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Modeling Heat-Resistance of *Alicyclobacillus acidoterrestris* in Different Fruit Juices: Combined Effects of pH and Temperature

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HIGHLIGHTS

- The food juice matrix influences thermal resistance of *A. acidoterrestris*.
- *Alicyclobacillus* thermal resistance is affected by pH x temperature combinations.
- Mathematical models predict heat and pH effect on D-values and endospore survival.

Abstract: This study evaluated the efficacy of pH and temperature on the thermal resistance of *Alicyclobacillus acidoterrestris* endospores in different fruit juices. A polynomial equation was used to describe the pH x temperature impact on the D-values of endospores. Endospore resistance was influenced by fruit juice matrices. Maximum heat resistance was found in passion fruit juice (D value 24.7±2.8 min, 90 ºC, pH 4.5) and the greatest thermal destruction was observed in papaya juice (D value 1.7±2.8 min, 95 °C, pH 2.5). Regardless of the fruit juice, endospore thermal destruction was more effective at 95 °C. In both temperatures analyzed, the effect of pH on the reduction of heat resistance was more pronounced in papaya juice. According to the mathematical models, the interaction pH x temperature had the greatest impact on endospores thermal reduction. These results emphasize the relevance of time⁄temperature binomials to prevent spoilage by *A. acidoterrestris* in thermal treated fruit juices.

Keywords: inactivation model; D-values; spoilage; thermoacidophilic bacteria.

INTRODUCTION

Deterioration and reduced shelf life of fruit juices cause considerable economic losses in the food industry, and bacteria of the genus *Alicyclobacillus* are often associated with the spoilage of fruit juices [1]. The first association between spoilage of fruit juices and *Alicyclobacillus* growth was described almost four decades ago for the deterioration of apple juice in Germany [2]. Since then, several cases of fruit juice spoilage caused by *Alicyclobacillus* have been reported worldwide [3,4].

The deterioration caused by *Alicyclobacillus* is often difficult to detect due to the lack of gas production and little change in turbidity during bacterial growth. Generation of off-flavors is the main change caused by *Alicyclobacillus* in fruit juices, which has been related to the production of guaiacol (2-methoxyphenol) and halophenols (2,6-dibromophenol and 2,6-dichlorophenol) [1].

Alicyclobacillus is a heterotrophic, aerobic, non-pathogenic, endospore-forming, and thermoacidophilic bacterium that is capable of growing at temperatures between 20 °C and 70 °C (optimum range from 42 °C to 60 °C), and in a pH range from 2.5 to 6.0 [5]. The resistance of *Alicyclobacillus* to environmental stresses appears to be related to the presence of ω-alicyclic fatty acids as a major component of the membrane lipids [6].

Among all *Alicyclobacillus* spp., *A. acidoterrestris* is the most frequently associated with fruit juice spoilage [7], and contamination can occur at any point of the supply chain, from farm to the final product. Because of the economic losses that these microorganisms cause to the food industry, different approaches have been proposed to control *A. acidoterrestris* germination and outgrowth [4,8,9].

Given the high thermal resistance of *Alicyclobacillus* endospores and the fact that harsh heat treatments can alter the organoleptic properties of foods, two strategies have been used to improve the efficiency of endospore inactivation: (1) combination of methods: heat treatment combined with water activity reduction, addition of organic acids (low pH), reduction of oxygen inside the packages, addition of antimicrobial compounds, and storage under chilled conditions [10]; (2) non-thermal approaches: high hydrostatic pressure (HHP), supercritical carbon dioxide, high-voltage pulsed electric fields, ultraviolet radiation, gamma irradiation, ultrasound, and nonconventional chemical reagents [10,11,12].

The combination of heat and low pH has been traditionally used by the food industry to reduce thermal resistance and prevent the germination of endospores [13]. However, previous studies focused mostly on apple and orange juices, and the understanding of the thermal resistance of *Alicyclobacillus* endospores in other fruit juices is less understood. Moreover, the use of mathematical models to evaluate and predict how intrinsic factors in fruit juices and processing conditions impact the survival of *A. acidoterrestris* endospores could be useful to control and optimize industrial thermal processes.

