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Thermal Inactivation Kinetics of *Bacillus coagulans* Spores in Tomato Juice

JING PENG,[1] JAE-HYUNG MAH,[2] ROMEL SOMAVAT,[3] HUSSEIN MOHAMED,[4] SUDHIR SASTRY,[3] AND JUMING TANG[1]*

[1]*Department of Biological Systems Engineering, Washington State University, Pullman, Washington 99163, USA;* [2]*Department of Food and Biotechnology, Korea University, Jochiwon, Chungnam 339-700, South Korea;* [3]*Department of Food, Agricultural and Biological Engineering, The Ohio State University, Columbus, Ohio 43210, USA; and* [4]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt*

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ABSTRACT

The thermal characteristics of the spores and vegetative cells of three strains of *Bacillus coagulans* (ATCC 8038, ATCC 7050, and 185A) in tomato juice were evaluated. *B. coagulans* ATCC 8038 was chosen as the target microorganism for thermal processing of tomato products due to its spores having the highest thermal resistance among the three strains. The thermal inactivation kinetics of *B. coagulans* ATCC 8038 spores in tomato juice between 95 and 115°C were determined independently in two different laboratories using two different heating setups. The results obtained from both laboratories were in general agreement, with *z*-values (*z*-value is defined as the change in temperature required for a 10-fold reduction of the *D*-value, which is defined as the time required at a certain temperature for a 1-log reduction of the target microorganisms) of 8.3 and 8.7°C, respectively. The *z*-value of *B. coagulans* 185A spores in tomato juice (pH 4.3) was found to be 10.2°C. The influence of environmental factors, including cold storage time, pH, and preconditioning, upon the thermal resistance of these bacterial spores is discussed. The results obtained showed that a storage temperature of 4°C was appropriate for maintaining the viability and thermal resistance of *B. coagulans* ATCC 8038 spores. Acidifying the pH of tomato juice decreased the thermal resistance of these spores. A 1-h exposure at room temperature was considered optimal for preconditioning *B. coagulans* ATCC 8038 spores in tomato juice.

Bacillus coagulans, a facultative anaerobic spore-forming bacterium, is acid tolerant and grows well in foods at pH 4.0 to 4.5 at ambient temperature. This is the single organism most frequently isolated from spoiled canned vegetables acidified to pH 4.0 to 4.5 and has been considered the primary cause of economically important spoilage in thermally processed tomatoes and tomato products *(8, 19)*. It results in a type of spoilage commonly referred to as flat sour in tomato-based products *(14, 17, 19, 23)*. Although *B. coagulans* is a nonpathogenic microorganism, it may cause a food safety hazard due to its ability to increase the pH of acidic foods, processed with a reduced treatment, to a level that can allow the germination of surviving *Clostridium botulinum* spores *(1, 2)*.

Thermal processing is the most common and effective method for inactivation of microorganisms and extending the shelf life of tomato juice. Most published data related to the inactivation of *B. coagulans* spores in food media are based on studies performed with moderate heat only *(17, 23)* or by combining heat treatment with other technologies, such as high pressure *(5, 12, 21)*. For high-temperature heating, Palop et al. *(13, 14)* studied heat resistance in food medium (pH 4 and 7) between 105 and 130°C, using a strain isolated from canned asparagus, and Mallidis et al. *(8)* obtained the *z*-value (*z*-value is defined as the change in temperature required for a 10-fold reduction of the *D*-value, which is defined as the time required at a certain temperature for a 1-log reduction of the target microorganisms) of *B. coagulans* spores in tomato serum (pH 4.24) at temperatures in a narrow range (95 to 105°C). Several studies also reported on the inactivation of *B. coagulans* spores by hydrodynamic cavitation *(10),* supercritical CO_2 microbubble treatment *(4),* and high pressure with nisin *(3)*.

