

Inactivation Kinetics of *Alicyclobacillus acidoterrestris* Spores in Orange Juice by Ohmic Heating: Effects of Voltage Gradient and Temperature on Inactivation

AYSE HANDAN BAYSAL^{1*} AND FILIZ ICIER²

¹Department of Food Engineering, İzmir Institute of Technology, Urla, 35430, İzmir, Turkey; and ²Department of Food Engineering, Ege University, Bornova, 35100, İzmir, Turkey

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ABSTRACT

The effectiveness of ohmic and conventional heating for reducing spores of *Alicyclobacillus acidoterrestris* was investigated in commercial pasteurized orange juice. The kinetic parameters (*D*- and *z*-values) were determined during ohmic and conventional heating. The effects of temperature (70, 80, and 90°C) and heating time (0, 10, 15, 20, and 30 min) on inactivation of *A. acidoterrestris* spores during ohmic heating in orange juice were significant ($P < 0.05$). For 70°C, the voltage gradient also had an effect on inactivation kinetics. At 30 V/cm, *D*-values at 70, 80, and 90°C were 58.48, 12.24, and 5.97 min, respectively. *D*-values at corresponding temperatures for conventionally heated spores were 83.33, 15.11, and 7.84 min, respectively. Results showed significantly higher lethality for spores treated with ohmic heating than for spores treated with conventional heating. Conventional heating was ineffective for pasteurizing orange juice, whereas the maximum ohmic heating treatment applied at 30 V/cm was sufficient to inactivate 5 log units of *A. acidoterrestris* spores.

In the 1980s, an acidophilic *Bacillus* species was isolated from apple juice and identified as a new type of spoilage bacterium named *Bacillus acidoterrestris* (9, 13). This organism was later reclassified in a new genus *Alicyclobacillus* (30) because ω-allylic fatty acid was the major membrane fatty acid component of its cells. *Alicyclobacillus acidoterrestris* is a motile, spore-forming, rod-shaped organism with a central, subterminal, or terminal oval spore that grows at pH values of 2.5 to 6.0 at temperatures of 25 to 60°C (31, 32). Because its spores have resist high temperatures and acidic environments, *A. acidoterrestris* has become an important potential spoilage concern for hot-fill fruit and vegetable juices. The fruit juices and fruit juice-containing drinks that are most susceptible to this bacterium are either fresh (not heat treated) or pasteurized (but not ultrahigh-temperature treated) (15, 20, 21). The organism causes a flat sour type of spoilage and produces an offensive-smelling compound, guaiacol, and other taint chemicals (15, 21). Recently, *A. acidoterrestris* has been implicated in fruit juice spoilage incidents in the United Kingdom, Germany, and the United States (15, 21, 27). Cerny et al. (9) and Splittstoesser et al. (28) reported *D*-values for *A. acidoterrestris* at 90°C of 15 and 16 to 23 min, respectively. Splittstoesser et al. (28) also reported *D*-values of 2.4 to 2.8 min at 95°C. These data suggest that spores survive the typical juice pasteurization process, which consists of holding at 86 to 96°C for 2 min. Given its ability to grow at pH values of <3.8 and to survive the typical juice pasteurization process, *A. acidoterrestris* has

caused great concern in the fruit juice industry (3, 20, 28). A 1-log inactivation of *A. acidoterrestris* spores in orange juice (pH 4.1) is achieved through thermal treatment at 95°C for 3 min, and the *z*-value of these spores is 9.5°C (5). Therefore, it is difficult to eliminate these spores using the standard heat treatment (e.g., 95°C for <10 min) without causing damage to the commercial product.

Ohmic heating is an alternative heating system for pumpable foods. This method can be used as a continuous in-line heating method for cooking and sterilization of viscous liquids and mixtures containing particulate food products. The ohmic heating technique is based on the passage of electrical current through a food product that provides electrical resistance (16). Heat is generated instantly inside the food, and the amount of heat generated is directly related to the current induced by the voltage gradient in the field and the electrical conductivity (2, 16). During conventional thermal processing, significant product quality damage may occur because of slow conduction and convection heat transfer. However, ohmic heating volumetrically heats the entire mass of the food material, and the resulting product maintains its of higher quality than its thermally processed counterpart (2). Like thermal processing, ohmic heating inactivates microorganisms by heat. A large number of potential applications exist for ohmic heating, including blanching, evaporation, dehydration, fermentation, and extraction.

