Thermal inactivation of *Alicyclobacillus acidoterrestris* spores under different temperature, soluble solids and pH conditions for the design of fruit processes

Filipa M. Silva[a,*], Paul Gibbs[b], Margarida C. Vieira[c], Cristina L.M. Silva[a]

[a]*Escola Superior de Biotecnologia-UCP, Rua Dr. António Bernardino de Almeida, P-4200 Porto, Portugal*
[b]*Department of Food Safety and Preservation, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, UK*
[c]*Escola Superior de Tecnologia, Universidade do Algarve, Campus da Penha, 8000 Faro, Portugal*

Abstract

Alicyclobacillus acidoterrestris, a thermoacidophilic, non-pathogenic and spore-forming bacterium has been detected in several spoiled commercial pasteurised fruit juices. *A. acidoterrestris* spores, besides being resistant to the pasteurisation treatment conditions normally applied to acidic fruit products, can germinate and grow causing spoilage. Therefore, this microorganism was suggested as the target to be used in the design of adequate pasteurisation processes. The objectives of this work were to investigate the influence of temperature (T: 85–97°C), total soluble solids (SS: 5–60°Brix or % by weight) and pH (2.5–6.0) on D-values (decimal reduction time) of *Alicyclobacillus acidoterrestris* (type strain, NCIMB 13137) spores, and to fit a model using response surface methodology. A central composite face-centred experimental design was used, and the response, D-value determined in malt extract broth, ranged between 0.498 ± 0.045 and 94.9 ± 6.7 min. Within the factor ranges studied, temperature was the parameter that most affected the D-value. Following this was the SS and, lastly, the pH value. A linear decrease in D-value was observed with decreasing SS and pH, and a non-linear decrease in D-value was noticed with increasing temperature. A second order polynomial was successfully fitted to the data ($R^2 = 0.98$). In general, D-values measured in real fruit systems, such as orange, apple and grape juices, blackcurrant concentrates, cupuaçu (exotic fruit) extract and orange juice drink, were higher than those predicted by the malt extract broth model. This result emphasises the importance of experimental validation of any model-derived process.

Keywords: *Alicyclobacillus acidoterrestris*; Spores; Thermal inactivation; Heat resistance; Kinetics; Fruit products; Response surface methodology

1. Introduction

Generally, fruit products, such as juices, nectars, concentrates or purées, are acidic ($pH < 4.6$) and,

*Corresponding author. Tel.: +351-2-558-0058; fax: +351-2-509-0351.
E-mail address: filipa@esb.ucp.pt (F.M. Silva)

therefore, a pasteurisation treatment in the temperature range of 85–95°C should be adequate for their stabilisation at ambient temperature. Such a process inactivates all nonspore-forming microorganisms that are able to spoil the product and, usually, surviving bacterial spores will not germinate and grow under these acidic conditions (Blocher and Busta, 1983).

However, *Alicyclobacillus acidoterrestris* (AAT), a thermoacidophilic, non-pathogenic, spore-forming bacterium has been detected in several spoiled commercial pasteurised fruit juices. Cerny et al. (1984) reported a new type of spoilage bacterium in aseptically packaged apple juice, subsequently named *Bacillus acidoterrestris* by Deinhard et al. (1987). Later, Wisotzkey et al. (1992) proposed a new genus, *Alicyclobacillus*, characterised by ω-alicyclic fatty acids as the major natural membrane lipid component. Yamazaki et al. (1996) isolated and identified AAT in spoiled acidic juices. AAT spore germination and growth to 10^6 cfu/ml under acidic conditions has also been reported in orange juice stored at 44°C for 24 h (Pettipher et al., 1997). Acidic spore-forming bacillus spores (VF strain) were isolated from a spoiled apple juice (Splittstoesser et al., 1994) and identified later by Pontius et al. (1998) as AAT.