The equilibrium pH of tomatoes varies from 4.0 to 4.7, depending on the variety and ripeness *(20)*. The practice of acidification of canned tomato juice to a pH lower than 4.5 before treatment prevents the outgrowth of spores surviving heat treatment, particularly spores of *C. botulinum (11)*. Although the heat resistance of *B. coagulans* spores in tomatoes has been studied at acid pH *(8, 14, 16, 17, 23),* information about heat resistance at different acidic pH levels between 4.0 and 4.5 (the commonly controlled pH range for canned tomato products) is still limited, especially under high temperatures. Since the thermal resistance of *B. coagulans* spores is strain dependent, three strains of this microorganism (ATCC 8038, ATCC 7050, and 185A) were investigated in this study, although the first two strains have been used more frequently in previously published work *(10, 15, 16, 22, 23)*. The objective of this study was to

* Author for correspondence. Tel: 509-335-2140; Fax: 509-335-2722; E-mail: jtang@wsu.edu.

characterize the thermal resistance of *B. coagulans* spores in tomato juice products at pHs between 4.0 and 4.4 using different heating setups in two different laboratories. The influence of some environmental factors, including cold storage, pH, and preconditioning, upon the thermal resistance of this microorganism was also investigated. The current study provides theoretical support for developing and validating thermal pasteurization processes of tomato products.

MATERIALS AND METHODS

Microorganisms. *B. coagulans* strains ATCC 7050 and ATCC 8038 were purchased from the American Type Culture Collection (Manassas, VA). *B. coagulans* strain 185A was obtained from Dr. V. M. Balasubramaniam at The Ohio State University (OSU) *(6)*. These strains were grown aerobically in nutrient broth (Difco Laboratories, Inc., Detroit, MI) for 48 h at 37°C and then resuspended in nutrient broth containing 20% glycerol. The stock culture was divided into sterile cryogenic vials (Fisher Scientific, Pittsburgh, PA) and then stored in a freezer (−20°C) until further use.

Preparation of *B. coagulans* spores. To induce sporulation of vegetative cells of *B. coagulans,* the procedures described by Palop et al. *(14)* and modified by Milly et al. *(10)* were used. Briefly, vegetative cells of *B. coagulans* were grown in nutrient broth aerobically for 48 h at 37°C and transferred into nutrient broth at least three times before spore preparation. Spores of *B. coagulans* were prepared by distributing 1 ml of actively growing vegetative cells (48 h at 37°C) onto a plate containing nutrient agar (Difco, BD, Sparks, MD) fortified with 500 mg/liter of dextrose (Bacto Dextrose, Difco) and 3 mg/liter of manganese sulfate (Fisher Scientific). The inoculated plates were incubated at 50°C for 7 days, when more than 90% sporulation was obtained as verified by observing the refractive spores under phase-contrast microscopy. Spores were harvested by flooding plates with 5 ml of cold, sterile, deionized water and dislodging spores from the agar surface with a sterile disposable inoculation loop. After harvesting, the spores were washed three times by centrifugation at 14,000 × *g* at 4°C for 10 min, resuspended in sterile, deionized water, and stored at 4°C until used.

Preparation of tomato juice. Two forms of tomato juice were used: commercial tomato juice (Campbell Soup Co., Camden, NJ) was used in the Washington State University (WSU) study involving oil bath heating, and fresh Roma tomatoes bought from a local grocery store (Kroger, Inc., Columbus, OH) were used in the OSU study. Tomatoes of bright red color (with an ''a'' value of 20) were cut into quarters and then blended to prepare the tomato juice medium.

Evaluation of cold-storage time on the viability of *B. coagulans* in sterile distilled water and its thermal resistance in tomato juice. Cultured *B. coagulans* spores (ATCC 8038 and ATCC 7050) were suspended in sterile distilled water and stored at 4°C. The viable numbers of vegetative cells and spores were counted after 10, 23, and 31 days of storage to study the effect of refrigerated storage on the viability of this microorganism. For enumeration, the spore suspensions were heat shocked at 80°C for 15 min, cooled in a crushed-ice-water bath, and checked microscopically to ensure the absence of vegetative cells. The spore count was obtained by preparation of 10-fold serial dilutions in sterile 0.1% peptone water. One hundred microliters of each dilution was spread plated onto nutrient agar and incubated for 7 days (ATCC strains) or 2 days (185A) at 37°C. The spore numbers were calculated from three replicates. The vegetative cells in the spore suspension were counted by the same procedures but without the heat-shocking step.

To further investigate the response of this microorganism to cold storage, the thermal resistance of prepared *B. coagulans* ATCC 8038 spores in tomato juice (pH 4.0) was measured at 100°C after 0, 10, and 28 days of cold storage. The thermal resistance of *B. coagulans* spores in tomato juice was determined by following the procedures described below.

