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Effect of sporulation medium on wet-heat resistance and structure of *Alicyclobacillus acidoterrestris* DSM 3922-type strain spores and modeling of the inactivation kinetics in apple juice

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ABSTRACT

Alicyclobacillus acidoterrestris is a spoilage bacterium in fruit juices leading to high economic losses. The present study evaluated the effect of sporulation medium on the thermal inactivation kinetics of *A. acidoterrestris* DSM 3922 spores in apple juice (pH 3.82 ± 0.01; 11.3 ± 0.1 °Brix). *Bacillus acidocaldarius* agar (BAA), *Bacillus acidoterrestris* agar (BATA), malt extract agar (MEA), potato dextrose agar (PDA) and *B. acidoterrestris* broth (BATB) were used for sporulation. Inactivation kinetic parameters at 85, 87.5 and 90 °C were obtained using the log-linear model. The decimal reduction times at 85 °C ($D_{85\,°C}$) were 41.7, 57.6, 76.8, 76.8 and 67.2 min; $D_{87.5\,°C}$-values were 22.4, 26.7, 32.9, 31.5, and 32.9 min; and $D_{90\,°C}$-values were 11.6, 9.9, 14.7, 11.9 and 14.1 min for spores produced on PDA, MEA, BATA, BAA and BATB, respectively. The estimated *z*-values were 9.05, 6.60, 6.96, 6.15, and 7.46, respectively. The present study suggests that the sporulation medium affects the wet-heat resistance of *A. acidoterrestris* DSM 3922 spores. Also, the dipicolinic acid content (DPA) was found highest in heat resistant spores formed on mineral containing media. After wet-heat treatment, loss of internal volume due to the release of DPA from spore core was observed by scanning electron microscopy. Since, there is no standardized media for the sporulation of *A. acidoterrestris*, the results obtained from this study might be useful to determine and compare the thermal resistance characteristics of *A. acidoterrestris* spores in fruit juices.

1. Introduction

Alicyclobacillus acidoterrestris is a thermoacidophilic, non-pathogenic, rod-shaped spore-forming bacterium with a central, subterminal, or terminal oval spore. It grows at pH values ranging from 2.5 to 6.0 at temperature range of 25 to 60 °C (Silva and Gibbs, 2001; Bahçeci and Acar, 2007). ω-Alicyclic fatty acids are the major lipid components of *A. acidoterrestris* membranes and they are associated with the resistance of the organism to acidic conditions and high temperatures (Hippchen et al., 1981).

Fruit juices are generally treated at temperatures of about 95 °C for 2 min (Komitopoulou et al., 1999). However, spores have been shown to survive in such heat treatments (Splittstoesser et al., 1998) and surviving spores can germinate and grow at pH < 4 in fruit juices, leading to spoilage (Walker and Phillips, 2008). *A. acidoterrestris* has been detected in several spoiled commercial pasteurized fruit juices like apple, tomato, orange, grapefruit, mango, pear, passion fruit, and white grape juices. Detection of *A. acidoterrestris* in fruit juices is difficult because the spoiled juice appears normal or has light sediment (Walker and Phillips, 2005). Spoilage is characterized by a phenolic off-flavor associated with the presence of guaiacol, 2,6-dibromphenol and 2,6-dichlorophenol (Chang and Kang, 2004) and an important problem for manufacturer due to difficulties to eliminate the microorganism from the processing environment (Jensen, 1999). Therefore, *A. acidoterrestris* has been suggested as the target to be used in the design of adequate pasteurization processes of acidic food products (Silva et al., 1999).

Several studies have revealed the thermal inactivation parameters of *A. acidoterrestris* in fruit juices. *D*-values of *A. acidoterrestris* spores were reported as 16–23 min at 90 °C and 2.4–2.8 min at 95 °C (Splittstoesser et al., 1994). $D_{95°C}$-values in apple, grape, berry, orange, Concord grape, grapefruit, clarified lemon and non-clarified lemon juices and fruit drink, fruit nectar, cupuaçu extract range from 1.00 to 9.98 min. The $D_{90\,°C}$-values in apple, grape, Concord grape, orange, grape fruit juices, a clear apple drink, an orange drink, apple nectar with or without ascorbic acid, and mango pulp range from 5.95 min to 23.10 min. Also, the *z*-values range from 6.90 to 21.27 °C in different fruit products (Smit et al., 2011).