Generally, AAT growth characteristics in the recommended culture medium (often called *Bacillus acidocaldarius* medium, BAM, since it was initially used with this microorganism) is limited to temperatures ranging between 25 and 60°C and pH values between 2.55 and 5.5 (Deinhard et al., 1987; Previdi et al., 1995; Yamazaki et al., 1996; Pinhatti et al., 1997). These microorganisms can have a slow growth cycle (up to five days) and are responsible for off-flavours. Pettipher et al. (1997) concluded that 10^5–10^6 cells/ml in apple and orange juices formed enough guaiacol (ppb) to produce sensory taint. Growth of heat-activated VF strain spores was detected in tomato, apple, orange, grapefruit, pineapple and white grape juices during two days of storage at 43°C, by plating (potato dextrose agar), counting and comparing the viable spores of the initial inoculated juice and the same juice kept for two days at 43°C (Splittstoesser et al., 1994). The same work reported growth inhibition with a high content of total soluble solids (SS). The visual detection of spoilage is very difficult because AAT does not produce gas during growth and incipient swelling of containers does not occur. Due to these facts, spoilage during storage of retail product can occur without visible changes (Walls, 1997).

Concerning heat resistance, Pontius et al. (1998) concluded that the type of organic acid (malic, citric, tartaric) did not significantly affect *D*-values. A review on *D*-values (decimal reduction time) and *z*-values was carried out (Table 1). The $D_{95°C}$ ranged from 0.1 to 16 min and *z* was between 7.2 and 10.8°C. There are few published data and no systematic work that investigated simultaneously the influence of pH, SS and temperature (*T*) on the heat resistance of AAT spores.

In this work, AAT spores are suggested as target for pasteurisation of high-acidic food products due to their thermoacidophilic properties and the occurrence in several spoiled pasteurised products. The main objective of this work was to investigate the effects of pH (2.5–6.0), total soluble solids (SS: 5–60°Brix or % by weight of sucrose) and temperature (*T*: 85–97°C) on *D*-values of *A. acidoterrestris* type strain spores using response surface methodology (RSM).

2. Materials and methods

2.1. Bacterial strain and growing enumeration medium

A. acidoterrestris type strain NCIMB 13137 (National Collections of Industrial and Marine Bacteria, Auris Business Centre, Aberdeen, UK) was enumerated using agar medium composed by three solutions mixed after sterilisation (121°C for 10 min): (i) $CaCl_2 \cdot 7H_2O$, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 0.2 g; yeast extract, 2 g; glucose, 5 g; KH_2PO_4, 3 g, and distilled water, 500 ml, adjusted to pH 4.0 with H_2SO_4 (pH meter ORION, model 520 A); (ii) 1 ml of SL-6 trace elements solution ($ZnSO_4 \cdot 7H_2O$, 0.1 g; $MnCl_2 \cdot 4H_2O$, 0.03 g; H_3BO_3, 0.3 g; $CoCl_2 \cdot 6H_2O$, 0.2 g; $CuCl_2 \cdot 2H_2O$, 0.01 g; $NiCl_2 \cdot 6H_2O$, 0.02 g; $Na_2MoO_4 \cdot 2H_2O$, 0.03 g; distilled water, 1 l); (iii) agar, 15 g; distilled water, 500 ml. The inoculated plates were incubated for 3 days at 45°C.

2.2. Spore production

Sporulation agar medium: yeast extract, 1 g; $(NH_4)_2SO_4$, 0.2 g; $CaCl_2$, 0.25 g; $MgSO_4$, 0.5 g;

Table 1
Literature values of heat resistance of *A. acidoterrestris* spores[a]

Heating medium	pH	SS (°Brix or % by weight of sucrose)	*T* (°C)	*D*±SD[b] (min)		*z* (°C)	Reference
				D	SD		
Apple juice	3.5	11.4	85	56	14	7.7	Splittstoesser et al., 1994
			90	23	7.5		
			95	2.8	0.7		
Grape juice	3.3	15.8	85	57	13	7.2	
			90	16	4.1		
			95	2.4	0.9		
Berry juice	3.5	nr	88	11	nr	7.2	Walls, 1997
			91	3.8	nr		
			95	1	nr		
Wine	nr	nr	75	33	nr	10.5	Splittstoesser et al., 1997
			85	0.57			
Orange juice drink	4.1	5.3	95	5.3	nr	9.5	Baumgart et al., 1997
Fruit drink	3.5	4.8	95	5.2	nr	10.8	
Fruit nectar	3.5	6.1	95	5.1	nr	9.6	
Citrate buffer	2.5–6.9	nr	95	1	nr	nr	Yamazaki et al., 1997
Model fruit juice	3.1	nr	91	31	nr	10.0	Pontius et al., 1998
			97	7.9			
	3.7	nr	91	54	nr	7.7	
			97	85			

[a] nr = not reported.
[b] SD = standard deviation.

glucose, 1 g; KH_2PO_4, 0.6 g; distilled water, 500 ml, adjusted to pH 4.0 with H_2SO_4, and agar, 20 g; distilled water, 500 ml. The two solutions were sterilised separately (121°C for 10 min), mixed and dispensed in medical flats (Bernard Muller, London, UK) when still warm.