Preparation and preconditioning of a mixture of spore suspension and tomato juice. The pH of tomato juice (Campbell Soup Co.), initially ranging from 4.0 to 4.1, was adjusted to different values by adding 1 M sodium citrate or citric acid to evaluate the influence of the pH of the heating medium on microbial heat resistance. To precondition spores in tomato juice (adjusted to pH 4.3), capillary tubes with a mixture of 50 μl of tomato juice inoculated with the spore suspension were placed at 4°C or room temperature for time periods of 1, 2, 3, and 4 h. The *D*-values at 100°C ($D_{100°C}$-values) of preconditioned mixtures were determined by following the procedures described below.

Evaluation of heat resistance of *B. coagulans* spores using oil bath. The thermal resistance of test microorganisms was determined by thermal death time tests and reflected by *D*- and *z*-values. Since the thermal destruction of *B. coagulans* generally follows a first-order reaction based on most published data *(14, 17, 19),* the *D*-values of *B. coagulans* spores were obtained by taking the negative reciprocal of the slope from linear regression of the survivor curves. The *z*-value was estimated by plotting the log *D*-values versus heating temperatures and taking the negative reciprocal of the slope from linear regression. Fifty microliters of tomato juice inoculated with spore suspension (initial spore concentration was 10^8 CFU/ml) was injected into a glass capillary tube with an inner diameter of 1.8 mm and an outer diameter of 3 mm (Corning, Inc., Corning, NY) using a pipette, and the open ends of the tubes were heat sealed. The tubes were immersed completely in a circulating oil bath (Thermo Electron Corporation, Waltham, MA) and heated to between 95 and 115°C for different time intervals. The come-up time (the time for sample to reach within 0.5°C of the target temperature) was around 5 s. After heating, the tubes were removed from the oil bath, cooled immediately in a crushed-ice-water bath, and washed in 70% ethyl alcohol. Both tube ends were cut aseptically, and the suspension was flushed out with 3 ml of sterile 0.1% peptone water. The treated samples were then 10-fold serially diluted in sterile 0.1% peptone water and spread plated onto nutrient agar medium. Based on our preliminary test results, the ATCC strains were incubated for 7 days and the 185A strain for 2 days at 37°C, and then colonies were manually counted.

Evaluation of heat resistance of *B. coagulans* spores using a capillary tube setup. The evaluation of heat resistance of *B. coagulans* spores using a capillary tube setup was performed in the Department of Food, Agricultural and Biological Engineering at OSU using *B. coagulans* ATCC 8038 spores prepared in the same way as described above. Tomato juice (inherent pH ranging from 4.1 to 4.3) was adjusted to a standard value of 4.4 using sodium citrate to prevent the varying acidity from affecting the thermal resistance of the organism. Tomato juice samples inoculated with *B. coagulans* spores were heated in conventional capillary cells *(18)* and treated at temperatures ranging from 95 to 110°C for

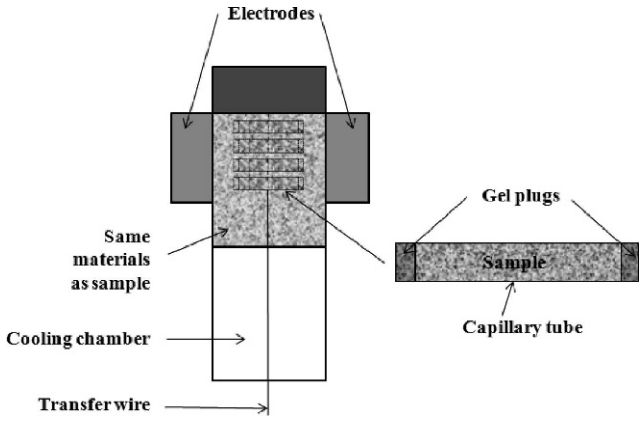

FIGURE 1. *Schematic of kinetics treatment chamber, with spore suspensions within samples in capillary tubes. Gel plugs in the capillary tubes are nonconductive, so that the sample heats by heat transfer from an outer ohmic heater. Samples are withdrawn into a cooling chamber after processing.*

different time intervals. The come-up times were 158, 170, 180, and 192 s when heating from room temperature to 95, 100, 105, and 110°C, respectively. All zero-time samples were allowed to reach the process temperature and then immediately cooled to provide initial count data. Two sample-containing capillary cells mounted on each capillary tube holder were used for each holding time. All tests were replicated three times.