Here we investigate the effects of different combinations of pH and temperature on the thermal resistance of *Alicyclobacillus* endospores in different tropical fruit juices. Mathematical models were also developed using polynomial regression analysis to predict how heat and pH impact the D-values and survival of *Alicyclobacillus* endospores in fruit juices, an approach that could be useful to optimize industrial processes [14,15].

MATERIAL AND METHODS

Composition of the tropical fruit juices

Commercial pineapple, guava, orange, papaya, mango, and passion fruit juices belonging to the same industrial batch were purchased from a local market (Viçosa, MG, Brazil). Fruit juice composition was characterized via physicochemical analysis and determination of the following parameters: pH, titratable acidity, soluble solids (° Brix), water activity (aw), reducing sugars and minerals (K, Na, Ca, Mg, P and S). All procedures were performed according to the methods recommended by the AOAC [16].

Microorganism and growth conditions

Alicyclobacillus acidoterrestris DSM 2498 isolated from juice samples was obtained from the André Tosello Foundation (Campinas, São Paulo, Brazil). Cultures were routinely grown for 24 h in BAM (*Bacillus acidocaldarius* medium) under aerobic conditions [15]. The incubation was carried out at 43 °C and the flasks were maintained under orbital agitation (180 rpm). Stock cultures were kept at -80 °C in BAM medium containing 10% glycerol.

Preparation of *A. acidoterrestris* endospore suspension

The endospore suspensions were obtained from cultures of *A. acidoterrestris* DSM 2498 grown under the conditions described above. Stationary-phase cultures (3% inoculum, v/v) were transferred to BAM medium and maintained for 120 hours at 45 °C under aerobic conditions (120 rpm); the culture was then kept at 4 °C for 48 h [15]. Culture samples were collected, and cells containing endospores were visualized under an optical microscope after staining the samples with malachite green dye [17]. Cultures were centrifuged (3,700 x g, 20 min, 5 °C) when approximately 80-90% of the cells contained endospores, and the resulting pellet was resuspended in 20 mL of sodium phosphate solution (5 mM, pH 4.0).

Before starting the heat treatment, *A. acidoterrestris* endospores were activated at 80 °C for 10 min. The suspension containing endospores was then centrifuged three times (3700 x g, 20 min, 5 °C), and resuspended in 10 mL of sodium phosphate solution (5 mM, pH 4.0) to eliminate the remaining vegetative cells. Endospores were counted by plating on BAM agar, and the suspension was maintained at 4 °C until use.

Heat treatment and determination of D values

Alicyclobacillus acidoterrestris endospores inoculated in different fruit juices were submitted to heat treatment (90 °C and 95 °C for 25 min). The thermal resistance was examined at different pH values for each tropical fruit juice used in this study. Stainless steel tubes (AISI 304 – 7.4 x 127 mm and 0.25 mm thickness) containing 5 mL of each fruit juice at different pH values (2.5, 3.0, 3.3, 3.6, 4.0, and 4.5, adjusted with 1 M HCl or NaOH solution) were heated to 90 °C or 95 °C.

After reaching the desired temperature, the endospore suspension was added to a final concentration of approximately 10^6 CFU/mL and immediately homogenized in a vortex for no longer than 10 s. Samples (200 µL) were collected at different time intervals during the heat treatment (0, 5, 10, 15, 20, and 25 min), transferred to an ice bath, and then plated (10 µL) in triplicate, using BAM agar and the drop plate technique [18].

The decimal reduction time (D value), or the time required to reduce the number of endospores by 90% at a given temperature, was calculated as the negative reciprocal of the angular coefficient from the linear regression equation of the survival curve sampled between 0 to 25 min of heat treatment. At least 5-time points were used to calculate the D values reported in this study. The z_{pH} value, or pH range resulting in a 10-fold reduction in the D value, was calculated from the logarithmic regression lines of the D-values of each temperature plotted against the corresponding pH values. All experiments were performed with two biological replicates using at least duplicate samples.