Sporulation broth medium: the same formula but without the agar in 1000 ml of distilled water, adjusted to pH 4.0 (pH meter ORION, model 520 A).

A portion of AAT was cultured in sporulation broth at 45°C overnight. These cultures were spread onto sporulating agar inside medical flats and incubated at 45°C for two–three days. Spores were removed by agitating each bottle after adding sterile glass beads and 10 ml of water (repeating this procedure five times to ensure total removal of spores). The spore crop was centrifuged (15 min, 21,000 *g*, 4°C), the supernatant was decanted and the pellet was resuspended in 50% (v/v) aqueous ethanol, to destroy any vegetative cells. After 30–60 min, the spore suspension was recentrifuged, the supernatant was discarded and the pellet was resuspended in sterile water (repeated a further three times). The final pellet was resuspended in sterile distilled water, stored at 4°C for at least one week before use and enumerated with and without previous heat treatment (10 min at 80°C).

2.3. Experimental design

The *D*-value of AAT spores as a function of *T* (85–97°C), SS (5–60°Brix) and pH (2.5–6) was determined by heating a solution that simulated a

fruit system at a specified temperature. This solution was composed by MEB (malt extract broth, Oxoid, reference code CM57, Basingstoke, UK) adjusted to a specific Brix value (or % by weight of sucrose) by refractometry at 20°C and pH (pH meter ORION, model 520 A) before sterilisation, with fructose and HCl, respectively. Although sucrose solutions were used for the calibration of the refractometer, the SS of the MEB heating solutions was obtained by adding fructose. Fructose was used because this is the major sugar of apple juice, and is also present in citrus juices ($\approx 25\%$) and tropical fruit purées (Varnam and Sutherland, 1994; Zadernowski et al., 1997). When using the same weight of sucrose or fructose, similar final Brix values were obtained. Assuming that the system could behave as a second-order polynomial model,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

a three-factor ($X_1 = T$, $X_2 = \mathrm{SS}$, $X_3 = \mathrm{pH}$) at two levels central composite face-centred design (Montgomery, 1991; Stat-Ease, 1992) with four centre points (18 random experiments) was used and the response ($Y = D$-value) was determined for each experiment (Table 2).

2.4. Thermal treatments and enumeration procedure

Cole and Jones (1990) conceived an apparatus (Sherwood Instruments, Lynnfield, MA, USA) comprising a narrow stainless-steel coil (9.5 ml total volume, 3.175 mm outer diameter, 0.5 mm wall thickness), submerged in a thermostatic water bath and an automatic sampler with the sampling frequency controlled by a computer program. This apparatus was used for the thermal inactivation experiments (Fig. 1). The sample come up time (time to reach the water bath temperature) was about 1 s. For each run, cleaning was performed by injecting first Quadralene (0.5%, v/v; neutral liquid, Divosan), then disinfectant (Quatdet, 1%, w/w), industrial alcohol and finally sterile water. The stored spore suspension was centrifuged and then the pellet was resuspended in 15 ml of a specific pH–SS MEB to yield an initial count of $\approx 10^7$ spores cfu/ml

Table 2
Factorial experimental design and *D*-values of *A. acidoterrestris* spores measured in malt extract broth

Design	pH	SS[a] (°Brix or % by weight of sucrose)	*T* (°C)	$D \pm \mathrm{SE}$[b] (min)
Cube	2.5	5	85	35.5±2.3
Cube	2.5	5	97	0.771±0.037
Cube	2.5	60	85	60.3±2.4
Cube	2.5	60	97	2.15±0.14
Cube	6	5	85	52.6±1.6
Cube	6	5	97	0.498±0.045
Cube	6	60	85	94.9±6.7
Cube	6	60	97	4.35±0.27
Star	4.25	32.5	85	65.9±2.5
Star	4.25	32.5	97	1.39±0.06
Star	4.25	5	91	5.75±0.17
Star	4.25	60	91	25.3±1.6
Star	2.5	32.5	91	4.35±0.3
Star	6	32.5	91	9.76±0.32
Centre	4.25	32.5	91	11.2±0.9
Centre	4.25	32.5	91	11.1±0.3
Centre	4.25	32.5	91	10.2±0.2
Centre	4.25	32.5	91	15.5±0.6

[a] Added as fructose.
[b] SE = standard error.