DPA (pyridine-2,6-dicarboxylic acid) is a major chemical component found in the inner core of bacterial spores (5–14% of spore dry weight) but not in vegetative cells (Tabor et al., 1976). DPA is usually present in 1:1 molar ratio with Ca^{2+}. DPA release from spores occurs during the

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first minute of germination, spore activation by a lethal heat treatment or wet-heat induced spore inactivation (Kort et al., 2005). For DPA analysis, spectrophotometry (Janssen et al., 1958), ultraviolet spectroscopy (Scott and Ellar, 1978), Fourier transform infrared spectroscopy (Goodacre et al., 2000), liquid chromatography (Paulus, 1981), luminescence (Fell et al., 2001), electron monochromator mass polarography spectroscopy (Beverly et al., 2000), fluorescence (Hindle and Hall, 1999), and pyrolysis-gas chromatography/ion mobility electrophoresis spectroscopy (Goodacre et al., 2000) have been used.

The effect of sporulation conditions on spore heat resistance is important in the validation of thermal processes (Olivier et al., 2012). Therefore, the main objectives of this study were to determine the effect of sporulation medium on wet-heat resistance, dipicolinic acid composition and spore structures of *A. acidoterrestris* DSM 3922-type strain after wet-heat treatment by scanning electron microscopy.

2. Materials and methods

2.1. Microorganism and spore production

A. acidoterrestris DSM 3922 was kindly provided by Karl Poralla (Deutsche Sammlung von Mikroorganismem und Zellkulturen's collection, Braunschweig, Germany) and was used as the test microorganism in this study. Cells of *A. acidoterrestris* were pre-cultured at 43 °C for 4 h in 10 ml BAT broth (Döhler, Germany) to achieve a cell density of approximately 10^6–10^7 CFU/ml. Then, 100 μl culture was spread onto *Bacillus acidoterrestris* agar (BATA, Merck), *Bacillus acidocaldarius* agar (BAA, Darland and Brock, 1971), potato dextrose agar (PDA, BD Difco), and malt extract agar (MEA, Oxoid). Additionally, 1 ml of the culture was inoculated into 100 ml BAT broth (BATB) and incubated with shaking at 120 rpm. The composition of the sporulation media is represented in Table 1. All inoculated plates and broth were incubated at 43 °C. Sporulation was determined by direct observation of free, fully refractile spores under the phase-contrast microscope (Olympus CX31, Japan). After reaching more than 85–90% of sporulation, spore suspensions were prepared as described by Murray et al. (2007) and stored at −20 °C for further use.

2.2. Apple juice

Concentrated apple juices (70.3 °Brix) were provided by ASYA Fruit Juice and Food Ind. Inc. (Isparta, Turkey). The concentrate was reconstituted by diluting in sterile deionized water to 11.3 ± 0.1 °Brix by a refractometer (Mettler Toledo, USA). Diluted juice samples were tested for the presence of *Alicyclobacillus* spp. by membrane filtration method. Briefly, the membrane (0.45 μm pore size, Sartorious) that had been used to filter the reconstituted juice was transferred aseptically onto BATA. Then, the membranes on the plates were incubated at 43 °C for up to 7 days. The pH of the reconstituted juice was measured as 3.82 ± 0.01 (Hanna Instruments, Hungary).

Table 1
Composition of the sporulation media used in this study.

Sporulation media	Composition
PDA	Potato extract 4.0 g/L; dextrose 20.0 g/L and agar-agar 15.0 g/L. Acidified to pH 3.50 ± 0.1 through a sterile solution of 10% (w/v) tartaric acid.
MEA	Malt extract 30 g/L, mycological peptone 5.0 g/L and agar-agar 15.0 g/L. Acidified to pH 3.50 ± 0.1 through a sterile solution of 10% (w/v) tartaric acid.
BATA	Yeast extract 2.0 g/L, D (+)glucose 5.0 g/L, $CaCl_2.2H_2O$ 0.25 g/L, $MgSO_4.7H_2O$ 0.5 g/L, $(NH_4)_2SO_4$ 0.2 g/L, KH_2PO_4 3.0 g/L, agar-agar 18 g/L and 1 ml trace element solution (included in the medium). Trace minerals solution contains $CaCl_2.2H_2O$ 0.66 g/L, $ZnSO_4.7H_2O$ 0.18 g/L, $CuSO_4$ 0.16 g/L, $MnSO_4.7H_2O$ 0.15 g/L, $CoCl_2$ 0.18 g/L, H_3BO_3 0.1 g/L, Na_2MoO_4. $2H_2O$ 0.3 g/L. Acidified to pH 4.0 ± 0.2 through a sterile solution of 1 N H_2SO_4.
BAA	Yeast extract 1.0 g/L, $(NH_4)_2SO_4$ 0.2 g/L, $MgSO_4.7H_2O$ 0.5 g/L, $CaCl_2$. $2H_2O$ 0.25 g/L, KH_2PO_4 3.0 g/L, glucose 5 g/L, agar-agar 15 g/L, and 1 ml trace element solution (1:1000, v/v). Trace element solution contains $FeSO_4.7H_2O$ (0.28 g/L), $MgCl_2.4H_2O$ (1.25 g/L), $ZnSO_4.7H_2O$ (0.48 g/L). Acidified to 4.0 ± 0.2 through a sterile solution of 1 N H_2SO_4.
BATB	Same with BATA but does not contain agar.

