MPN/g	3.8 ± 0.2 log CFU/g
75°C, 600 MPa, 10 min	<0.48 log MPN/g	<0.48 log MPN/g
85°C, 600 MPa, 4 min	<0.48 log MPN/g	<0.48 log MPN/g
85°C, 0.1 MPa, 180 min	<0.48 log MPN/g	<0.48 log MPN/g

[a] The spore viable counts were below the plate count detection limit (<1.0 log CFU/g) immediately after these treatments. The MPN detection limit was 0.48 log MPN/g.

that *B. cereus* KCTC 1012 spores suspended in McIlvaine buffer at pH 4.5 were more resistant to inactivation by high pressure treatment (≤600 MPa, 20 to 60°C, 15 min) than those at higher pH levels (6.0 to 8.0). Van Opstal et al. *(32)* reported >6-log reduction of *B. cereus* LMG 6910 in ultra-high temperature–treated skim milk by a combined pressure–heat treatment at 400 MPa and 60°C for 30 min. Scurrah et al. *(28)* reported 3.6- to 6.1-log reductions for various *B. cereus* strains (NZ 3, NZ 4, NZ 5, NZ 6, and NZ 7) in 9.5% reconstituted skim milk by a combined pressure–heat treatment (600 MPa, initial temperature 72°C, 1 min). Black et al. *(2)* achieved a 5.9-log reduction of *B. cereus* NIZO LB5 spores in 10% reconstituted skim milk by the addition of nisin to the milk at a concentration of 500 IU/ml and treatment at 500 MPa and 40°C for 5 min (cycled twice).

The viable count reduction of *B. cereus* spores during pressure come-up time in the current study was significant ($P < 0.05$) at all process temperatures tested. The spore viable counts were reduced by 2.2, 3.1, and 3.4 log during the 30-s pressure come-up time at 60, 75 and 85°C, respectively. Margosch et al. *(13)* reported inactivation of *B. cereus* TMW 2.383 spores in mashed carrots to undetectable level (>6.0-log reduction) during come-up time to reach the target process pressure of 800 MPa at 80°C. They also found considerable inactivation of other bacterial spores in mashed carrots during pressure come-up time for the same process conditions, including proteolytic *Clostridium botulinum* type B TMW 2.357 (1.5-log reduction), *Thermoanaerobacterium thermosaccharolyticum* TMW 2.299 (4-log reduction), and *Bacillus smithii* TMW 2.487 (3-log reduction). Remarkable spore inactivation was reported during come-up time to 700 MPa and 105°C for *Clostridium tyrobutylicum* ATCC 25755 (2.5-log reduction), *Clostridium sporogenes* ATCC 7955 (3.3-log reduction), *Bacillus*