MEB. The water bath was set at the design temperature, ten sampling times were programmed in the computer, 10 ml of inoculated MEB were injected into the submerged coil, and the starting time key in the computer was pressed; the remaining MEB was used for the time zero determination of viable spore count and final pH measurement. Times were selected in order to cover spore inactivation until ca. 10^2 cfu/ml remained. For each sampling time, 200 μl of the heated spores were collected and promptly cooled by dilution in a tube containing 5 ml of MEB resuscitation broth (3.85×10^{-2} dilution) at room temperature. The samples were left at ambient temperature to allow further cooling and recovery of the heat-shocked spores for approximately 30 min. Then, enumeration of the surviving spores was carried out by appropriate decimal dilutions in MRD (Oxoid, UK), surface plating on agar medium plates (see enumeration medium above) were incubated for three days at 45°C and colonies were counted.

2.5. Data analysis and data model fitting

A log-linear relation was observed between the spore concentration and time. For each thermal

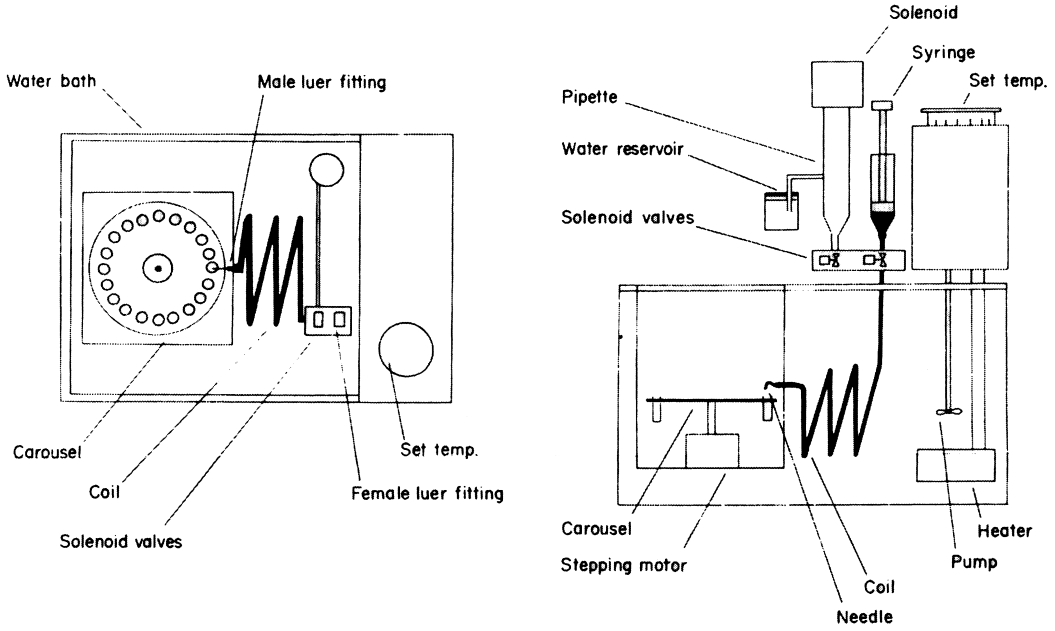

Fig. 1. Submerged coil heating apparatus. Plan (a) and side view (b) of water bath, coil and sampling device. The electronic system for sampling frequency control and solenoid valve switching is not shown [Reproduced by permission from M.B. Cole and M.V. Jones, Lett. Appl. Microbiol. 11, (1990) 234].

inactivation experiment (T, SS, pH), the $D \pm$SE (standard error) were estimated directly by performing a non-linear regression to the log (spore concentration) vs. time curve and using the statistical software Stata (Version 3.0). If linear regression was carried out, $(-1/D) \pm$SE would be estimated instead of the required $D \pm$SE. The RSM was used to analyse the relative importance of T–SS–pH and to fit a model able to predict D-values knowing the T, SS (brix or %, w/w, sucrose) and pH values (Eq. 1). The software Design-Expert (Version 4.0.2) was used for calculations (Stat-Ease, 1992).