2.3. Heat treatments

A temperature-controlled water bath (P-Selecta Precisdig) was used for the wet-heat treatment of spores. The water bath was adjusted to the intended temperature (85, 87.5 or 90 °C). After heat-shock at 80 °C for 10 min in the water bath and then cooling on ice, the spore stock suspension (10^6–10^7 CFU/ml) was centrifuged. After resuspending the pellet in 1 ml of apple juice, the suspension was transferred to a screw-cap test tube (160 × 100 mm glass tube with an inside diameter of 16 mm). Tube containing inoculated apple juice was then placed in the water bath. During the thermal treatments, a K-type thermocouple (Hanna Instruments, Hungary) was placed in another test tube containing the same volume of apple juice as a control. When the control was reached the target temperature, the other heated tube containing spore suspension was removed from the water bath immediately to determine N_0 for modeling. After each sampling time, viable spore counts were determined by serially diluting aliquots of juice in maximum recovery diluent (Oxoid) and then plating on PDA in duplicates (Baysal and Icier, 2010). A minimum of seven time intervals were used: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min at 85 °C; 5, 10, 15, 20, 25, 30, 40, 50, and 60 min at 87.5 °C and 5, 10, 15, 20, 25, and 30 min at 90 °C, respectively.

2.4. Modeling of the inactivation data

Survival curves were obtained by plotting the logarithm of the survival fraction (N/N_0) versus the treatment time (min). As the number of logarithm of the survivors declines linearly over treatment time (Schaffner and Labuza, 1997), the log-linear model (Bigelow and Esty, 1920) from GlnaFiT Excel add-in software was used to fit the experimental data (Geeraerd et al., 2005) using the following equation:

$$\log N/N_0 = -k_{max}.t/ln10 \quad (1)$$

where N is the spore count (CFU/ml) after exposure to the thermal treatment for a specific treatment time t (min), N_0 initial spore count (CFU/ml) measured immediately after come-up-time. D (decimal reduction time) is the time required to destroy 90% of the spores (min) and can be calculated as the reciprocal of the rate constant (k_{max}, min^{-1}) using the Eq. (2).

$$D = 2.303/k_{max}. \quad (2)$$

All thermal resistance determinations were repeated three times on separate days. For each data set, D-value was calculated using corresponding rate constant and the results were expressed as the mean ± standard deviation from three experiments. The z-value is the change of temperature (°C) required for 1-log cycle change in D-values. The z-values were determined by plotting the log$D$ values against heating temperatures and then taking the reciprocal of the slope from linear regression (Daryaei and Balasubramaniam, 2013).

2.5. Scanning electron microscopy

The untreated and treated spores were first centrifuged (16,000 ×g, 10 min). Next, the pelleted spores were repeatedly washed by centrifugation, and then suspended in sterile ultrapure water. Finally, the resultant spore suspension (10 μl) was fixed onto clean glass slides

and air-dried overnight. For SEM imaging, samples were coated with gold at a vacuum of 0.09 mbar, for 90 s, at 15 kV, in argon gas at a power of 15 mA and examined with a scanning electron microscope (Phillips XL-30S FEG).

2.6. DPA analysis

The DPA content of the spore suspensions during wet-heat was measured according to a colorimetric method with some minor modifications (Rotman and Fields, 1967). The untreated samples were autoclaved at 121 °C for 20 min to determine the total DPA content (Janssen et al., 1958). First, treated and autoclaved suspensions were centrifuged at 16,000 ×*g* for 15 min. After centrifugation, the supernatant (800 μl) was mixed with 200 μl solution containing 1% (w/v) $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ and 0.1% (w/v) L-cystein in 0.05 M sodium acetate buffer (pH 4.0) (Planchon et al., 2011). The absorbance of the solution was measured spectrophotometrically at 440 nm. The dry weight of each spore suspension was determined by completely drying the remaining spore pellet. A standard calibration curve was used to calculate the amount of DPA in supernatants. Finally, the DPA contents were expressed as μmol DPA/mg spore dry weight and the release (%) was calculated based on the relative proportion of DPA content of treated spores to their total DPA content.