Consumers of processed foods demand that the manufacturing industry maintain quality, reduce the use of chemical additives, and provide foods that are perceived to have received minimal physical processing. Therefore, an

* Author for correspondence. Tel: +90 232 7506187; Fax: +90 232 7506196; E-mail: handanbaysal@iyte.edu.tr.

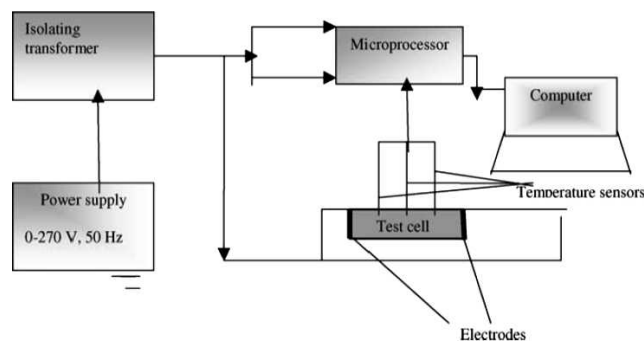


FIGURE 1. Schematic diagram of the ohmic heating system.

understanding of the ohmic heating process for sterilizing food products is essential for process validation and safety. Most published research work related to ohmic heating technology has focused on the thermal and rheological behavior of fluids containing particles, electrical conductivity measurements, ascorbic acid degradation, and enzyme deactivation kinetics (7, 16–19). However, data on the effects of voltage gradients and temperature on microbial inactivation in liquid foods are limited (8, 11, 12, 22).

The present study concerns primarily the application of ohmic heating to ensure uniform commercial sterility in the product, thereby maintaining product safety and quality by reducing overprocessing and retaining nutrients and sensory qualities. The objective of this work was to evaluate the effectiveness of ohmic heating for destroying spores of *A. acidoterrestris* in orange juice by determining the inactivation kinetics parameters (*D*- and *z*-values) and investigating the lethality effects of voltage gradients. The results obtained with ohmic heating also were compared with those obtained with conventional heating.

MATERIALS AND METHODS

Bacterial strain. *A. acidoterrestris* type strain DSM 3922 was used in this study (Prof. Dr. Karl Poralla, Fakultät für Biologie, Eberhard-Karls-Universität Tübingen, Tübingen, Germany). Cultures were grown for 2 days at 43°C on *Bacillus acidocaldarius* medium (BAM; 0.25 g of CaCl₂·2H₂O, 0.5 g of MgSO₄·7H₂O, 0.2 g of (NH₄)₂SO₄, 3.0 g of KH₂PO₄, 1 g of yeast extract, 5 g of glucose, 1 ml of trace element solution, and 1 liter of deionized water, pH 4.3) and then stored at 4°C as stock cultures. Trace element solution contains 0.28 g of FeSO₄·7H₂O (Merck), 1.25 g of MgCl₂·4H₂O (Merck, Darmstadt, Germany), 0.48 g of ZnSO₄·7H₂O (Merck), and 1 liter of deionized water.

Preparation of spore suspension. To induce sporulation, cells grown at 43°C for 2 days on BAM were spread onto BAM (pH 4.3) in petri plates and incubated at 43°C for 7 days until at least 80% of cells sporulated, as determined by phase contrast microscopy. Spores were harvested by depositing approximately 5 ml of sterile water onto the surface of the BAM culture plates; spores were dislodged by gentle rubbing with a sterile swab. Pooled suspensions from plates containing spores were centrifuged (4,000 × *g* for 20 min at 4°C), resuspended in sterile water, and centrifuged again (4,000 × *g* for 10 min at 4°C). This procedure was repeated four times. The final pellets were resuspended in sterile phosphate buffer (pH 7.0), combined, heated (80°C for 10 min) to kill vegetative cells, and stored at 4°C until used.