Regression model

To generate a mathematical model using the regression method, pH and temperature were used as independent variables, and the LogD value was used as the dependent variable. Testing was performed on six different types of juices inoculated with *A. acidoterrestris* DSM 2498 endospores. Two tubes were analyzed for each pH value, each processing temperature, and each type of matrix (juice), totaling six tubes per analysis. Thermal reduction times were calculated according to the procedures described above. The endospore count at each time of heat treatment was transformed into Log according to Salles and coauthors [19] using the equation: Log_{10} (x + 1), where x is CFU/mL. The value of 1 was added to the CFU/mL to avoid zero values in the analysis. Experimental data were analyzed using the regression method with a commercial statistical package (SAS System Software, version 9.0) and the following polynomial regression model:

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

where β_0, β_1, and β_2 represent the estimated regression coefficients, with β_0 as the constant; β_1 and β_2 represent the linear effects of pH and temperature, respectively; β_{12} is the interaction effect, and β_{11} and β_{22} represent the quadratic effect. When necessary, non-significant variables were gradually excluded from the model to improve the goodness of fit of the equation. Experiments were performed at least in duplicate with two biological replications, and the mean values were presented in Tables and Figures.

Regression analysis and analysis of variance were performed for fitting the model represented by the equation above (Equation 1) and to evaluate the statistical significance of the model terms. Response surface plots were generated using the software Matlab 16.0.

RESULTS

Composition of tropical fruit juices

The physicochemical analysis of commercial tropical fruit juices showed that the pH values ranged from 3.2 (passion fruit juice) to 3.9 (papaya juice) (Table 1). An inverse linear correlation ($R^2 = 0.899$) between pH and titratable acidity was observed, with the highest titratable acidity value found in passion fruit juice (0.6 g/100 mL). Guava juice had the lowest soluble solid compared to the other juices. The highest concentration of reducing sugars was observed in pineapple juice (81.7 mg/l), and the lowest in guava juice (33.7 mg/l). The total concentration of mineral salts also differed among the fruit juices (P<0.05), with the highest total concentration found in papaya juice (464.4 mg/l) and the lowest in passion fruit juice (194.3 mg/l). Papaya and orange juices had the highest concentrations of phosphorus. Potassium was also found at the highest concentration in papaya fruit juice. The water activity did not differ among the fruit juices (ranging from 0.991 to 0.995). Similarly, sodium, magnesium, calcium, and sulfur concentrations also did not differ significantly among the juices (Table 1).

Table 1. Physicochemical analysis of different tropical fruit juices.

Analysis	Pineapple	Guava	Orange	Papaya	Mango	Passion fruit
pH	3.7 ± 0.1bc	3.8 ± 0.1ab	3.5 ± 0.1c	3.9 ± 0.0a	3.6 ± 0.1bc	3.2 ± 0.2d
Titratable acid - g*	0.4 ± 0.0c	0.2 ± 0.0d	0.5 ± 0.0b	0.2 ± 0.0d	0.3 ± 0.0c	0.6 ± 0.1a
Soluble solids (°Brix) - %	11.9 ± 0.1a	10.3 ± 0.6b	11.8 ± 0.3a	11.7 ± 0.2a	12.4 ± 0.1a	11.8 ± 0.1a
Brix/titratable acid	33.1 ± 1.6c	43.2 ± 2.9b	24.8 ± 2.3d	51.0 ± 4.9a	36.5 ± 2.5bc	20.7 ± 2.1d
Aw	0.993 ± 0.0a	0.995 ± 0.0a	0.993 ± 0.0a	0.995 ± 0.0a	0.991 ± 0.0a	0.993 ± 0.0a
Reducing sugars – mg/mL	81.7 ± 0.2a	33.7 ± 0.3f	48.7 ± 0.0c	40.9 ± 0.2e	43.7 ± 0.0d	53.2 ± 0.5b
Sodium – mg/L	33.0 ± 10.9a	25.0 ± 3.0a	35.2 ± 5.4a	36.3 ± 6.7a	21.3 ± 1.3a	21.4 ± 0.2a
Potassium – mg/L	192.5 ± 36.6cd	279.1 ± 8.3b	215.5 ± 1.8c	346.3 ± 30.6a	220.2 ± 15.6c	150.9 ± 0.6d
Magnesium – mg/L	10.6 ± 7.3a	5.9 ± 0.4a	8.5 ± 0.4a	13.6 ± 1.9a	10.4 ± 1.0a	4.7 ± 0.1a
Calcium – mg/L	36.9 ± 36.6a	21.0 ± 2.0a	16.3 ± 1.7a	38.1 ± 6.1a	25.5 ± 1.3a	10.6 ± 0.8a
Phosphorus – mg/L	7.6 ± 2.6bcd	7.1 ± 0.62cd	12.9 ± 0.6a	10.5 ± 1.2ab	8.6 ± 0.7bc	5.1 ± 0.3d
Sulfur – mg/L	5.5 ± 3.5a	18.6 ± 11.8a	4.3 ± 4.3a	19.5 ± 14.9a	8.8 ± 4.4a	1.5 ± 0.5a
Mineral salts measured– mg/L	286.2 ± 97,8bc	356.7± 26.1ab	292,8± 14.2bc	464.5± 61.6a	294.8± 24.2bc	194.4± 0.9c