2.7. Statistical analysis

The mean values and standard deviations were calculated by Excel (Microsoft Corp., USA). The heat-resistance and DPA data were analyzed by one way analysis of variance (ANOVA) using Minitab 16.0 (Minitab Inc., UK). The Tukey–Kramer test was used to compare the means of treated groups. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Determination of inactivation kinetic parameters

The log-linear model was suitable for describing wet-heat thermal inactivation kinetics of *A. acidoterrestris* spores. The *Adj-*R^2 values were between 0.974 and 0.996. The effects of the sporulation medium and heating temperature on *D* and *z*-values were shown in Table 2. The results indicated that there were significant differences in the wet-heat resistance among spores formed on different sporulation media. Especially, the spores formed on mineral-containing media (BATA, BAA and BATB) were more resistant than those formed on non-mineral containing media (PDA and MEA). The *D*-values for all spore suspensions decreased significantly as the heating temperature increased ($p < 0.001$). The $D_{85\ °C}$-values were 41.7, 57.6, 76.8, 76.8 and 67.2 min; $D_{87.5\ °C}$-values were 22.4, 26.7, 32.9, 31.5, 32.9 min; and $D_{90\ °C}$-values were 11.6, 9.9, 14.7, 11.9 and 14.1 min for spores produced on PDA, MEA, BATA, BAA and BATB, respectively. There was a significant difference between the heat resistance of spores made in liquid (BATB) and on agar (BATA) at 85 °C (Table 2). On the other hand, $D_{87.5\ °C}$ and $D_{90\ °C}$ values of spores formed in BATB and on BATA were not statistically different (Table 2).

Also, the *z*-values were found to be 9.05 ± 0.17 ($R^2 = 0.999$), 6.60 ± 0.83 ($R^2 = 0.995$), 6.96 ± 0.18 ($R^2 = 0.999$), 6.15 ± 0.29 ($R^2 = 0.999$), and 7.46 ± 0.69 °C ($R^2 = 0.997$) for spores from PDA, MEA, BATA, BAA and BATB, respectively (Table 2).

3.3. Scanning electron microscopy

SEM micrographs were taken to visualize damage to the spore external structure after wet-heat treatment at 90 °C for 60 min. Spores with a rough surface have a greater resistance to heat than those with a smooth surface (Lindsay et al., 1990). In contrast, untreated spores from PDA (Fig. 1a) and MEA (Fig. 1b) were short, planiform elliptical rods having rough surfaces while spores showing high heat resistance from BATA (Fig. 1c) and BAA (Fig. 1d) had smooth surfaces. SEM images of autoclaved spore suspensions produced on BATA (Fig. 2) indicate that spores lost their internal volume most probably due to the release of intracellular components compared to untreated spores (Fig. 1). Since DPA and other small molecules constitute 5–15% of the dry weight of spores, the spores collapse after autoclaving because of the release of these compounds. Also, autoclaved spores have a wrinkled appearance resulting from the loss of internal volume (Perkins et al., 2004). The representative micrographs of the spores treated for the 60 min at 90 °C (Fig. 3) are similar to the SEM images of autoclaved spore suspensions (Fig. 2) indicating the loss of their internal volume most probably due to the release of DPA from their spore core.

3.4. DPA analysis

When spores are steam autoclaved, essentially all their DPA is released (Janssen et al., 1958). Therefore, the total DPA content of the spores produced on different media was determined after autoclaving. It was found that the DPA contents of spores produced on nonmineral containing media were significantly lower than mineral containing media ($p < 0.005$). DPA amounts from spores produced on PDA (0.21 ± 0.00 μmol) and MEA (0.26 ± 0.02 μmol) were found significantly lower than spores produced on BATA (0.47 ± 0.00 μmol) and BAA (0.49 ± 0.01 μmol) on the basis of the dry weight of the spores (Table 3). Based on these values, DPA content was also expressed as % spore dry weight ranging from 3.5% to 8.2%.

Also, the amount of DPA released from untreated and heat-treated spores (85 °C for 60 min, 87.5 °C for 30 min and 90 °C for 15 min) were determined. After 60 min at 85 °C, the spores produced on PDA and MEA released most of their DPA, approximately 93% and 83%, respectively. Whereas the spores formed on mineral containing media (BATA and BAA) released approximately 70% of their DPA. Also, the DPA release of spores from nonmineral containing media after 87.5 °C treatment for 30 min was higher than that of spores from mineral containing media. The correlation coefficient (R^2) between the DPA release and corresponding $D_{85\ °C}$-values and $D_{87.5\ °C}$-values was higher than 0.98 indicating the presence of a linear relationship. At 90 C°, the DPA release from spores produced on nonmineral containing media was higher but there was no clear correlation between the release and $D_{90\ °C}$-values.