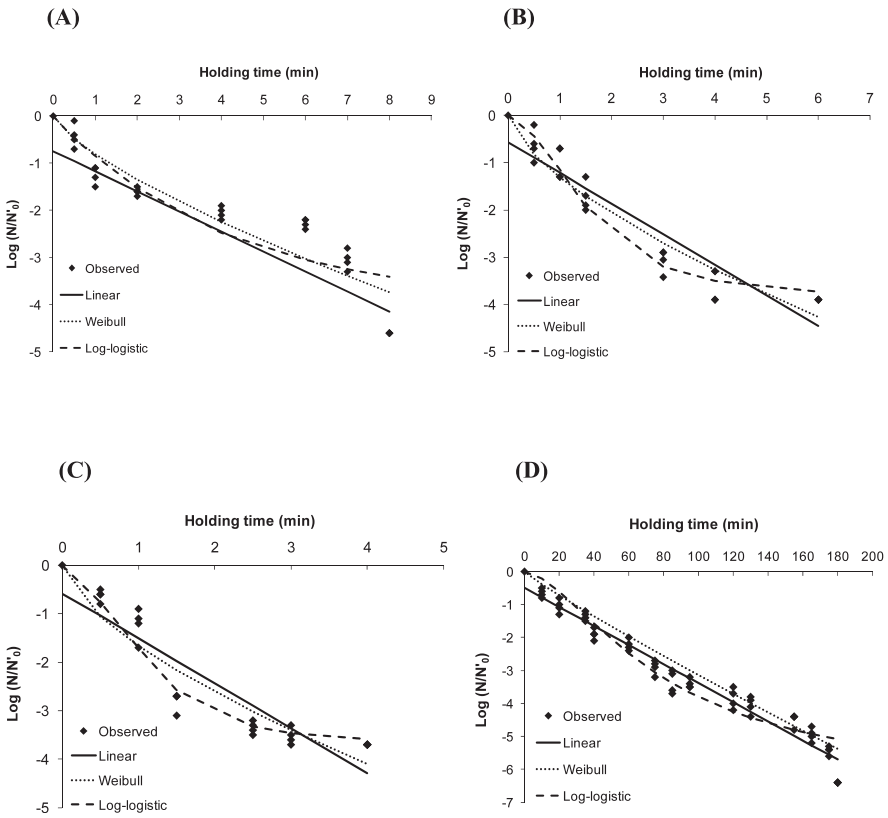

FIGURE 2. *Inactivation kinetics of* Bacillus cereus *ATCC 9818 spores in rice during treatment at 600 MPa and various process temperatures: 60°C (A), 75°C (B), 85°C (C), or 0.1 MPa and 85°C (D), fitted by linear, Weibull, and log-logistic models.* N'_0 *and* N *are the spore counts (in CFU per gram) immediately after come-up time and after holding time, respectively. The spores were inoculated into the product at an inoculum level of 1.1 × 10^8 CFU/g prior to treatments. Detection limit was 10 CFU/g.*

TABLE 2. *Estimation of the kinetic parameters of* Bacillus cereus *ATCC 9818 spore inactivation in rice by pressure–heat treatment (600 MPa, 60 to 85°C) or thermal processing (0.1 MPa, 85°C) by using linear and nonlinear models*[a]

Process	Temp (°C)	Pressure (MPa)	Log reduction during come-up time [$\log(N'_0/N_0)$][b]	Linear			Weibull				Log logistic					
				D (min)	MSE	R^2	A_f	b	n	MSE	R^2	A_f	A	σ	τ	MSE	R^2	A_f
Pressure–heat	60	600	2.2 ± 0.2	2.36 ± 0.04	0.31	0.86	1.34	0.81	0.73	0.26	0.86	1.30	−4.4	−3.34	0.58	0.35	0.79	1.30
Pressure–heat	75	600	3.1 ± 0.1	1.54 ± 0.03	0.22	0.87	1.29	1.31	0.66	0.14	0.92	1.24	−3.7	−4.90	0.20	0.09	0.96	1.23
Pressure–heat	85	600	3.4 ± 0.1	1.08 ± 0.02	0.27	0.83	1.29	1.66	0.65	0.20	0.88	1.26	−3.2	−5.10	0.08	0.08	0.98	1.14
Thermal	85	0.1	0.3 ± 0.0	34.6 ± 1.1	0.12	0.96	1.09	0.05	0.92	0.21	0.93	1.19	−6.4	−6.04	1.90	0.24	0.92	1.23

[a] Kinetic parameters were calculated after excluding log reductions during process come-up time. The spores were inoculated in the product at an inoculum level of 1.1×10^8 CFU/g prior to treatments.

[b] N'_0 and N are the spore counts (in CFU per gram) immediately after come-up time and after pressure holding time, respectively.

sphaericus NZ 14 (3.7-log reduction), *Bacillus amyloliquefaciens* ATCC 49763 (2.5-log reduction), and *Geobacillus stearothermophilus* ATCC 7953 (1.5-log reduction) *(1, 23)*.

A 180-min treatment time was required to inactivate the *B. cereus* ATCC 9818 spores in rice to an undetectable level (<10 CFU/g) at 85°C under atmospheric conditions in the current study, with no significant inactivation ($P > 0.05$) during the temperature come-up time.

Microbial stability of pressure–heat- and heat-treated rice during storage. Combined pressure–heat treatment can result in injury of a proportion of the microbial population (spores and vegetative bacteria) rather than inactivation. This might be a function of process parameters (pressure, temperature, and holding time) and substrate composition, pH, a_w, etc. The injury can cause an overestimation of the process lethality, since the injured cells might not be detected with selective conditions for the enumeration of survivors. Further, the injured bacteria can recover if present in a nutrient environment.

In the current study, in addition to the plating method, a standard three-tube MPN technique was used to evaluate the potential recovery of injured *B. cereus* if present in the treated rice product. The three-tube MPN method is recommended by the U.S. Food and Drug Administration for routine surveillance of products in which small numbers of spore-forming bacteria such as *B. cereus* are anticipated *(29)*. It was also used by other researchers to estimate the survivor spore population in the pressure-heat–treated foods in which the spores were not detected by direct plating method *(4, 9, 25, 26)*. The *B. cereus* counts in the processed rice samples in which an undetectable inactivation level (<10 CFU/g) was achieved on NA (i.e., pressure–heat treated at 600 MPa and 60°C for 8 min, 75°C for 6 min, or 85°C for 4 min; and heat treated at 85°C for 180 min) were also below the MPN detection limit (0.48 log MPN/g) immediately after treatment (data not shown). However, the MPN values increased significantly ($P < 0.05$) during storage at 25°C in the samples treated at 600 MPa and 60°C for 8 min, or 600 MPa and 75°C for 6 min, reaching 1.2 and 0.96 log MPN/g, respectively after 1 week (Table 1). The viable counts in these samples further increased to 4.8 and 3.8 log CFU/g after 4 weeks of storage at 25°C. In contrast, no recovery of injured spores was detected ($P > 0.05$) in any of the pressure–heat- or heat-treated samples stored at 4°C (data not shown). Extending the pressure–heat treatment holding time helped prevent the spore recovery in the samples treated at 600 MPa at lower process temperatures (60 or 75°C) during storage at 25°C (Table 1). Due to experimental constraints, we did not conduct studies at different process times to exactly identify the boundary separating the treatment conditions resulting in spore injury from those that caused no injury. However, our results do demonstrate that the recovery of injured *B. cereus* spores can be controlled by the process temperature and the extent of pressure holding time during combined pressure–heat treatment. Ratphitagsanti et al. *(25)* reported significant recovery and growth (>7 log CFU/ml) of *B. amyloliquefaciens* TMW 2.479 Fad 82 spores in pressure-assisted,