* g citric acid in 100 mL of juice. Values are means ± standard deviations (n=3). Values in the same line that are followed by the same letter do not differ significantly (ANOVA, Duncan test, P<0.05)

D values for *Alicyclobacillus acidoterrestris* endospores in fruit juices

The D values varied depending on the fruit juice analyzed and were also influenced by pH and temperature (Table 2). In general, the decrease in the pH of the juices led to a greater thermal inactivation of the endospores for both temperatures. For example, a decrease in pH from 4.5 to 2.5 led to a 2.9-fold change in the D value of the endospores in pineapple juice treated at 90°C. On the other hand, a 1.7-fold reduction in the D value was observed in guava juice in the same conditions. When treated at 95 ºC, D values of endospores varied from 1.97-fold (orange juice) to 1.12-fold (mango juice) from the pH reduction of fruit juices from 4.5 to 2.5. As expected, the increase in the heat treatment temperature of the juices led to a reduction in the D value of endospores.

The average D value, considering pH and temperature, was calculated and papaya juice presented the lowest value (3.1 min), while orange and passion fruit juices had the highest values, 9.6 and 9.4 min, respectively. In addition, considering the averages between the different temperatures, papaya juice also presented the lowest D values, being 4.2 min at 90°C and 1.8 min at 95°C, while the highest values were found in passion fruit juice and pineapple/orange with 14.4 and 7.7 min, respectively.

Papaya juice was the food matrix that allowed the greatest reduction (less resistance to heat) of viable endospores of *A. acidoterrestris* compared to other fruit juices for each pH evaluated at both temperatures. In the most drastic condition evaluated, pH 2.5 at 95 ºC, a D value of 1.7 was found in papaya juice, while in the other juices, the D values ranged from 3.7 to 4.9 min. On the other hand, in the mildest condition evaluated, pH 4.5 at 90 ºC, the D value found in papaya juice was 6.3 min., while in the other juices, the D value ranged from 14.4 to 24.7 min. (Table 2).

Mathematical models

The factors pH and the interaction pH and temperature were significant (P<0.05) for most of the models of thermal inactivation of *A. acidoterrestris* endospores tested in this study. The only exception was orange juice, where only independent effects were observed. The quadratic factor of temperature and pH was not significant in any of the models. The endospores inactivation considering pH and temperature in the different fruit juices followed linear models (Table 3).

Among the fruit juices analyzed in the current work, adjustment of the D values in the regression model presented determination coefficients (R^2) ranging from 0.83 (papaya juice) to 0.94 (guava juice). Coefficients of variation (CV) varied from 14.1 (mango juice) to 27.3 (papaya juice). The probability values were lower than 0.05 for all fruit juices, indicating that the terms of the model have significant effects (95% confidence level) on the response variable (D value) (Table 3). The lowest p-value of lack-of-fit was obtained for guava juice (0.07), and the highest for passion fruit juice (0.82). The predicted residual error sum of squares (PRESS) statistics was lowest in pineapple juice (0.09) and highest in papaya juice (0.24).

From the obtained models, the mean D value point was calculated considering a pH of 3.5 and a temperature of 92.5 (Table 3). The predicted values are in agreement with the experimental observations presented in the current work. The lowest mean D value was obtained with papaya juice (2.80 min), and the highest with orange juice (8.36 min). Similar mean D values were obtained for pineapple, guava, and mango juice (7.19, 7.54, and 6.9), which showed similar physicochemical properties, reinforcing the idea that the food matrix plays a major role in the thermal sensitivity of *A. acidoterrestris*.