2.6. Thermal inactivation of Alicyclobacillus acidoterrestris spores in cupuaçu pulp extract, orange juice and blackcurrant concentrates

The practical application of the model was investigated by performing the same thermal inactivation experiments with fruits within the range of temperatures, SS and pH modelled. Extracts of an exotic fruit named cupuaçu (*Theobroma grandiflorum*), orange juice and blackcurrant concentrates were used. The predicted D-values were compared with D-values measured in the real fruits. D-values obtained with other fruits referred to in the literature were also used for the validation process (Table 1).

3. Results and discussion

The viable counts for both heated and non-heated spore suspensions were similar. This result was also observed by Pontius et al. (1998). Also, as cited by other authors, the curves of log (survivor spores/ml) vs. time did not exhibit any 'shoulder' and, therefore, no deviation from linear behaviour was observed (Previdi et al., 1997; Pontius et al., 1998).

3.1. T–SS–pH effect on the D-values of A. acidoterrestris spores in malt extract broth

D-values determined in MEB ranged from 0.498 ± 0.045 ($T = 97$°C, SS = 5°Brix, pH = 6) to 94.9 ± 6.7 min ($T = 85$°C, SS = 60°Brix, pH = 6) (Table 2). A response surface analysis was performed to investigate significant factors and their combinations on D-values. In Fig. 2, the Pareto chart

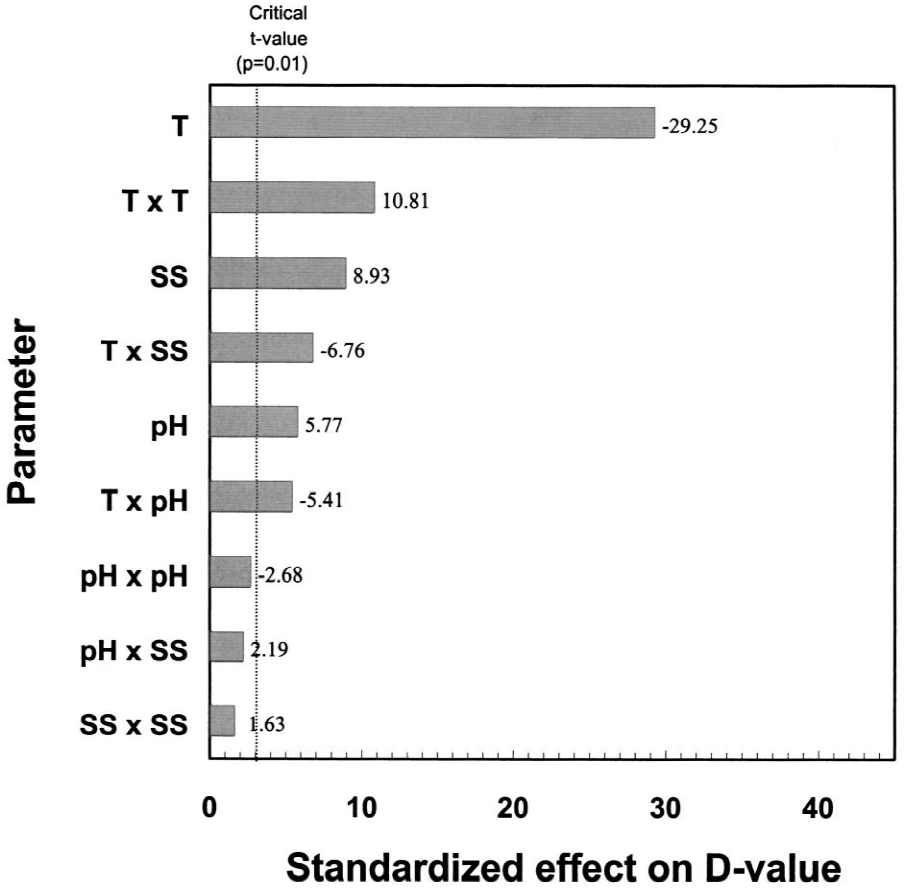

Fig. 2. Pareto chart for the evaluation of parameter effects on *D*-values of *A. acidoterrestris* spores.