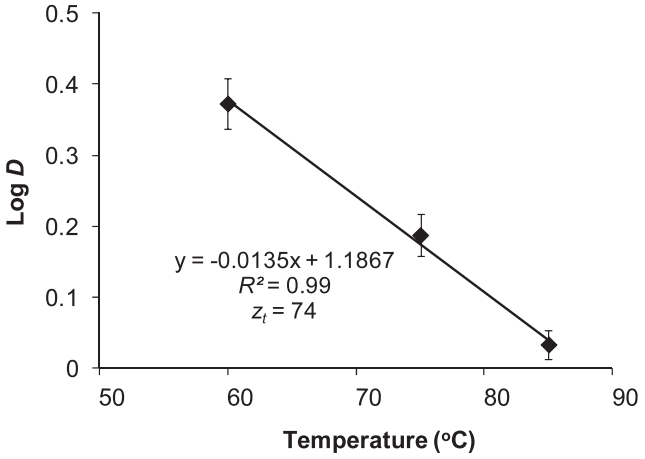

FIGURE 3. *Estimation of* z_T*-value of* Bacillus cereus *ATCC 9818 spore inactivation in rice by combined pressure–heat treatment (600 MPa, 60 to 85°C).*

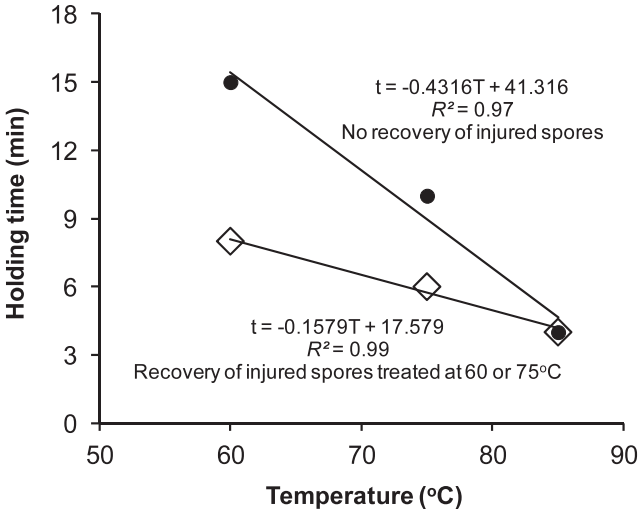

FIGURE 4. *Process time required to inactivate* Bacillus cereus *ATCC 9818 spores in rice to below the detection limit of 10 CFU/g (≥7.0-log reduction) at 600 MPa and various process temperatures* (T)*, with recovery of injured spores during subsequent storage of samples at 25°C (◇), or without recovery of injured spores (i.e., viable spores were below 0.48 log MPN/g) during storage at 25°C (●).*

thermally treated carrot purée (700 MPa, 105°C, 5 min) initially inactivated to the undetectable level of the plate-count method ($<10^2$ CFU/ml) during subsequent storage of the treated product at 32°C for 28 days. Increasing the treatment holding time from 5 to 15 min or adding organic acids to the inoculated carrot puree prior to treatment inhibited spore recovery during storage.