Electrical heating treatment. The static ohmic heating system used included an isolated power supply (10 kVA), microprocessor board, and a teflon test cell (0.025 by 0.025 by 0.040 m). A schematic illustration of the system is given in Figure 1 (18, 19). Two parallel constructed electrodes in the test cell were made of stainless steel. Teflon-coated electronic temperature sensors (Omega Engineering, Inc., Stamford, CT) with a compression fitting were used to measure the temperature at the different sections of the sample in the test cell. Temperature uniformity was checked during previous heating experiments by measuring the temperatures at seven locations in the test cell. Because the temperature variation at different points inside the test cell was ±1°C during heating, the ohmic heating process was assumed to be uniform. Therefore, only the temperature in the center of the test cell was measured. A microprocessor board was used to monitor the current and voltage applied at constant time intervals of 1 s. Each combined spore suspension was diluted with *Alicyclobacillus*-free commercial pasteurized orange juice (pH 3.637 ± 0.023; total soluble solid content [°Brix], 11.700 ± 0.346; water activity (*a_w*), 0.99 ± 0.00; titratable acidity, 0.33 ± 0.15%; total sugar content, 11.030 ± 0.234) provided by a local market to obtain a preparation containing approximately 10⁵ spores per ml. For each temperature studied, the orange juice sample (30 ml) was placed in the test cell and inoculated with 1 ml of the bacterial spore suspension. After the system was sealed, the sample was ohmically heated. Inoculated samples were ohmically heated to 70, 80, and 90°C by using a voltage gradient of 30, 40, and 50 V/cm and were held at this temperatures. A microprocessor-controlled voltage gradient on-off mechanism was used to control the temperature during holding periods. Five time intervals (0, 10, 15, 20, and 30 min) were used for each temperature. Because the main aim here was to keep the temperature constant after heating with different voltage gradients, the on-off control procedure during the holding time at each constant temperature was similar for the same voltage gradient. However, because the energy given per time unit by different voltage gradients was different, on-off seconds must have been different for different voltage gradients. In contrast, the energy given to the samples during the holding time at each prescribed temperature was not significantly different for different voltage gradients applied. Immediately after the designated temperature-holding time, 1 ml of the sample was taken from the test cell and poured into dilution tubes in an ice bath for microbiological analysis. Before and after each temperature treatment session, the ohmic heating test cell and thermocouple (diameter, 1.3 × 10⁻³ m; response time, 0.5 s) were cleaned and sanitized with a 200 mg/kg hypochlorite solution and rinsed with sterile distilled water. Sanitation efficiency was determined using the swab technique.

Conventional heating treatment. To determine the differences in the efficiency between ohmic heating and conventional heating, conventional heating was conducted by matching the thermal history of the samples treated with the 30 V/cm voltage gradient. For conventional heating, a screw-cap test tube (glass tube with an inside diameter of 1.6 × 10⁻³ m) containing the inoculated sample was completely immersed in a temperature-controlled water bath (SELECTA Precisdig, Barcelona, Spain) and heated to 70, 80, or 90°C. Holding times (0, 10, 15, 20, or 30 min) began (*t*₀) when the tubes reached the test temperature. For replication, three test cells were heated in the same cycle, and three tubes were subsequently removed at appropriate time intervals. All tubes containing the samples were immediately transferred to an ice bath until further analyses were performed. The thermal history of the samples was monitored with the microprocessor system

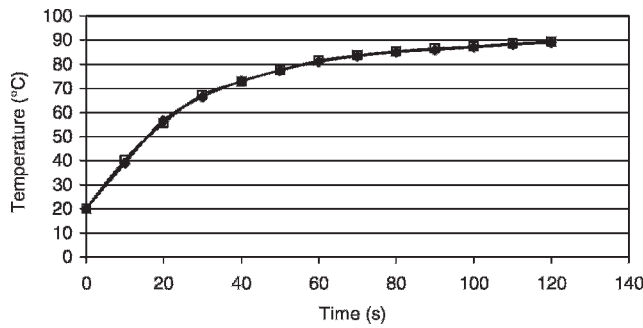


FIGURE 2. Thermal history of orange juice samples treated by conventional (□) and ohmic (◆) heating.

connected to a T-type thermocouple (Omega Engineering) placed in another test tube as a control. This procedure was repeated for all temperatures. Initial levels of *A. acidoterrestris* DSM 3922 spores in suspensions were 10^5 to 10^6 CFU/ml.