Table 2
Estimated parameters of the log-linear model.

Sporulation media	$k_{max85°C}$ (1/min)	$k_{max87.5°C}$ (1/min)	$k_{max90°C}$ (1/min)	$D_{85\ °C}$ (min)	$D_{87.5\ °C}$ (min)	$D_{90\ °C}$ (min)	*z* (°C)
PDA	0.06 ± 0.01[†]	0.11 ± 0.02	0.20 ± 0.02	41.7 ± 7.6^{Ca†}	22.4 ± 4.5^{Bb}	11.6 ± 1.2^{ABc}	9.05 ± 0.17^{A}
MEA	0.04 ± 0.0	0.09 ± 0.01	0.23 ± 0.01	57.6 ± 0.0^{BCa‡}	26.7 ± 1.9^{ABb}	9.9 ± 0.5^{Bc}	6.60 ± 0.83^{B}
BATA	0.03 ± 0.0	0.07 ± 0.0	0.16 ± 0.01	76.8 ± 0.0^{Aa}	32.9 ± 0.0^{Ab}	14.7 ± 0.6^{Ac}	6.96 ± 0.18^{B}
BAA	0.03 ± 0.0	0.07 ± 0.01	0.20 ± 0.07	76.8 ± 0.0^{Aa}	31.5 ± 2.4^{Ab}	11.9 ± 2.1^{ABc}	6.15 ± 0.29^{B}
BATB	0.04 ± 0.01	0.07 ± 0.0	0.16 ± 0.01	67.2 ± 11.1^{ABa}	32.9 ± 0.0^{Ab}	14.1 ± 0.4^{Ac}	7.46 ± 0.69^{B}

For each data set, the *D*-value was calculated using corresponding rate constant (k_{max}) and the results were expressed as the mean ± standard deviation from three experiments.
[†] Values with different capital letters in the same column are significantly different ($p < 0.05$).
[‡] Values with different lower case in the same row are significantly different ($p < 0.05$).

