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Survival kinetics of *Alicyclobacillus acidoterrestris* spores in thermally processed pineapple-mango blend juice

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Abstract

The presence of *Alicyclobacillus acidoterrestris* (AAT) spores in pineapple and mango juices has been reported in the industry. Thermal resistance of AAT spores to pasteurisation treatment poses a serious threat to the tropical juice industry. This study investigates the influence of formulation (pineapple, mango, 70% mango-30% pineapple and 50% mango-50% pineapple), total soluble solid (10 - 15°Bx), and pH (3.58-3.70) on AAT (ATCC®49025™) spores' survivor after processing at 85 - 95°C. As the temperature rises to 85°C and 90°C, 1 log reduction of the spores was observed after treatment for 5 mins. Spore concentration for each juice showed a sharp decrease when the temperature reached 95°C, which resulted in a significant reduction ($P<0.05$) of 3 to 4 log CFU/mL. Significant reductions in pH and total soluble solids of juice samples were noticed in all formulations and D-values of AAT spores heated at 85°C (14.1 - 16.6 mins) were significantly reduced by double when heated at 90°C (5.8 - 6.7 mins) ($P<0.05$). Weibull's model shows a good fit to AAT spores thermal inactivation behaviour in pineapple-mango juice blends tested in this study with the lowest value of Mean Squared Error (MSE) and Accuracy Factor (A_f) close to unity ($0.07 \leq MSE \leq 0.48$; $0.88 \leq A_f \leq 1.19$) compared to first-order model ($0.17 \leq MSE \leq 3.41$; $1.07 \leq A_f \leq 2.34$).

1. Introduction

As more people become aware of the nutritional and health benefits of tropical fruits, domestic and international tropical fruit markets are expanding (Bhat and Paliyath, 2016). Due to their unique nature and high nutrient content, tropical fruits offer local growers access to speciality markets that are of particular interest. By transforming them into dried fruit, purees, or juices, their availability on the market can be maintained. Increased fruit juice consumption has a direct positive effect on the economy, but it can have a negative effect when foodborne disease outbreaks and spoiling issues occur (Tribst *et al*., 2009). Generally, alterations in texture, flavour, and appearance indicate food spoilage, hence influencing consumer approval. This has resulted in manufacturers losing income (Rawat *et al*., 2015)

Alicyclobacillus acidoterrestris (AAT) has been linked to the spoilage of low-acid and pasteurised beverage products. AAT is a nonpathogenic, thermoacidophilic, Gram-positive, spore-forming bacteria (Duong and Jensen, 2000; Pettipher and Osmundson, 2000; Silva and Gibbs, 2004; Jovetta *et al.*, 2011; Groenewald *et al*., 2013; Pornpukdeewattana *et al.,* 2019). The decay of these spore-formers is difficult to detect. Without gas production and pH changes, only off-flavour and odour can indicate spoilage (Yamazaki *et al*., 1996). The degradation of mango juice is 'medicinal' and 'antiseptic,' whilst pineapple juice is 'cheesy' (Danyluk *et al*., 2011).

Cerny *et al.* (1984) initially reported AAT in aseptic apple juice deterioration. AAT comes from the soil, according to researchers. Fruit juice contamination arises from soil-polluted raw materials or inadequately washed or unwashed raw fruits (Chang and Kang, 2004; Groenewald *et al.*, 2009). Standard pasteurisation temperatures in these industries destroy germs like *Escherichia coli* and Salmonella, but not AAT spores

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(Splittstoesser *et al.*, 1994).

A number of factors affect the heat resistance of AAT spores in products, such as temperature, pH, total soluble solids content, water activity, species or strain, dipicolinic acid content, divalent cations, and some antimicrobial compounds (Silva *et al.*, 1999; Uchida and Silva, 2017; Pornpukdeewattana *et al*., 2019). *Alicyclobacillus* spp. can be difficult to control in fruit juice products because their spores survive pasteurisation and can germinate and thrive after processing if the circumstances are conducive. *Alicyclobacillus acidoterrestris* spores may germinate and grow in a pH range of 2.0 to 6.0 at temperatures ranging from 20 to 55°C.

Several studies show that temperatures below 85°C have no effect on AAT spore inactivation (Pornpukdeewattana *et al.*, 2019). D-values (time to inactivate 90% of a population) decline with temperature, especially over 85°C. The D-value of AAT spores in apple, grape, orange, and lemon juices of different pH and soluble solids was 4.9 to 36.5 mins at 90°C (Table 1). z-values (temperature needed to drop one log of D-value) ranged from 7.1 to 17.9 degrees Celsius (Table 1).