Microbiological analysis. The numbers of the survivors were determined by the spread plate method on BAM (pH 4.3). The heated spore suspensions were cooled in ice water immediately after treatment. The surviving population of *A. acidoterrestris* spores was enumerated after duplicate spread plating of 0.1 ml of appropriate 10-fold serial dilutions in sterile phosphate buffer onto BAM and incubation at 43°C for 2 to 5 days. *D*-values were based on the reciprocal of the slopes obtained when the log CFU of survivors was plotted against time. For example, a *D*-value of 1 min at 80°C means that for each minute of processing at 80°C the target bacterial population will be reduced by 90% (equation 1):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \quad (1)$$

where N_0 is the number of surviving spores at holding time zero after reaching the prescribed temperature, N is the number of surviving spores at any time (t), and D is the time required to reduce the number of surviving spores by 90% at a selected temperature.

The z -values (temperature increase required to reduce the *D*-value by 1 log unit; equation 2) represents the reciprocal of slopes when the log-transformed *D*-values were plotted against temperature (T). The reference temperature in equation 2 was defined as 70°C:

$$\log\frac{D}{D_{ref}} = \frac{T_{ref} - T}{z} \quad (2)$$

Statistical analysis. All experiments were duplicated and repeated three times. Data from three samples subjected to each treatment in each of three independent replicate experiments were analyzed. For statistical evaluation, significant differences ($P < 0.05$) between mean values of treatments were identified and regression analyses were performed using the SPSS 11.0.1 statistical package (30). The statistical criteria applied to discriminate among the models were the correlation coefficient (R^2) and the standard errors (SE) for each coefficient. The confidence level used to determine statistical significance was 95%. Kinetic modeling was performed for three holding temperatures (70, 80, and 90°C) and three voltage gradients (30, 40, and 50 V/cm).

RESULTS AND DISCUSSION

Ohmic heating was investigated as an inactivation process that could be used as alternative to conventional heating, and the effects of voltage gradient, temperature, and holding time on the ohmic heating process were determined.

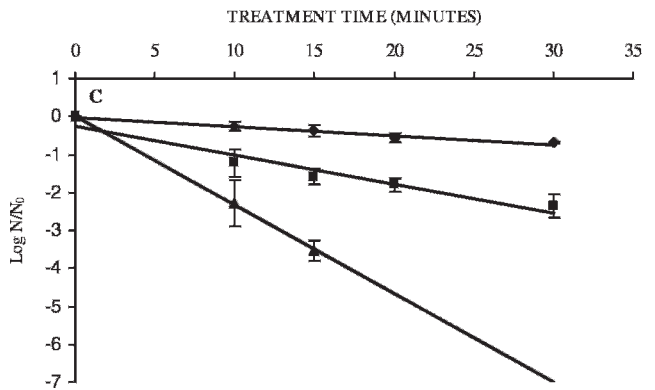
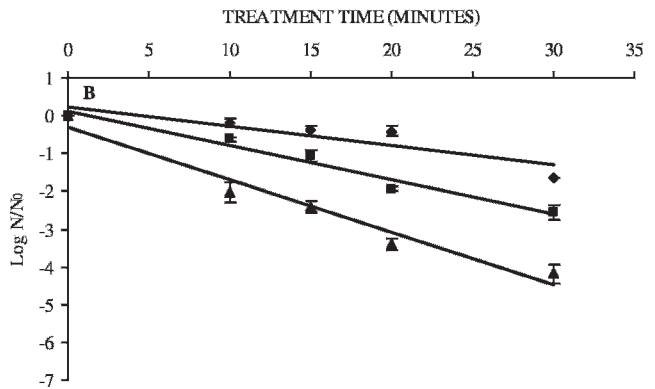
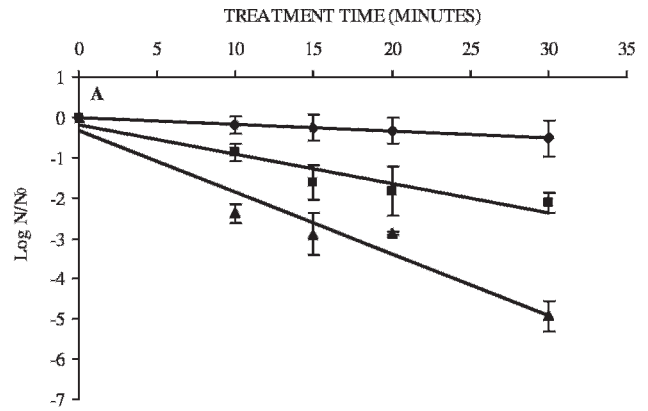


FIGURE 3. Survival curves for *A. acidoterrestris* DSM 3922 spores in orange juice (pH 3.637) treated with ohmic heating at 30 V/cm (A), 40 V/cm (B), and 50 V/cm (C) and at 70°C (◆), 80°C (■), and 90°C (▲). The results are means from three experiments, and error bars indicate standard errors.