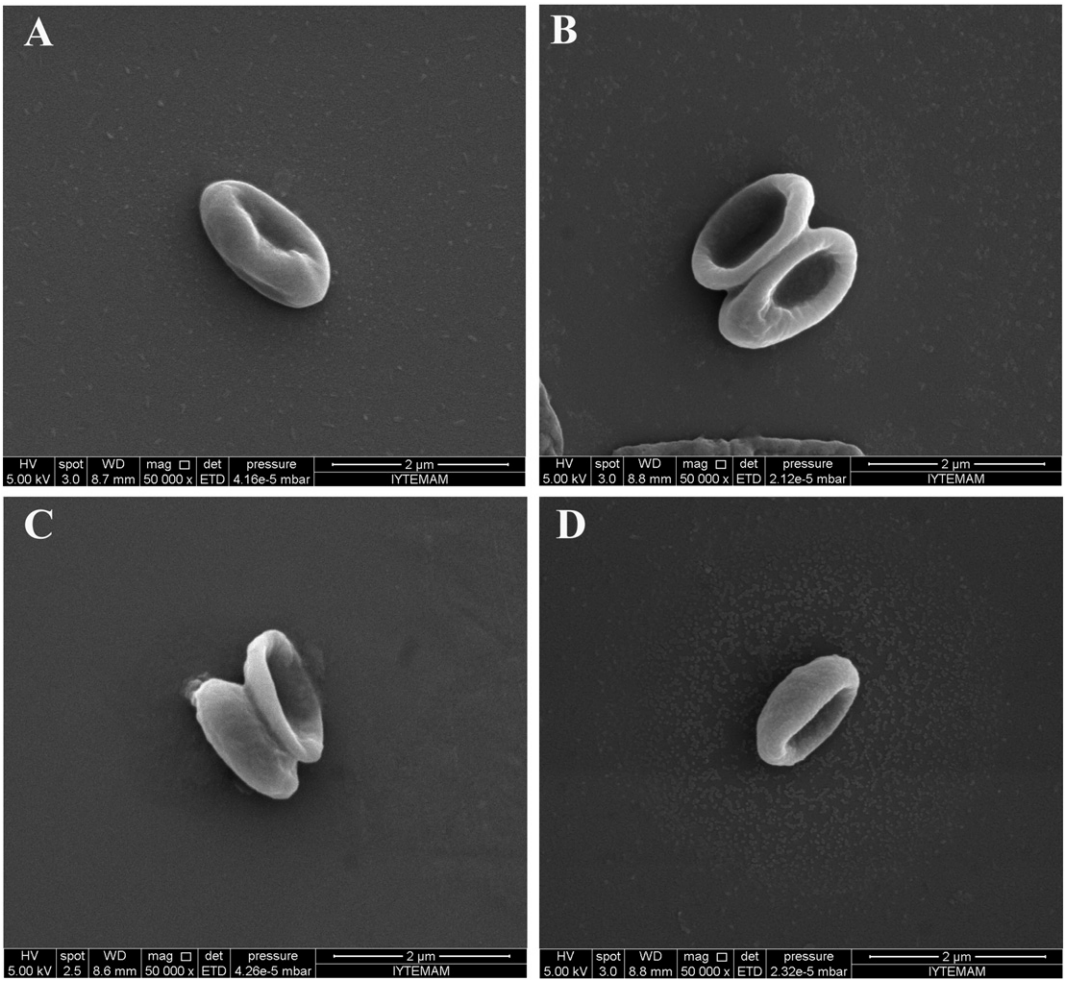

Fig. 1. Scanning electron microscopy images of untreated spores produced on (a) PDA, (b) MEA, (c) BATA and (d) BAA.

4. Discussion

Heat is the most efficient method for spore inactivation (Bigelow and Esty, 1920; Gould, 2006). The major factors that contribute to the wet heat resistance of spores are the core water content, mineralization of the spore core due to accumulation of high levels of divalent cations and DPA and the presence of high levels α/β type small acid soluble proteins (SASP) in the spore core (Melly et al., 2002). Also, the levels of Mn and the maturation of released spores during sporulation affect wet-heat resistance (Ghosh et al., 2011; Sanchez-Salas et al., 2011).

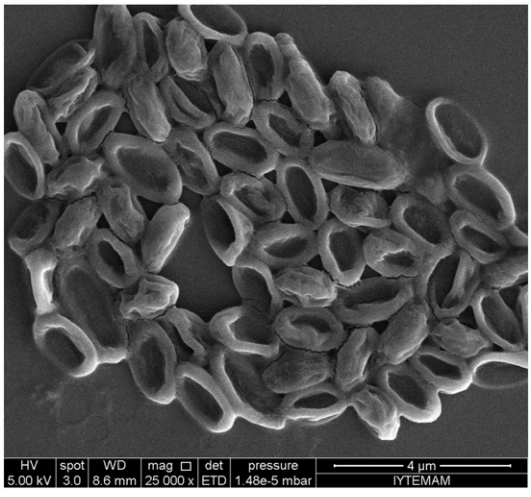

Fig. 2. Scanning electron microscopy images of autoclaved spores produced on BATA.

Although novel models could be used to describe *A. acidoterrestris* spore inactivation kinetics, the Bigelow model (first-order kinetics) was still being used to calculate *D* and *z*-values and compare them with the previous published data (Silva et al., 2012). In this study, $D_{85\ °C}$ values were between 41.7 and 76.8 min and $D_{90\ °C}$ values ranged from 9.9 to 14.7 min. Thermal resistance parameters of *A. acidoterrestris* spores in apple juice have been determined by other researchers. Splittstoesser et al. (1994) found $D_{85\ °C}$ as 56.0 ± 14 min for the strain VF. $D_{90\ °C}$ values in apple juice were found as 15.0 min (Cerny et al., 1984); 23.0 ± 7.5 min (Splittstoesser et al., 1994), 7.8 ± 0.85 min (Komitopoulou et al., 1999); and 11.1 ± 1.6 min (Bahçeci and Acar, 2007). The differences between the *D*-values may be due to the differences in the test strains, inoculum levels, incubation temperatures, sporulation temperature, differences in nutrient composition and pH of the heating medium, water activity, presence or absence of divalent cations and antimicrobial compounds (Bahçeci and Acar, 2007). Similarly, Mazas et al. (1995) determined the thermal resistance characteristics of *B. cereus* spores sporulated on different types of sporulation media (nutrient agar supplemented with Mn^{2+}, fortified nutrient agar, Angelotti medium and milk agar). They found clear differences between the *D*-values for spores produced in the four media. In another study, the effect of temperature and pH during sporulation on heat resistance of *B. weihenstephanensis* and *B. licheniformis* spores were studied (Baril et al., 2012). They found that a decrease in heat resistance was observed for spores produced either at low or high temperature or at acidic pH.