Table 2. D_T value (min) of *Alicyclobacillus acidoterrestris* DSM 2498 in different tropical fruit juices and at different pH values.

pH	**2.5**		**3.0**		**3.3**		**3.6**		**4.0**		**4.5**	
Juice/ temperature	**90 °C**	**95 °C**	**90 °C**	**95 °C**	**90 °C**	**95 °C**	**90 °C**	**95 °C**	**90 °C**	**95 °C**	**90 °C**	**95 °C**
Pineapple	5.5 ± 0.0d	4.4 ± 1.1e	7.6 ± 0.8d	4.7 ± 0.1e	8.3 ± 0.8d	5.8 ± 1.3d	8.4 ± 1.0d	6.2 ± 0.2d	10.0 ± 1.0c	6.9 ± 1.1d	16.0 ± 1.1b	7.7 ± 0.3d
Guava	9.2 ± 0.5c	4.9 ± 0.6e	10.2 ± 1.3c	4.2 ± 0.4e	11.2 ± 0.4c	4.9 ± 0.1e	15.9 ± 0.3b	3.3 ± 0.8f	13.4 ± 0.0b	5.4 ± 0.7e	14.5 ± 2.0b	4.5 ± 0.8e
Orange	9.9 ± 2.2c	3.9 ± 0.7e	10.9 ± 1.0c	4.4 ± 0.8e	11.8 ± 0.7c	5.3 ± 0.3e	13.8 ± 1.7b	4.7 ± 0.3e	12.5 ± 0.0c	6.9 ± 1.2d	23.3 ± 0.3a	7.7 ± 0.6d
Papaya	3.5 ± 0.8e	1.7 ± 0.1f	3.2 ± 0.3f	2.0 ± 0.3f	3.3 ± 0.3f	2.0 ± 0.2f	4.1 ± 0.1e	1.9 ± 0.2f	4.8 ± 0.9e	2.3 ± 0.8f	6.3 ± 2.8d	1.8 ± 0.0f
Mango	7.5 ± 0.8d	4.7 ± 0.8e	10.2 ± 0.7c	3.8 ± 0.9e	9.5 ± 1.1c	4.9 ± 0.0e	14.1 ± 0.6b	5.1 ± 0.2e	14.2 ± 0.5b	4.5 ± 0.2e	14.4 ± 1.5b	5.3 ± 0.7e
Passion fruit	10.5 ± 2.0c	3.7 ± 0.2e	11.7 ± 2.4c	4.5 ± 1.7e	11.7 ± 0.2c	4.1 ± 1.5e	12.2 ± 1.4c	4.3 ± 1.2e	15.5 ± 2.5b	4.4 ± 0.1e	24.7 ± 2.8a	4.9 ± 0.6e

* Values are means ± standard deviations (n = 4). Values in the same column that are followed by the same letter do not differ significantly (ANOVA, Scott-Knott test, P<0.05)

Table 3. Statistical parameters to assess the adequacy of the effects of pH and temperature and the equations of interaction models for different tropical fruit juices.

Juice	**R^2**	**R^2adj**	**Lack-of-fit**	**P > F***	**PRESS**	**Mean point****	**Equation**
Pineapple	0.89	0.88	0.64	< 0.05	0.09	7.19	$\text{Log}D_{\text{model}} = -1.04857 + (1.51351 \times pH) + (0.0142546 \times {}^\circ C) - (0.0145495 \times pH \times {}^\circ C)$
Guava	0.94	0.93	0.07	< 0.05	0.10	7.54	$\text{Log}D_{\text{model}} = 1.8032 + (1.94928 \times pH) - (0.0121528 \times {}^\circ C) - (0.0204607 \times pH \times {}^\circ C)$
Orange	0.93	0.92	0.18	< 0.05	0.12	8.36	$\text{Log}D_{\text{model}} = 7.63586 + (0.159492 \times pH) - (0.0786136 \times {}^\circ C)$
Papaya	0.83	0.80	0.75	< 0.05	0.24	2.80	$\text{Log}D_{\text{model}} = -1.19516 + (2.17265 \times pH) + (0.0149009 \times {}^\circ C) - (0.0226729 \times pH \times {}^\circ C)$
Mango	0.92	0.91	0.09	< 0.05	0.11	6.90	$\text{Log}D_{\text{model}} = 0.505748 + (2.1317 \times pH) + (0.0002530 \times {}^\circ C) - (0.0220157 \times pH \times {}^\circ C)$
Passion fruit	0.92	0.90	0.82	< 0.05	0.19	7.64	$\text{Log}D_{\text{model}} = 1.7881 + (2{,}41997 \times pH) - (0.0140179 \times {}^\circ C) - (0.0249495 \times pH \times {}^\circ C)$

*95% confidence interval; **Mean point D value considering pH 3.5 and temperature

The graphical representations of the regression models are presented in Figure 1. As expected, the decrease in LogD was accentuated by the increase in temperature during heat treatment. However, the overall influence of pH on the LogD value was more pronounced at 90 ºC than at 95 ºC (Figure 1).