For the system design, the capillary tubes used at OSU containing 37 μl of tomato juice inoculated with *B. coagulans* spores were plugged at both ends with nonconductive capillary tube sealant. The capillary tubes were placed inside an external ohmic heating device to enable heating under pressurized conditions. The samples in the capillaries had the insulating gel plugs at the ends to prevent their heating ohmically; thus, all capillaries heated by heat transfer from the external, ohmically heated medium. To hold the capillary cells in place, they were mounted on cell holders (two cells per holder) attached to the treatment chamber (Figure. 1). Temperatures were measured in selected, thermocouple-containing capillary cells. The system also facilitated rapid posttreatment cooling through pulling of the treated samples into the cooling section with the help of an attached thread. A detailed description of the setup and procedures is provided by Somavat et al. *(18).*

To enumerate spore survival, treated capillary cells were washed with a cold, 1,400-ppm hypochlorite solution and rinsed with cold, sterile water. The capillary washing protocol developed by Somavat et al. *(18)* was followed to prevent any residual hypochlorite from affecting the final plate count. The clean capillary cells were then crushed inside sterile polypropylene tubes containing 0.1% peptone water using sterile glass rods. A heat shock of 80°C for 15 min was given to inactivate all vegetative cells. Ten-fold serial dilutions in peptone water were prepared and spread plated onto tryptic soy agar plates. Inoculated plates were incubated for 48 hours at 37°C, and colonies enumerated.

RESULTS AND DISCUSSION

Effect of cold-storage time on the viability of *B. coagulans* in sterile distilled water and its thermal resistance in tomato juice. To investigate whether storage at 4°C influences the viability of *B. coagulans,* viable numbers of vegetative cells and spores in sterile distilled water were counted over a period of 1 month. The results in Table 1 show that there were few changes in the viable numbers of both vegetative cells and spores of *B. coagulans* ATCC 8038 during 4°C storage. In contrast, viable vegetative cells of ATCC 7050 experienced a 1-log reduction after 10 days of cold storage, and no viable vegetative cells could be detected after 23 days of storage (detection limit, ≤5 log CFU/ml). Because the spores produced by the ATCC 8038 strain (ca. 10^8 CFU/ml) were much more numerous than those obtained from the ATCC 7050 strain (below the detection limit, ≤5 log CFU/ml) when grown under the same conditions and, also, due to the stable viability of ATCC 8038 stored in sterile distilled water at 4°C, *B. coagulans* ATCC 8038 was chosen for further study.

In addition, since the sporulation temperature of 50°C was relatively high and the sporulation time of 7 days was relatively long, maintaining the moisture content of the sporulation medium was taken into consideration. The viability of spores produced in a moist chamber was compared with those cultured without a moist chamber, along with the viability of their vegetative cells (Table 1). It was found that maintaining the moisture content of the sporulation medium decreased the viability of both spores and vegetative cells, and the reduction of viability was more evident with spores than with vegetative cells. Therefore, *B. coagulans* was grown on sporulation medium without the use of a moist chamber in order to produce high-viability spores.

To further investigate the effect of cold storage on this microorganism, *B. coagulans* ATCC 8038 spores stored at 4°C for up to 28 days were used to inoculate tomato juice (pH 4.0), after which $D_{100°C}$-values were determined. $D_{100°C}$-values of 2.56 ± 0.00, 2.09 ± 0.34, and 2.87 ± 0.03 were found after 0, 10, and 28 days of storage, respectively. No significant differences ($P > 0.05$) in *D*-values were found. These results demonstrate the relative stability of thermal resistance of those spores under cold storage conditions. Therefore, a temperature of 4°C was used for storing *B. coagulans* ATCC 8038 spores. Similar results were observed in our previous study *(7),* which showed that storing *Clostridium sporogenes* PA 3679 spores at 4°C is satisfactory for maintaining the viability and heat resistance of those spores during short-term storage.