Samples also were treated with conventional heating to compare the two heating processes. To assess the possible electrical effect of ohmic heating on microbiological inactivation, the thermal history during both heating processes was determined. In this respect, the thermal history using conventional heating could be matched only by ohmic heating at 30 V/cm (Fig. 2). The possible differences from conventional heating may be related to the effect of the electric field.

The electrical conductivity of the orange juice increased linearly as the temperature increased during ohmic heating. The electrical conductivity of the orange juice ranged from

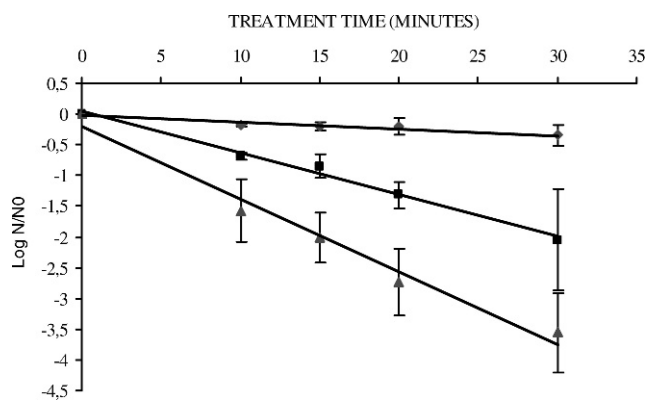


FIGURE 4. Survival curves for *A. acidoterrestris* DSM 3922 spores in orange juice (pH 3.637) treated with conventional heating at 70°C (◆), 80°C (■), and 90°C (▲). The results are means from three experiments, and error bars indicate standard errors.

0.457 to 1.564 S/m for the temperature range of 20 to 90°C. The pH values before and after heating experiments were not significantly different ($P > 0.05$).

Figure 3 provides data for populations of *A. acidoterrestris* spores suspended in orange juice treated with ohmic heating at different voltage gradients (30, 40, and 50 V/cm), and Figure 4 provides population data for orange juice samples treated with conventional heating at different holding temperatures (70, 80, or 90°C). The number of surviving spores at ohmic holding time zero was taken as the initial value. As the temperature increased, the number of spores sharply decreased. The regression coefficients of the survival curves were greater than 0.90 in most cases. Ohmic heating reduced the viability of spores appreciably (Fig. 3). The effects of temperature and heating time on logarithmic reduction of *A. acidoterrestris* DSM 3922 spores during ohmic heating in orange juice were significant ($P < 0.05$). The voltage gradient \times temperature and temperature \times time interactions in relation to logarithmic reduction of *A. acidoterrestris* spores were significant at $P < 0.05$ (Tables 1 and 2).

In this study, the difference in resistance of the *A. acidoterrestris* DSM 3922 strain in orange juice during ohmic (30 V/cm) and conventional heating was evaluated by inactivation kinetics constants (D - and z - values). D -values at 70, 80, and 90°C for ohmic (30 V/cm) and conventional heating were 58.48, 12.24, and 5.97 min (z -value of 18.80°C) and 83.33, 15.11, and 7.84 min (z -value of 17.89°C), respectively (Table 2). For the thermal

TABLE 2. D - and z -values for *A. acidoterrestris* DSM 3922 spores in orange juice treated with conventional heating at different temperatures^a

Temp (°C)	D -value (min)	R^2
70	83.33 \pm 0.99 A	0.923
80	15.11 \pm 0.27 B	0.990
90	7.84 \pm 0.44 C	0.9714
z -value (°C)	17.89 \pm 0.25	0.926

^a Conventionally heated samples had the same thermal history as samples treated with ohmic heating at 30 V/cm. Values are the mean \pm SE from three replicate heating experiments. Means with different letters are significantly different ($P < 0.05$).

inactivation of *A. acidoterrestris* DSM 3922 spores, D -values for ohmic heating were considerably lower than those for conventional heating (Table 2 and Fig. 5). The analysis of variance revealed that the differences between two heating methods based on D -values were significant ($P < 0.05$). However, z -values were not significantly different for the two heating treatments ($P > 0.05$).