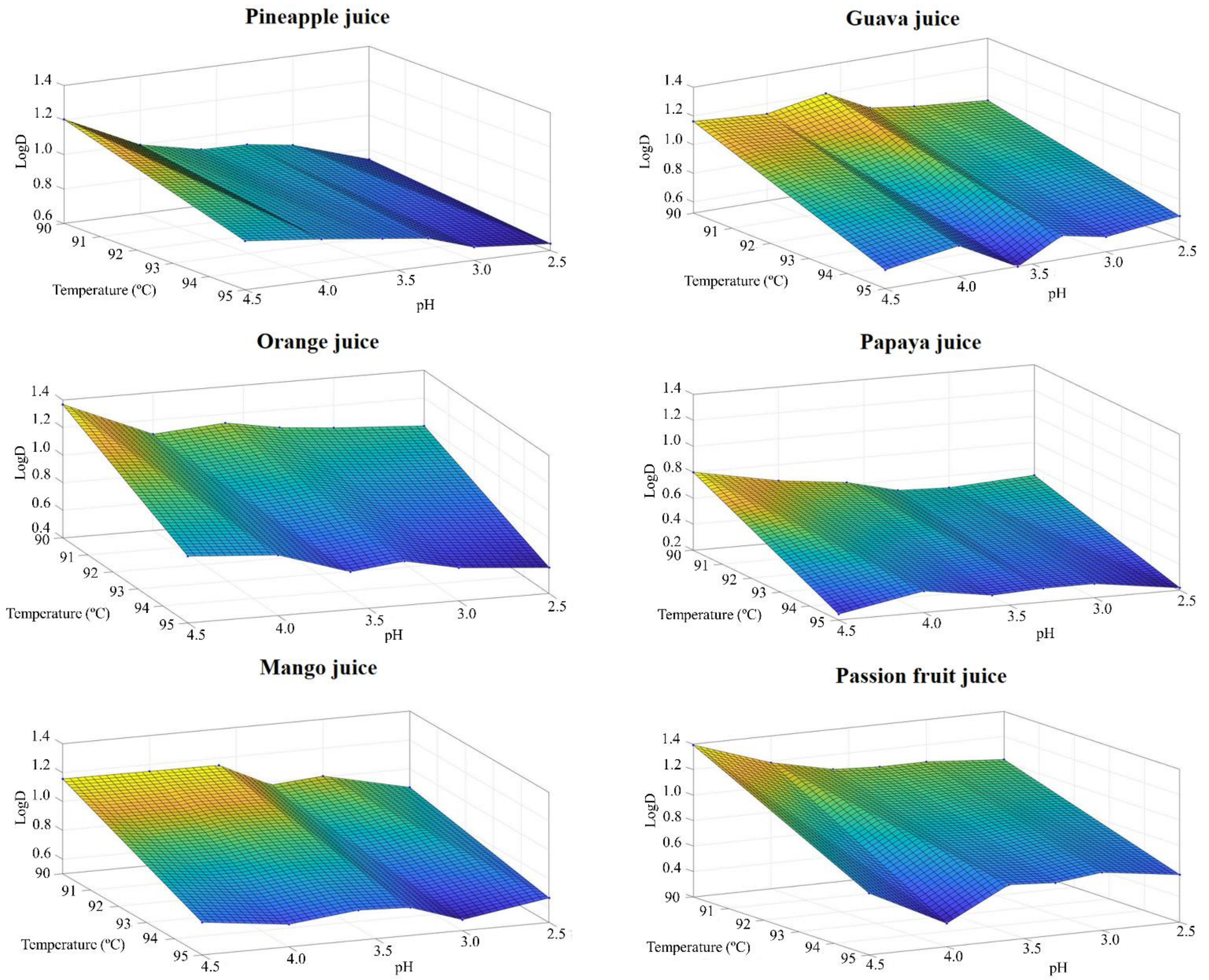

Figure 1. Response surface graphs for the effect of temperature and pH on LogD-value of *A. acidoterrestris* spores in fruit juices.