Effect of pH on the thermal resistance of *B. coagulans* ATCC 8038 spores. According to published data, most authors have revealed that acidification of the heating medium causes a decrease in microbial heat resistance. Palop et al. *(14)* investigated the thermal resistance of *B. coagulans* spores in homogenized tomato and asparagus at pH 7 and 4 at temperatures between 105 and 130°C and found that the spores were less heat resistant in both food media at pH 4. Similar results were obtained by Mazas et al. *(9),* who reported sharp *D*-value reductions for spores of three *Bacillus cereus* strains by acidifying the pH of the heating medium from 7.0 to 4.0. In the current study, the effect of pH on the thermal resistance of *B. coagulans* ATCC 8038 spores in commercial tomato juice was

TABLE 1. *Effect of 4°C storage on the viability of vegetative cells and spores of* B. coagulans

Strain and sporulation environment	Count (log CFU/ml) at indicated storage time (days)[a]							
	Vegetative cells				Spores			
	0	10	23	31	0	10	23	31
ATCC 8038, not in moist chamber[b]	8.94 ± 0.00	8.49 ± 0.06	8.71 ± 0.25	8.77 ± 0.16	8.81 ± 0.05	8.82 ± 0.03	9.35 ± 0.08	9.02 ± 0.14
ATCC 8038, in moist chamber[c]	8.57 ± 0.07	8.10 ± 0.10	8.07 ± 0.66	8.27 ± 0.05	7.84 ± 0.18	7.96 ± 0.04	8.68 ± 0.03	8.55 ± 0.04
ATCC 7050, not in moist chamber[b]	6.69 ± 0.12	5.63 ± 0.43	ND[d]	ND	ND	ND	ND	ND
ATCC 7050, in moist chamber[c]	6.54 ± 0.09	5.60 ± 0.46	ND	ND	ND	ND	ND	ND

[a] Data are the means ± standard deviations of replicates.
[b] Incubated without moist chamber: plates sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI) only.
[c] Incubated in moist chamber: plates sealed with Parafilm and petri dish containing water were put into a plastic bag to prevent moisture loss of the sporulation medium.
[d] ND, colonies not detectable; detection limit 5 log CFU/ml.

FIGURE 2. $D_{100°C}$-*values of* B. coagulans *ATCC 8038 spores exposed to different pH levels in tomato juice. Data are the means ± standard deviations of three replicates. Data obtained at WSU.*

examined by determining the $D_{100°C}$-values of the spores in commercial tomato juice adjusted to pH 3.8, 4.0, and 4.3 by adding sodium citrate. Significant differences in $D_{100°C}$-values were found at different pH levels ($P < 0.05$). As shown in Figure 2, the $D_{100°C}$-values increased from 2.85 min at pH 3.8 to 3.85 min at pH 4.3, which indicates that the thermal resistance of *B. coagulans* ATCC 8038 spores is influenced by tomato juice pH, decreasing with increased acidification.

Effect of preconditioning on the thermal resistance of *B. coagulans* ATCC 8038 spores. The influence of the preconditioning time of commercial tomato juice on the thermal resistance of *B. coagulans* ATCC 8038 spores was evaluated by exposing the spores to tomato juice for 1, 2, 3, and 4 h at room temperature and at 4°C, respectively. As shown in Figure 3, there were no significant differences ($P > 0.05$) in the $D_{100°C}$-values for the different preconditioning times at each treatment temperature. However, a significant difference ($P < 0.05$) in $D_{100°C}$-values was found between the two temperatures. The $D_{100°C}$-values of *B. coagulans* ATCC 8038 spores exposed to tomato juice at room temperature were higher than the $D_{100°C}$-values of those exposed to 4°C (3.5 versus 2.86 min, respectively). Therefore, a 1-h exposure at room temperature was considered to be optimum for preconditioning *B. coagulans* ATCC 8038 spores in tomato juice.