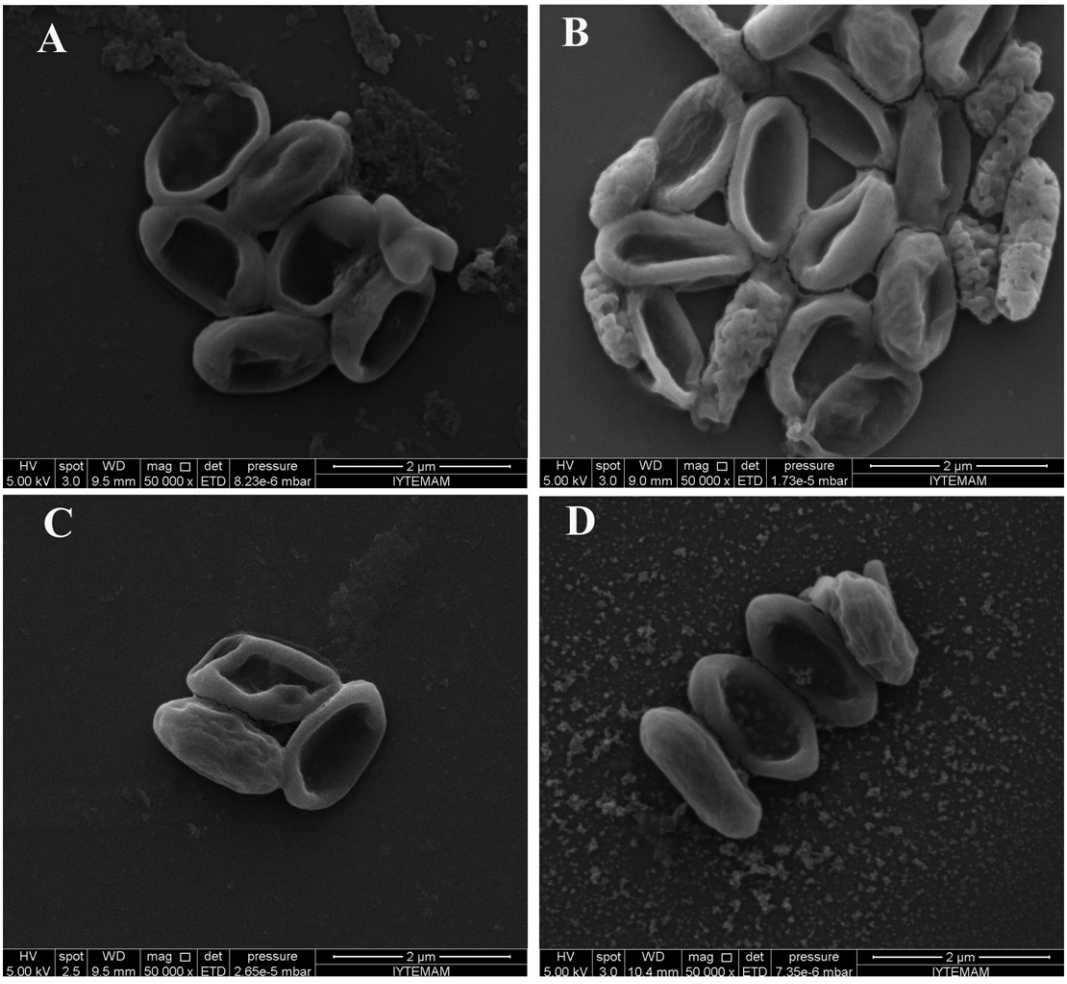

Fig. 3. Scanning electron microscopy images of heat-treated spores at 90 °C for 60 min produced on (a) PDA, (b) MEA, (c) BATA and (d) BAA.

Sporulation environment affects spore heat resistance, on the other hand it is not taken into account in heat process calculations (Carlin, 2011). When the thermal resistances were compared using *D*-values, spores produced on mineral containing media exhibited greater resistance to the wet-heat. This may be due to the mineralization of endospores with divalent cations found in BATA, BAA and BATB. In fact, mineralization of endospores with divalent cations such as Ca^{2+} or Mn^{2+}, contributes to the stabilization of endospores against heat. Ca^{2+} also chelate DPA to form Ca-DPA, which then stabilizes endospores and contributes to heat resistance. *A. acidoterrestris* endospores bind Ca^{2+} and Mn^{2+} more strongly at a low pH compared to *Bacillus* spp. and are also able to keep Ca-DPA levels constant. Thus, stabilization of Ca-DPA concentrations and the ability to strongly bind divalent cations contribute to the heat resistance of *A. acidoterrestris* spores (Yamazaki et al., 1997).