Figure 2 shows the relationship between the measured D values and the values predicted by the proposed models, considering the two temperatures and pH range (2.5 to 4.5) evaluated in this study, for each fruit juice in a combined heat treatment simulation with the pH. The measured D values show good agreement with the predicted values in all treatments performed, indicating that the model provides a valid description of the experimental data. The D values of all juices approached the best fit curve, but the residuals for D values up to 7 min are lower than at longer decimal reduction time. Comparing all the juices, it is noticed that the residues to the best fit curve are lower for papaya juice. Due to the observed similarities between the measured and predicted D values, the proposed models can be used directly to estimate the thermal resistance of *A. acidoterrestris* in fruit juices.

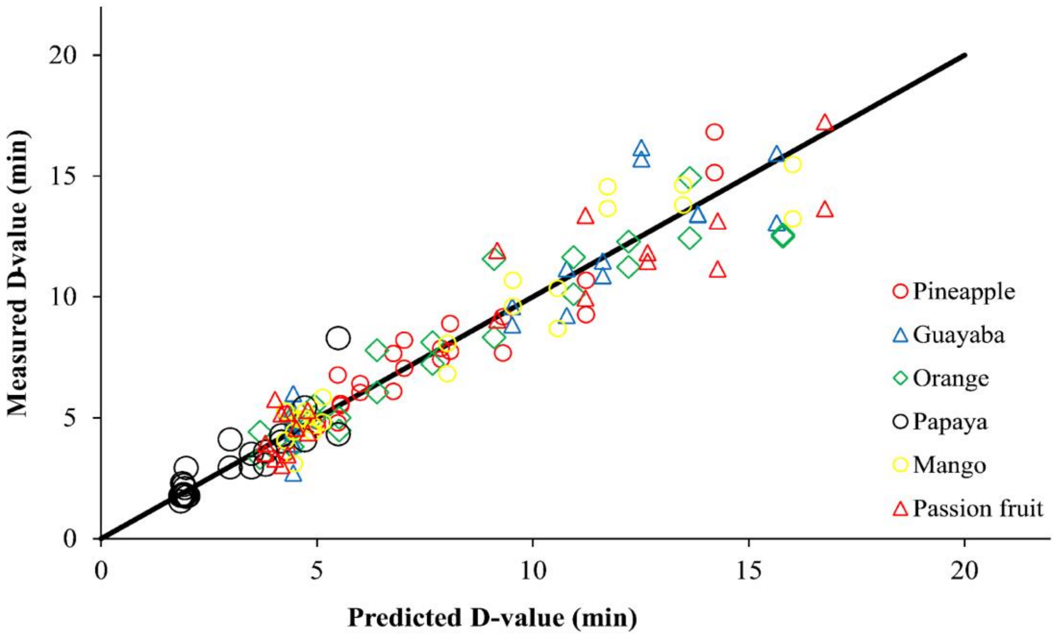

Figure 2. Comparison of predicted and measured D values of *A. acidoterrestris* endospores in different fruit juices.

DISCUSSION

In this study, the association between pH and temperature was evaluated as processing hurdles against endospores of *A. acidoterrestris* in tropical fruit juices. Our results indicate that the physicochemical properties of the fruit juices affect the thermal resistance of *A. acidoterrestris* endospores and both pH and temperature had a major impact on reducing the D value of endospores in tropical fruit juices.

Previously, it was shown that thermal mathematical models showing good agreement between measured and predicted D values were proposed, which could be useful to estimate the thermal resistance of *A. acidoterrestris* in tropical fruit juices [1,20-22]. This strategy is particularly relevant in the fruit juice industry, where the combination of high temperature and low pH is widely used during the processing of fruit juices to maintain the quality and extend the shelf life of the final product [20]. Moreover, less drastic heat treatments could be applied to media with lower pH values without losing the microbial killing efficiency [20]. The acidification of fruit juices is usually performed with organic acids, preferably those naturally present in the fruit. The antimicrobial activity of weak acids towards vegetative cells is explained by the ability of the non-dissociated acid to diffuse through the cytoplasmic membrane and dissociate in the cytoplasm, releasing anions and protons that will interfere with cell homeostasis and inhibit metabolism [23].