Thermal resistance of *B. coagulans* ATCC 8038 spores in tomato juice. The thermal resistance of *B. coagulans* ATCC 8038 spores at different temperatures in commercial tomato juice heated in an oil bath was determined by means of *D*-value measurements. Figure 4 shows typical thermal survivor curves of *B. coagulans* ATCC 8038 spores in tomato juice (pH 4.0). The *D*-values of *B. coagulans* ATCC 8038 spores decreased with increasing heating temperature. *D*-values of 7.05 min at 95°C, 2.56 min at 100°C, 1.18 min at 105°C, and 0.20 min at 110°C were obtained. The calculated *z*-value of *B. coagulans* ATCC

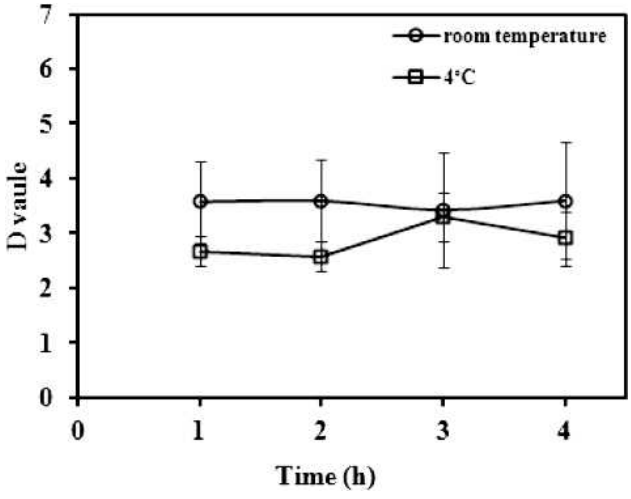

FIGURE 3. *Effect of preconditioning time on* $D_{100°C}$*-value of* B. coagulans *ATCC 8038 spores in commercial tomato juice at pH 4.3.* $D_{100°C}$*-value of control (spores in tomato juice without preconditioning) was <3 min. Data are the means ± standard deviations of duplicates. Data obtained at WSU.*

8038 spores in commercial tomato juice at pH 4.0 was 10.0°C. This value is higher than that obtained by Milly et al. *(10),* who obtained a *z*-value of 8°C when the spores were treated in tomato juice at pH 4.1. Since only the $D_{100°C}$-value and *z*-value were reported in that study, the difference in *z*-values between the two studies might be due to the different pH of tomato juice used in both studies. Sandoval et al. *(17)* determined the *D*-values of a strain of *B. coagulans* spores in double-concentrated tomato paste (pH 4.0) at 75, 80, 85, and 90°C and reported a corresponding *z*-value of 9.5°C. The different *z*-value obtained by Sandoval et al. could be due to the use of different strains, sporulation temperatures, and water activity.

The *D*- and *z*-values of spores in commercial tomato juice at pH 4.3 are shown in Table 2, along with the thermal inactivation data at pH 4.0. The *D*-value of *B. coagulans* ATCC 8038 spores in tomato juice at pH 4.3 was 4.56 min at 100°C, 1.20 min at 105°C, 0.27 min at 110°C, and 0.07 min at 115°C, with a corresponding *z*-value of 8.3°C. As seen in Table 2, all the *D*-values obtained at pH 4.3 under the same heating temperature were higher than the corresponding values obtained at pH 4.0, which demonstrated that acidifying the pH of the heating medium could reduce the thermal resistance of bacterial spores. This is in agreement with our previous results shown in Figure 2. Meanwhile, the *D*-values of the spores in tomato juice dropped from 4.56 min (pH 4.3) to 2.56 min (pH 4.0) at 100°C, whereas this value decreased only from 0.27 to 0.20 min at 110°C. This illustrates the reduced influence of acidification of the heating medium to depress the thermal resistance of bacterial spores at higher temperatures. However, an increase in the *z*-values of *B. coagulans* ATCC 8038 spores with acidification was observed from 8.3°C at pH 4.3 to 10.0°C at pH 4.0. Similar trends were observed by Palop et al. *(14),* who studied the heat resistance of *B. coagulans* spores (STCC 4522) in homogenized tomato at pH 7 and 4.