Heat resistance studies have indicated that *Alicyclobacillus* spores can survive the usual hot-fill processes that are used with commercial juices (4, 5, 14, 24–27). Data also indicate that increasing the °Brix makes the spores more resistant to heat. Although the reason *Alicyclobacillus* spores survive pasteurization and hot-fill and hold processes is still unclear, environmental factors such as pH, soluble solid content, and temperature influence the heat resistance of the spores (10, 25, 27). D -values for spores of different strains of *A. acidoterrestris* at 80, 85, 90, and 95°C in conventionally heated fruit juices were 41.1 to 54.3, 50 to 65.6, 7.4 to 23, and 2.3 to 5.3 min, respectively, with z -values of 7.7 to 12.9°C (3–5, 14, 25–27). Several authors have studied the thermal resistance of *A. acidoterrestris* strains and evaluated the ability of these spore-forming bacteria to resist conventional heating in fruit juices from 80 to 100°C (3–5, 14, 25–27). The D -values reported for 87.9, 91.1, and 95°C were 11, 3.8, and 1.0 min, respectively (10).

In present study, although D -values of the spores heated ohmically at 70°C at 30, 40, and 50 V/cm were significantly different ($P < 0.05$), D -values at 80 and 90°C were not significantly different for samples heated at 40 and 50 V/cm ($P > 0.05$). For the same voltage gradient, as the temperature increased D -values decreased significantly ($P < 0.05$). At the lowest temperature studied, D -values were

TABLE 1. D - and z -values for *A. acidoterrestris* DSM 3922 spores in orange juice treated with ohmic heating at different voltages and temperatures^a

Temp (°C)	30 V/cm		40 V/cm		50 V/cm	
	D -value (min)	R^2	D -value (min)	R^2	D -value (min)	R^2
70	58.48 \pm 0.49 AP	0.996	51.81 \pm 0.93 AQ	0.913	40.49 \pm 0.71 AR	0.975
80	12.24 \pm 1.06 BP	0.894	11.79 \pm 0.48 BP	0.963	11.43 \pm 1.21 BP	0.912
90	5.97 \pm 0.42 CP	0.929	6.51 \pm 0.48 CP	0.949	4.29 \pm 0.10 CQ	0.999
z -value (°C)	18.80 \pm 0.68	0.948	20.45 \pm 0.36	0.931	20.00 \pm 0.35	0.994

^a Values are the mean \pm SE from three replicate heating experiments. Within the same column, means with different letters A, B, or C are significantly different. Within the same row, means with different letters P, Q, or R are significantly different ($P < 0.05$).

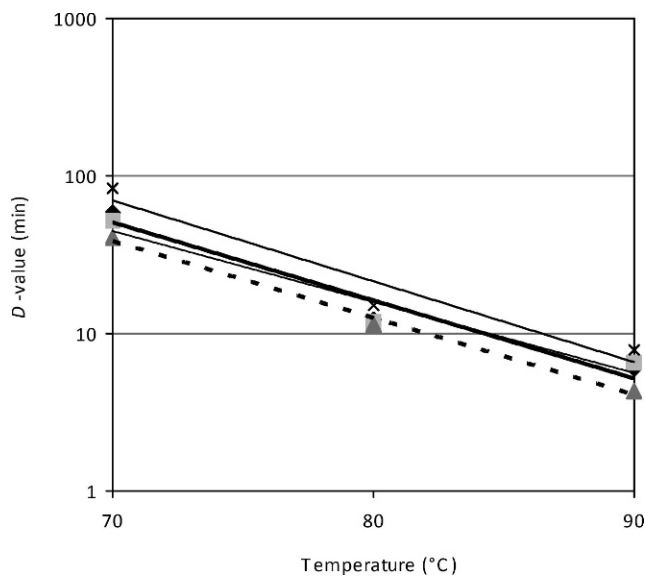


FIGURE 5. Thermal death time (TDT) curves of *A. acidoterrestris* DSM 3922 spores in orange juice that was ohmically heated at 30 V/cm (◆), 40 V/cm (■), and 50 V/cm (▲) and conventionally heated (×).