shows the standardised effects of different parameters on *D*-values. The standardised effect is defined as the estimated coefficient divided by its standard error (β_i/SE_i). An effect that exceeds the vertical line (critical *t*-value, $P=0.01$) may be considered significant. The following ranked parameters, in decreasing order of importance, have a significant effect ($P<0.01$) on *D*-values within the ranges studied: T, T^2, SS, $T\times$SS, pH, $T\times$pH. Figs. 3–5 present the response surface charts for *D* vs. (*T*, SS) for pH = 4.25, *D* vs. (*T*, pH) for SS = 32.5°Brix and *D* vs. (SS, pH) for $T=91$°C, respectively. As expected, temperature was the factor with the major impact on *D*-value: a small temperature increase caused a considerable decrease in *D*. Then, *D*-value increased slightly with increasing SS and pH, especially at lower temperatures ($T\times$SS and $T\times$pH were significant). In fact, close to 97°C, the effects of SS and pH were not visible (Figs. 3 and 4). Regarding the effect of pH on *D*-values, the opinions of other authors diverge: Yamazaki et al. (1997) reported no effect in the range of pH from 2.5 to 6.9, while Pontius et al. (1998) detected a significant effect in the range of pH from 2.8 to 4.0 at lower temperatures. The curvature observed in Figs. 3 and 4 surfaces was due to the quadratic effect of temperature on *D* (T^2 was significant). The *D*-value varied linearly with SS and pH (Fig. 5).

3.2. Model fitting and practical application

A second order polynomial was successfully fitted ($R^2=0.98$) to data determined using malt extract broth, by response surface regression (Fig. 6). Relative residuals (residual/D_{model}) ranged between 0.443 and 0.216.

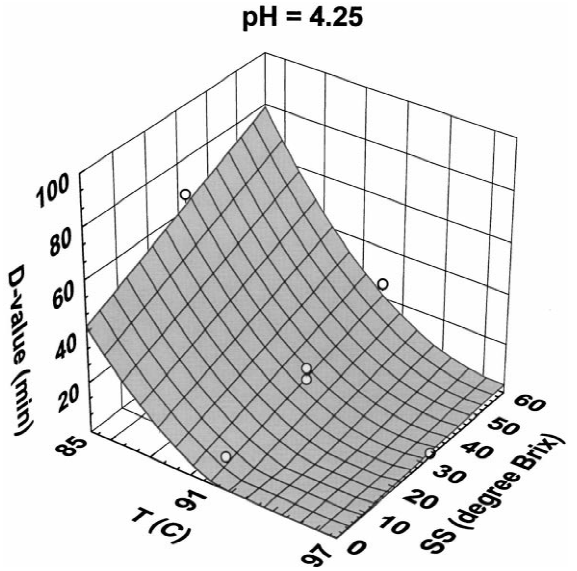

Fig. 3. Response surface chart of D vs. T and SS (pH = 4.25).

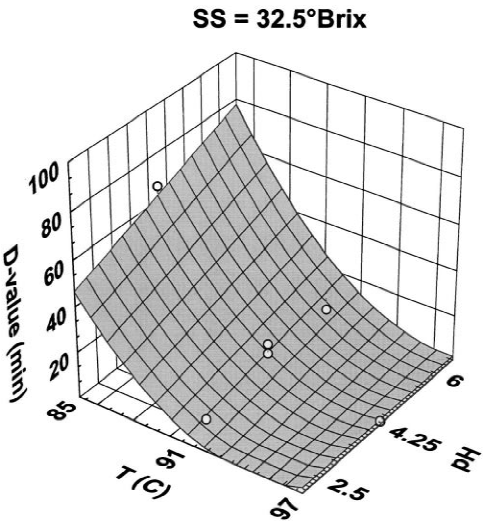

Fig. 4. Response surface chart of D vs. T and pH (SS = 32.5°Brix).

$$D_{\text{Model}} = 4715.1 - (102.96 \times T) + (0.56042 \times T^2) + (4.6096 \times \text{SS}) - (0.04699 \times T \times \text{SS}) + (57.147 \times \text{pH}) - (0.59083 \times T \times \text{pH}) \quad (2)$$

In MEB, predicted D-values compared well with measured D-values (see open circles in Fig. 6) and, thus, the model provides a valid description of the data used to generate it.