Thermal resistance of *B. coagulans* 185A spores at pH 4.3. Thermal resistance of *B. coagulans* 185A can only be found associated with thermal-assisted pressure processing *(6)* among published research. In the present study, the *D*- and *z*-values of *B. coagulans* 185A spores were determined following the same procedures as for *B. coagulans* ATCC 8038 spores and are shown in Table 2. Similar to the ATCC strains, the *D*-values of strain 185A spores decreased with increasing heat treatment temperature, showing *D*-values of 1.41 min at 100°C, 0.53 min at

FIGURE 4. *Thermal survivor curves for* B. coagulans *ATCC 8038 spores at different temperatures in commercial tomato juice at pH 4.0. Data are the means ± standards deviation of three replicates. Data obtained at WSU.*

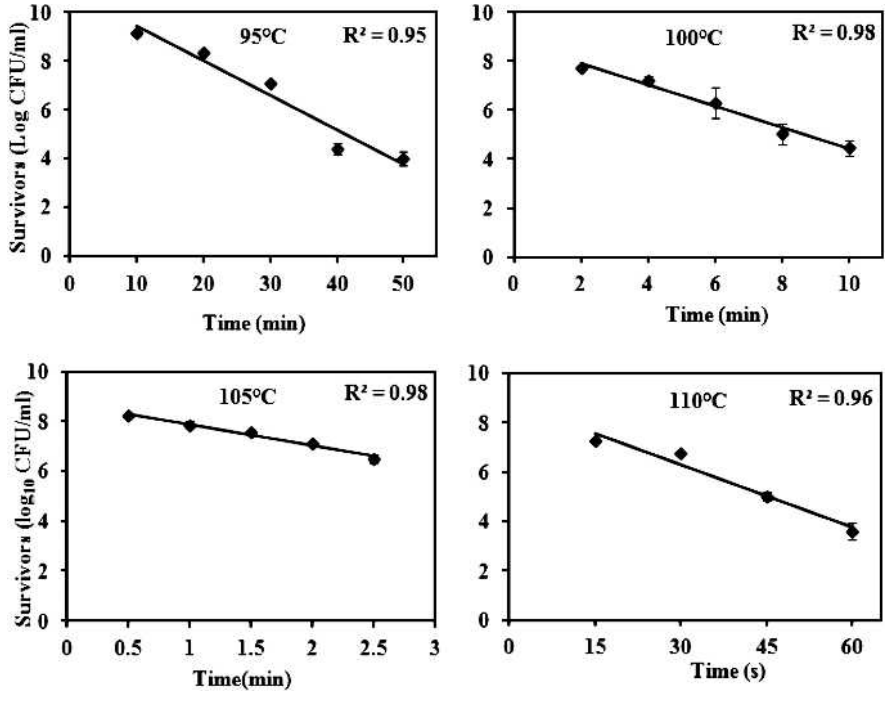

TABLE 2. *Comparison of* D- *and* z-*values of* B. coagulans *ATCC 8038 and 185A spores in commercial tomato juice*

Strain	*D*-value (min) at indicated temp[a]					*z*-value (°C)	pH
	95°C	100°C	105°C	110°C	115°C		
ATCC 8038	7.05 ± 0.14	2.56 ± 0.15	1.18 ± 0.02	0.20 ± 0.01	—[b]	10.0	4.0
	—	4.56 ± 0.25	1.20 ± 0.01	0.27 ± 0.01	0.07 ± 0.01	8.3	4.3
185A	—	1.41 ± 0.30	0.53 ± 0.10	0.14 ± 0.00	—	10.2	4.3

[a] Data are the means ± standard deviations of replicates.
[b] —, not tested because of too long or too short heat treatment time.

105°C, and 0.14 min at 110°C. The $D_{100°C}$-value of strain 185A spores subjected to heat only was higher than that of spores processed by combining heat with high pressure ($D_{100°C}$-value = 0.5 min under 600 MPa) *(6)*. The $D_{115°C}$-value was not measured due to the exceptionally brief treatment time required. The *z*-value of strain 185A spores was calculated to be 10.2°C.

As shown in Table 2, when exposed to the same pH and treatment temperature, *B. coagulans* ATCC 8038 spores had a much greater thermal resistance than 185A spores, with corresponding *D*-values 1 to 3 times greater. Therefore, *B. coagulans* ATCC 8038 spores were chosen as the major target bacterium for further thermal inactivation experiments.