The sporulation conditions in liquid or solid medium, with various sources of carbon or minerals have been shown to affect spore heat resistance (Mah et al., 2008). In the present study, it was found that the preparation in liquid may yield spores with lower thermal resistance as those made on agar media depending on the heating temperature. Similar to our findings, Rose et al. (2007) also found that *Bacillus subtilis* spores made in liquid had lower resistance to heat at 90 °C and several chemicals, and germinated more readily with several agents. They also observed differences in the composition of the inner membrane of spores. However, they could not find differences in the levels of DPA, core water, SASP and individual coat proteins or the cross-linking of a coat-protein. It has been suggested that this difference might arise from other factors such as the strength of their peptidoglycan cortex of spores prepared in liquid or on agar (Gerhardt and Marquis, 1989; Popham et al., 1995).

Table 3
Dipicolinic acid content and release during wet-heat treatment at 85 °C, 87.5 °C and 90 °C.

Sporulation media	DPA content		DPA release (%)		
	μmol/mg spore	% dry weight	85 °C–60 min	87.5 °C–30 min	90 °C–15 min
PDA	0.21 ± 0.00 ^{A†}	3.51 ± 0.00 ^{A†}	93.27 ± 2.47^{Ab†}	87.35 ± 0.49^{Ac}	98.5 ± 0.28^{Aa}
BATA	0.47 ± 0.00^{B}	7.85 ± 0.00 ^{B}	70.00 ± 1.41^{Ca‡}	52.0 ± 1.41^{Db}	75.65 ± 5.87^{BCa}
BAA	0.49 ± 0.01^{B}	8.19 ± 0.17 ^{B}	71.79 ± 2.43^{Ca}	60.5 ± 0.71^{Cb}	68.2 ± 4.53^{Cab}
MEA	0.26 ± 0.02^{C}	4.35 ± 0.34 ^{C}	82.65 ± 0.49^{Ba}	76.45 ± 0.78^{Bb}	84.2 ± 1.13^{Ba}

Data were expressed as the mean ± standard deviation from three experiments.
† Values with different capital letters in the same column are significantly different ($p < 0.05$).
‡ DPA release (%) values with different lower case in the same row are significantly different ($p < 0.05$).

The estimated *z*-values were within the range reported previously (6.4–12.2 °C) for apple juice (Splittstoesser et al., 1994; Previdi et al., 1997; Komitopoulou et al., 1999; Bahçeci and Acar, 2007). In the related literature, the *z*-value was shown to be independent of sporulation media (Mazas et al., 1995), sporulation pH (Mazas et al., 1997; Baril et al., 2012), and sporulation temperature (Condon et al., 1992; Gonzales et al., 1999; Raso et al., 1995; Baril et al., 2012). On the other hand, the *z*-value of spores produced on PDA was significantly ($p < 0.05$) different from the *z*-values of spores produced on BATA, BAA and MEA.

DPA content is the major factor to wet heat (Paidhungat et al., 2000). The DPA contents of spores produced on non-mineral containing media (PDA and MEA) were lower than mineral containing media (BATA and BAA). Therefore, the increase in the DPA content of spores formed on mineral containing media could contribute to the increase in the wet-heat resistance. Indeed, the role of DPA in spore resistance is to lower the core water content, probably by replacing some core water. This process can increase wet-heat resistance by protecting core proteins from inactivation or denaturation (Baweja et al., 2008). However, the role of DPA release in spore killing by wet-heat is not clear. The heat inactivation of spore does not instantaneously result in the release of its DPA content. Some factors other than DPA release are involved in heat inactivation (Kort et al., 2005). In *Bacillus stearothermophilus* spores, it has been suggested that DPA release occurs before spore killing but in *Bacillus megaterium* and *B. subtilis* spores, DPA release follows spore killing rather than precedes it (Mallidis and Scholefield, 1985; Belliveau et al., 1992; Coleman et al., 2007, 2010). The SEM micrographs also suggested that DPA release results in the loss of internal volume and the presence of solid inner core among the heat resistant spores occurs because of the slow release.

In conclusion, since *A. acidoterrestris* has been suggested as the target to be used in the design of adequate pasteurization processes of acidic food products (Silva et al., 1999), the effect of sporulation media for comparing thermal fruit juice processing technologies should be considered in the fruit juice industry to ensure the safety and prolong the shelf-life of fruit juices.

Conflict of interest

There is no conflict of interest in this study.

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