The practical application of the model was investigated by comparing predicted D-values (Eq. 2) with those determined experimentally in real fruit products such as orange juice, cupuaçu extract and

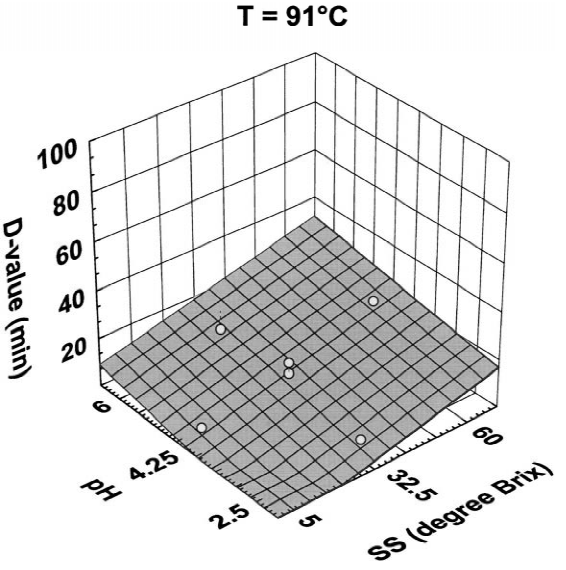

Fig. 5. Response surface chart of D vs. SS and pH (T = 91°C).

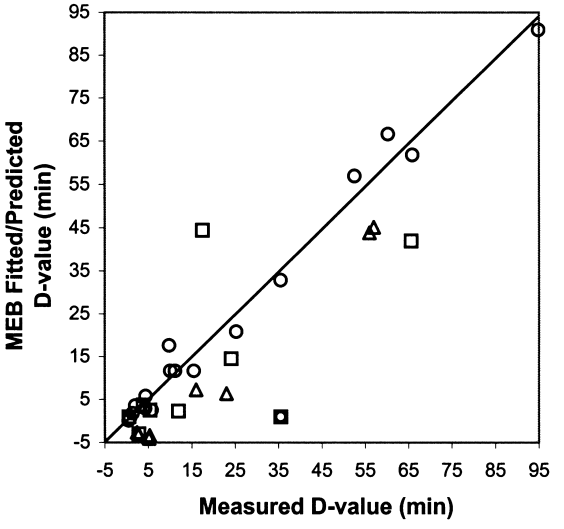

Fig. 6. Comparison of measured *A. acidoterrestris* spores' D-values with those fitted and predicted: malt extract broth (○), fruit products (□), literature data (△).

Table 3
D-values of *A. acidoterrestris* spores measured and predicted in cupuaçu extract, orange juice and two blackcurrant concentrates

Heating medium	pH	SS (°Brix or % by weight of sucrose)	*T* (°C)	Predicted *D*-value (min)	Measured *D*-value		
					D±SE (min)	95% CI[a]	*z* (°C)
Cupuaçu extract	3.6	11.3	85	44.4	17.5±1.1	13.9–21.1	9.0
			91	2.52	5.35±0.57	3.88–6.81	
			95	−2.99	2.82±0.27	2.17–3.48	
			97	0.966	0.569±0.034	0.491–0.647	
Orange juice	3.5	11.7	85	41.9	65.6±5.5	52.2–79.1	7.8
			91	2.32	11.9±0.6	10.7–13.2	
Light Blackcurrant concentrate	2.5	26.1	91	3.74	3.84±0.49	2.59–5.09	–
Blackcurrant concentrate	2.5	58.5	91	14.5	24.1±2.7	17.7–30.6	–

[a] CI = confidence interval.

blackcurrant concentrates (Table 3) and also with others reported in the literature (Table 1). Fig. 6 presents a comparison between measured vs. MEB fitted/predicted *D*-values. In this chart, there are three different symbols corresponding to fitted *D*-values in MEB (○), predicted *D*-values in fruit products (□) and predicted *D*-values using data reported in the literature (△). A problem arose in validation of the model because, with the exception of the cupuaçu extract at 85 and 97°C, all of the *D*-values predicted were smaller than the corresponding values measured in fruit products. This might be due to other constituents present in the fruit products and not in the MEB that have effects that are different from those of the HCl and fructose used for the model generation and that might increase the heat resistance of the spores.

Due to the differences observed between predicted and measured *D*-values in fruit products, the model obtained cannot be used directly to estimate heat resistance in real fruit systems, but is useful for predicting the trends and relative changes in *D*-values due to *T*, fructose and HCl variations. This result indicates that it is still necessary to perform some challenge tests before the predictions can be used in industry. In future studies, the water activity may be considered instead of SS, since different sugars generate different water activities at the same concentrations and might have different effects on *D.*

4. Conclusions

The heat resistance of *A. acidoterrestris* spores was greatly affected by temperature (85–97°C), followed by the SS (5–60°Brix) and pH (2.5–6.0); *D*-values decreased on increasing the temperature, decreasing SS and decreasing pH. Although *D* was linearly dependent on SS and pH, a non-linear relationship was observed with the temperature. Using the response surface polynomial model, *D*-values determined in malt extract broth were, in most cases, lower than those determined in fruit products. Challenge tests are recommended before the *D*-value predictions can be used in industry.