Thermal resistance of *B. coagulans* ATCC 8038 spores at pH 4.4 (OSU experiments). The thermal survivor curves (*D*-values) for *B. coagulans* ATCC 8038 spores in tomato juice (pH 4.4) from experiments conducted at OSU are shown in Figure 5. A comparison of the *D*- and *z*- values for the OSU and WSU data is shown in Table 3. With selected temperatures of 105 and 110°C, the *D*-values obtained from oil bath and electrical heating methods showed no significant differences ($P > 0.05$). The $D_{105°C}$-values obtained from the oil bath and electrical heating methods were 1.20 and 1.32 min, respectively, and the corresponding $D_{110°C}$-values were 0.27 and 0.17 min. Although the $D_{100°C}$-values obtained from the two heating methods deviated from one another somewhat, the agreement is quite remarkable, given that the data were obtained independently using two different heating methods in two different laboratories. The *z*-values obtained for the spores were 8.3 and 8.7°C for the WSU and OSU data, respectively. Two key reasons for the differences appear to be the slightly different pH levels (4.4 at OSU versus 4.3 at WSU) and the source of juice (commercial juice at WSU versus freshly blended tomatoes at OSU).

In conclusion, *B. coagulans* ATCC 8038 spores have greater thermal resistance (*D*-values) than strain 185A and are therefore considered ideal target bacteria for developing and validating thermal processes for tomato products. A storage temperature of 4°C is appropriate for maintaining the viability and thermal resistance of *B. coagulans* ATCC 8038 spores during short-term storage. Both pH and the preconditioning temperature influence the *D*-values of these spores. The thermal resistance data for *B. coagulans* ATCC 8038 determined independently at two different laboratories were in general agreement, with differences explainable by slightly different pH levels and juice sources.

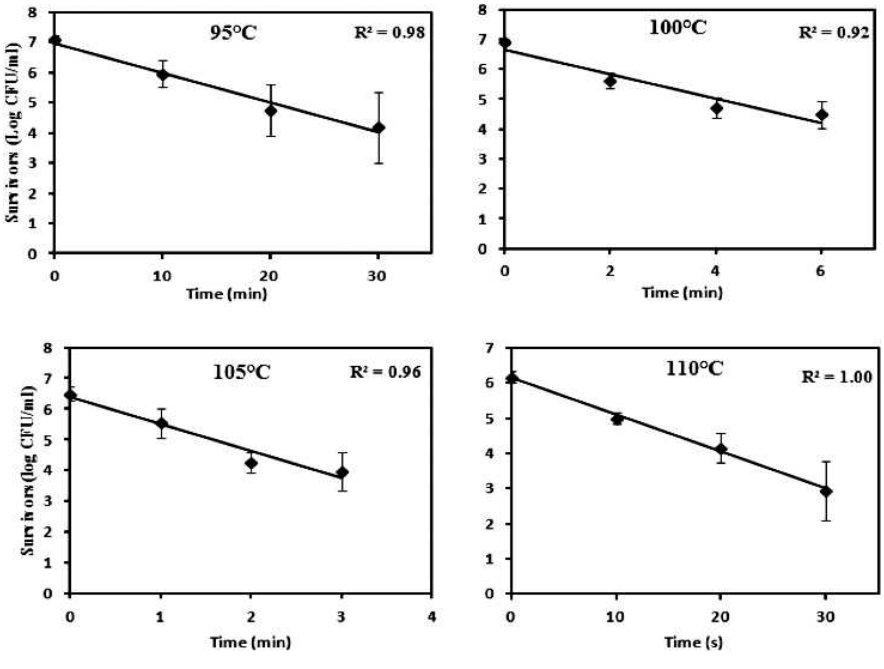

FIGURE 5. *Thermal survivor curves for* B. coagulans *ATCC 8038 spores heated at different temperatures in tomato juice adjusted to pH 4.4. Data are the means ± standard deviations of three replicates. Data obtained at OSU.*

TABLE 3. *The effect of heating method on the thermal resistance of spores of* B. coagulans *ATCC 8038*

Heating method	pH	*D*-value (min) at indicated temp[a]					*z*-value
		95°C	100°C	105°C	110°C	115°C	
Oil bath (WSU)	4.3	—[b]	4.56 ± 0.25	1.20 ± 0.01	0.27 ± 0.01	0.07 ± 0.01	8.3
Electrical (OSU)	4.4	10.13 ± 0.31	2.52 ± 0.14	1.32 ± 0.02	0.17 ± 0.05	—	8.7

[a] Data are the means ± standard deviations of replicates.
[b] —, not tested because of too long or too short heat treatment time.

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