Kinetics of *B. cereus* spore inactivation in rice. In the current study, an increase in the process temperature at the constant pressure of 600 MPa significantly enhanced ($P < 0.05$) the inactivation of *B. cereus* ATCC 9818 spores in rice (Fig. 2). The patterns of the inactivation kinetics of *B. cereus* spores in rice by combined pressure–heat treatment in the current study were nonlinear at all process temperatures tested. The tailing characteristics were observed in all pressure–heat survival curves (Fig. 2). Table 2 presents the kinetic parameters of spore inactivation, estimated with linear and nonlinear models. At the lowest process temperature (i.e., 60°C), the nonlinear Weibull model provided a slightly better fit with the spore inactivation data than the first-order kinetics, as indicated by lower MSE and A_f values. The log-logistic model provided best fitting at higher process temperature (75 and 85°C) among the three kinetic models tested, as demonstrated by the highest R^2 and lowest MSE values, as well as A_f values closer to 1. Daryaei and Balasubramaniam *(8)* reported better curve fitting of the log-logistic model to the pressure–heat inactivation data of *Bacillus coagulans* 185A spores in tomato juice at 95 to 105°C than at lower process temperatures. Wang et al. *(33)* found best fitting of the log-logistic model with all the survival curves of *B. coagulans* IFFI 10144 spore inactivation in phosphate buffer (pH 6.7) and ultrahigh temperature–treated whole milk by combined pressure–heat treatment (400 to 600 MPa at 70 and 80°C), followed by the Weibull model.

Inactivation of *B. cereus* spores by thermal processing at 85°C was nearly linear (Fig. 2D). The lowest MSE, highest R^2, and an A_f value of close to 1.0 was calculated for the first-order kinetics for thermal inactivation of spores among the three models tested (Table 2). Further, the shape factor (n) of the Weibull model was close to 1.0, indicating the linearity of the survival curve in the absence of high pressure.

The D-value of spores decreased significantly ($P < 0.05$) with increasing process temperature during pressure–heat treatment (Table 2). The D-value of *B. cereus* spores inactivation at 85°C under atmospheric conditions (D = 34.6 min) was noticeably higher than that of combined pressure–heat treatment (D = 1.08 min), showing the significant lethal contribution of high pressure to the spore inactivation. The accuracy of pressure–heat inactivation D-values obtained in this study is still questioned due to the nonlinearity of the survival curves. The thermal-inactivation D-values of *B. cereus* ATCC 9818 spores were reported to be remarkably higher than those of *B. cereus* ATCC 4342 and *B. cereus* ATCC 7004 spores in low-acid substrates (milk and pH 7.0 buffer) and acidic media (orange juice and pH 4.5 buffer) in the temperature range of 70 to 100°C *(10, 14, 17)*.

The Weibull model scale factor (b) increased and was inversely related to the D-value, and the shape factor (n) decreased with increasing temperature (Table 2). This was consistent with the findings reported by Daryaei and Balasubramaniam *(8)* for the inactivation of *B. coagulans* 185A spores in tomato juice at 600 MPa and 75 to 105°C, and by Rajan et al. *(22)* for the inactivation of *B. amyloliquefaciens* spores in egg patty mince at 600 MPa and 95 to 121°C. All n values in the current study were less than 1 (Table 2), indicating that the survival curves were concave upward.

The estimated z_T-value of *B. cereus* ATCC 9818 spores at the constant pressure of 600 MPa, determined by plotting the log D-values versus process temperatures and taking the reciprocal of the slope from linear regression, was about

74°C (R^2 = 0.99) (Fig. 3). The z_T-value for thermal inactivation of *B. cereus* ATCC 9818 spores was reported by other researchers at ranges of 10.5 to 36.5°C *(17, 19)*. The larger z_T-value of the spores under pressure–heat, as obtained in the current study, could indicate that pressure could reduce the lethal effect of temperature on spores in a combined pressure–heat treatment. In other words, the spores are possibly more sensitive to the temperature change at atmospheric pressure than at elevated pressures. Rajan et al. *(22)* similarly found that the z_T-value of *B. amyloliquefaciens* spores in egg patty mince ranged from 8.2°C at 0.1 MPa to 26.8°C at 700 MPa within the process temperature range of 95 to121°C. Miglioli et al. *(15)* reported z_T-values of 11.8°C at 0.1 MPa and 17.1°C at 700 MPa for *C. sporogenes* spores.

Figure 4 shows the plot of pressure holding time required to inactivate the *B. cereus* ATCC 9818 spores in rice (initial count of 1.1×10^8 CFU/g) to below the detection limit of 10 CFU/g (≥7.0-log reduction) at 600 MPa and various process temperatures with or without recovery of injured spores during subsequent storage of the treated product samples at 25°C. It is interesting to note that in both cases the relationship was linear. This relationship may be used to estimate the required holding time to achieve ≥7.0-log reduction in the product.

The current study demonstrates that combined pressure–heat treatment of rice (pH 6.0 and a_w of 0.95) at 600 MPa and process temperatures as low as 60°C for 15 min could effectively inactivate and prevent the outgrowth of *B. cereus* spores in the product. Alternatively, a 600-MPa pressure treatment at 85°C process temperature with a pressure holding time as short as 4 min was also effective and would permit a greater throughput. *B. cereus* spores seemed to be much more sensitive to combined pressure–heat treatment than thermal processing alone. This enables application of pressure–heat to the product as a final preservation step to reduce the risk of spore growth during storage if the spores possibly survived cooking.

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