significantly different for the different voltage gradients, possibly because of the comparable electrical effect in addition to the thermal effect ($P < 0.05$). However, at higher temperatures the possible electrical effect was not observed, possibly because it was masked by the dominant thermal effect. The effect of the voltage gradient was not significant at the high temperatures ($P > 0.05$). D -values for conventional treatment obtained in this study (15.11 min for 80°C and 7.84 min for 90°C) were lower than the D -values previously reported for inactivation of *A. acidoterrestris* spores in orange juice with a similar pH range (4, 5, 14, 26, 27). The z -values for conventional treatment (17.89°C) also are higher than those previously reported (4, 5, 14, 26, 27). These differences may be due to strain differences, differences in spore preparation and/or sporulation methods, and differences in the overall heating conditions. Cho et al. (12) studied the kinetics of inactivation of *Bacillus subtilis* ATCC 6633 spores by continuous or intermittent ohmic and conventional heating and found that spore inactivation was greater with ohmic than with conventional heating. Ohmic heating produced a higher rate of spore death during the first stage of heating and greater decrease in count of viable spores immediately after the incubation period. Therefore, Cho et al. concluded that spore inactivation during ohmic heating was primarily due to the thermal effect but there was an additional killing effect caused by the electric current (12). The results obtained in the present study are in agreement with these findings; conventional heating was less effective for reducing *A. acidoterrestris* DSM 3922 spores. The voltage gradient also had a greater effect as the temperature increased (Table 1). Because no data are currently available on the effect of ohmic heating on spores of *A. acidoterrestris* or other *Alicyclobacillus* species, results obtained in this study could not be compared with those of other studies.

In previous studies, much lower D -values have been recorded for other thermophilic microorganisms than for *A. acidoterrestris* spores (12). Although the mechanisms of resistance to pasteurization and hot-fill and hold processes of *Alicyclobacillus* spp. are still unclear, the thermal resistance of other bacterial spores is influenced by several environmental factors such as pH, a_w , and menstroom composition (6, 10, 27). The most significant parameter in the inactivation of microorganisms is the thermal effect itself, regardless of the type of thermal treatment. In another study, *B. subtilis* spores heated by conventional or ohmic methods with identical temperature histories at 92.3°C had significantly lower D -values when heated using ohmic rather than the conventional method (12). Pereira et al. (23) evaluated the influence of ohmic heating on the heat resistance of *Escherichia coli* in goat milk and *Bacillus licheniformis* in cloudberry jam and compared the results with those obtained after conventional heating. These authors concluded that spore inactivation during ohmic heating was more efficient than that obtained with conventional heating primarily because of the thermal effect with an additional killing effect caused by the electric current (23). In the present study, conventional heating was less effective for *A. acidoterrestris* DSM 3922 spore reduction than was ohmic heating, and moderate increases in the voltage gradient seemed to enhance the inactivation effect of ohmic heating. The inactivation of was attributed primarily to heat, but there was an additional killing effect caused by the voltage gradient.

Ohmic heating technology has been proposed as a method of preserving fruit juices. However, to meet regulatory criteria, ohmic heating treatments should inactivate 5 log units of any bacteria associated with foodborne illness (1). A review of published data on thermal inactivation of *E. coli* O157:H7 revealed no strong evidence that heat treatment at 70°C for 2 min (or the equivalent) would fail to produce a 6-log reduction in viable cells (6). According to the data on thermal resistance of *A. acidoterrestris*, this bacterium is much more heat resistant than *E. coli* O157:H7 (29). Although 30 min of ohmic heating at 30 V/m and 90°C was enough for a 5-log decrease in viable spores, only a 3.5-log reduction was obtained by conventional heating at the same voltage gradient and temperature. Therefore, conventional heating was considered ineffective for pasteurizing orange juice, and the maximum ohmic heating treatment applied at 30 V/cm was sufficient to achieve a 5-log reduction of *A. acidoterrestris* spores. A more systematic study should be conducted to investigate the influence of spore strain, initial spore levels, pH, a_w , and other characteristics of fruit products such as phenols on ohmic heat resistance of *Alicyclobacillus* species. Combination techniques that include ohmic heating should be investigated with the aim of developing effective alternative spore inactivation methods.

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