In the food industry, microbial safety and quality often rely on the application of hurdle technology [24], and one of the main challenges is to extend shelf life while keeping to a minimum the loss of nutrients and sensorial quality of the product. Therefore, temperature, pH, and active packaging approaches, or combinations of these treatments have been used to control microorganisms causing spoilage such as the alicyclobacilli [1,7,8]. In the current study, the greater inactivation of *A. acidoterrestris* endospores at 95 °C was expected, since the extent of the damage often depends on the intensity of the heat treatment [25].

The influence of the food matrices on the heat resistance of vegetative cells and endospores has been reported for different bacteria [26-28]. The higher thermal sensitivity of *A. acidoterrestris* endospores in papaya juice and higher thermal resistance in passion fruit juice may be related to differences in mineral concentration among the juices. Papaya juice had the highest concentration of minerals among the fruit juices analyzed, whereas passion fruit juice had the lowest. Potassium content was particularly high in papaya juice, compared to the other fruit juices tested in this study. Importantly, the mineral content appears to contribute to the germination of endospores through a nutrient-induced germination pathway [29], thus leading to an increased thermal sensitivity of endospores [30-32]. These results support the hypothesis that mineral content in tropical fruit juices is associated with spore resistance during thermal processing, which should be further investigated in future studies.

It is known that soluble solids, expressed in ° Brix, are one of the most important parameters in the food industry, given their influence on microbial resistance to pressure and heat [15]. The soluble solids content is the total of all the solids dissolved in the water, such as sugar, salts, proteins, and acids; thus, the higher the ° Brix, the greater the protection offered to microbial cells and endospores in a fruit juice. In our study, guava juice has the lowest ° Brix compared to the other juices, although this parameter has not been associated with a greater thermal resistance of *A. acidoterrestris* endospores, since the D values obtained in guava juice did not statistically differ from juices with higher ° Brix values.

The idea that the heat resistance of *A. acidoterrestris* is affected extrinsically by the food matrix was also apparent when the decimal reduction time (D value) of endospores was evaluated in tropical fruit juices at different pH values. For most fruit juices, the lowest D values were found at pH 2.5, indicating that acidity increases the thermal sensitivity of *A. acidoterrestris* endospores (Table 2). This fact could be due to a gradual loss of cations from the spore protoplast induced by the acidic conditions, leading to spore demineralization and lower heat resistance of the bacterial endospores [33].

Mathematical models based on the inactivation of *A. acidoterrestris* endospores in fruit juices allow extrapolation to the inactivation of other pathogenic and/or spoilage microorganisms. *Alicyclobacillus acidoterrestris* has been considered an indicator microorganism for fruit juices, and its high heat resistance indicates that it may pose risks for the spoilage of pasteurized, hot-packed, and UHT (*Ultra High Temperature*) juices [7]. Considering that pH showed a major effect on the thermal sensitivity of *A. acidoterrestris* endospores in fruit juices, the reduction of pH (juice acidification) could be useful to ensure more efficient control strategies during the processing of fruit juices and greater quality of the final product.

The interaction between temperature and pH becomes even more important for inactivating the germination of *A. acidoterrestris* endospores given its high heat resistance and the capacity to grow at pH values below 4.5 [34]. These facts highlight the potential applications of food manufacturing processes in improving processing conditions and ensuring microbiological food safety [20,35,36]. In addition, decreasing the time and temperature of thermal processing due to the temperature and pH interaction would reduce energy consumption and the dependency on chemical additives [37].

In conclusion, because the characteristics associated with product quality (color, taste, texture, and nutrients) are more heat-sensitive than the spores of contaminating microorganisms, the use of drastic thermal processing methods in the fruit juice industry may not be possible or desirable. Therefore, the results shown here are relevant not only for reducing production costs but also for improving the quality and safety of the final product to consumers. Taken together, our results show that the thermal resistance of *A. acidoterrestris* endospores is affected by the composition of tropical fruit juices, and based on mathematical modeling, the interaction between pH and temperature contributes the most to reducing the heat-resistance of *A. acidoterrestris* spores during thermal processing of fruit juices.

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