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References

Baumgart, J., Husemann, M., Schimdt, C., 1997. *Alicyclobacillus acidoterrestris*: occurrence, importance, and detection in beverages and raw materials for beverages. Flussiges Obst. 64, 178–180.

Blocher, J.C., Busta, F.F., 1983. Bacterial spore resistance to acid. Food Technol. 37, 87–99.

Cerny, G., Hennlich, W., Poralla, K., 1984. Fruchtsaftverderb durch bacillen: isolierung und charakterisierung des verderbserregers. Z. Lebensmitt. Unters. Forsch. 179, 224–227.

Cole, M.B., Jones, M.V., 1990. A submerged-coil heating apparatus for investigating thermal inactivation of micro-organisms. Lett. Appl. Microbiol. 11, 233–235.

Deinhard, G., Blanz, P., Poralla, K., Altan, E., 1987. *Bacillus acidoterrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. System. Appl. Microbiol. 10, 47–53.

Montgomery, D.C., 1991. Response surface methods and designs. In: Design and Analysis of Experiments, third ed, Wiley, Singapore, p. 547.

Pettipher, G.L., Osmundson, M.E., Murphy, J.M., 1997. Methods for the detection and enumeration of *Alicyclobacillus acidoterrestris* and investigation of growth and production of taint in fruit juice and fruit juice-containing drinks. Lett. Appl. Microbiol. 24, 185–189.

Pinhatti, M.E.M.C., Variane, S., Eguchi, S.Y., Manfio, G.P., 1997. Detection of acidothermophilic bacilli in industrialized fruit juices. Fruit Processing 7, 350–353.

Pontius, A.J., Rushing, J.E., Foegeding, P.M., 1998. Heat resistance of *Alicyclobacillus acidoterrestris* spores as affected by various pH values and organic acids. J. Food Prot. 61, 41–46.

Previdi, M.P., Colla, P., Vicini, E., 1995. Characterization of *Alicyclobacillus*, a spore-forming thermophilic acidophilic bacterium. Indust. Conserve 70, 128–132.

Previdi, M.P., Quintavalla, S., Lusardi, C., Vicini, E., 1997. Heat resistance of *Alicyclobacillus* spores in fruit juices. Indust. Conserve 72, 353–358.

Splittstoesser, D.F., Churey, J.J., Lee, C.Y., 1994. Growth characteristics of aciduric sporeforming bacilli isolated from fruit juices. J. Food Prot. 57, 1080–1083.

Splittstoesser, D.F., Lee, C.Y., Churey, J.J., Control of *Alicyclobacillus* in the juice industry, Session 36-3 presented at the Institute of Food Technologists Annual Meeting in Orlando, FL, 14–18 June 1997

Stat-Ease, 1992. Design-Expert® Software Version 3.0 User's Guide, Stat-Ease, Incorporated, USA.

Varnam, A.H., Sutherland, J.P., 1994. Fruit juices. In: Beverages — Technology, Chemistry and Microbiology, Chapman and Hall, London, pp. 60–63.

Walls, I., Alicyclobacillus — an overview, Session 36-1 presented at 1997 Institute of Food Technologists Annual Meeting in Orlando, FL, 14–18 June 1997

Wisotzkey, J.D., Jurtshuk, P., Fox, G.E., Deinhard, G., Poralla, K., 1992. Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. Int. J. Syst. Bacteriol. 42, 263–269.

Yamazaki, K., Teduka, H., Shinano, H., 1996. Isolation and identification of *Alicyclobacillus acidoterrestris* from acidic beverages. Biosci. Biotechnol. Biochem. 60, 543–545.

Yamazaki, K., Isoda, C., Tedzuka, H., Kawai, Y., Shinano, H., 1997. Thermal resistance and prevention of spoilage bacterium *Alicyclobacillus acidoterrestris*, in acidic beverages. J. Jpn. Soc. Food Sci. Technol. 44, 905–911.

Zadernowski, R., Markiewicz, K., Nesterowicz, J., Pierzynowska-Korniak, G., 1997. Physical and chemical characteristics of pulpy juices. Fruit Processing 7, 441–444.