Juice concentrates are unlikely to be spoiled by *Alicyclobacillus* due to their high soluble solid contents that prevent the germination and outgrowth of spores and natural compounds present in some of them may decrease spore count. When a concentrate is diluted to produce single-strength juice or used to produce juice-based beverages, the spores may find in such product a favourable environment for germination and growth that under certain conditions, can lead to product deterioration.

AAT is unable to thrive and grow in pineapple juice or beverages containing pineapple juice, according to a few research (Splittstoesser *et al.*, 1998; Walls and Chuyate, 2000). Pineapple juice may contain substances that impede its growth or survival. Bromelain, an endogenous enzyme in pineapple, may be antibacterial against *Alicyclobacillus* spp. (McKnight *et al.*, 2010). However, Danyluk *et al.* (2011) found *A. acidoterrestris* achieved population levels of >6 log CFU/mL within 7 days of incubation in uninoculated single-strength juices of pineapple and mango.

First-order kinetics, the most used model for microbial thermal inactivation, allows the computation of decimal reduction times (D) and z-values to identify acceptable thermal treatments. In some cases, first-order inactivation kinetics deviate, and alternate models have been developed (Manas *et al.,* 2003). The first-order

Table 1. D-value (min) and z (°C) values for spore of *A. acidoterrestris* in fruit juices as reported in the literature.

Fruit Juice	Strain	pH	TSS	T (°C)	D value (min)	z value (°C)	References
Grape juice	K47	4.05	15.5	95	2.25	NR	Groenewald *et al.* (2013)
	FB2				2.58	NR	
Orange juice	DSM 3922	3.64	[c]NR	70	83.33	17.89	Baysal and Icier (2010)
				80	15.11		
				90	7.84		
Passion fruit juice	DSM 2498	3.5	13	87	21.40	7.10	McKnight *et al.* (2010)
				90	4.90		
				95	1.50		
Orange juice	DSM2498	3.5	10	85	93.50	10.9	Ceviz *et al.* (2009)
				90	36.50		
				95	20.80		
Apple juice	DSM2498	3.5	10	85	117.60	10.9	Ceviz *et al.* (2009)
				90	32.80		
				95	20.80		
Clarified lemon juice	CIATIac	3.5	9.8	95	8.55	NR	Maldonado *et al.* (2008)
			50	95	6.20	NR	
			6.2	95	9.38	NR	
Apple juice	DSM 2498	3.68	12.2	90	11.10	8.5	Bahçeci and Acar (2007)
				93	4.20		
				96	2.10		
Orange juice	DSM 2498	3.15	9	85	50.00	7.9	Eiroa *et al.* (1999)
				90	16.90		
				95	12.70		

TSS: Soluble solids content, NR: Not reported, CIATIac: Centro de investigacio´n y asistencia te´cnica a la industria.

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inactivation model is generally accepted in food processing because of its simplicity and effectiveness in calculating treatment periods needed to attain commercial sterility (van Boekel, 2002). According to a semi-log plot of a first-order kinetics model, the ratio of microorganisms surviving heat treatment depends linearly on treatment duration.

Survivor curves can have shoulders, tails (biphasic curves), and upward or downward concavity (Shull *et al.,* 1963; Juneja and Marks 2005; Bialka *et al.,* 2008). Thermal spore destruction depends on strain, treatment duration, and temperature (Tremoulet *et al.,* 2002). Shoulders in survivor curves correlate with spore activation, while tails show resistance heterogeneity (Xiong *et al.,* 1999; Manas and Pagan, 2005). To characterise non-linear survivor curves, logistic (Kamau model), vitalistic, and Weibull distribution models have been developed (Xiong *et al.,* 1999; Guan *et al.,* 2006).

Therefore, the goals of this research were to determine the effect of temperature (85℃, 90℃ and 95℃), pH and total soluble solids for AAT spores' inactivation in pure mango, pure pineapple, 50% pineapple: 50% mango and 70% pineapple: 30% mango juice and to apply the First-order Model and Weibull model to the inactivation data obtained for AAT spores exposed to thermal treatment followed by assessment on the adequacy of fit of the both models.

2. Materials and methods

2.1 Strain

The strain of *Alicyclobacillus acidoterrestris* (ATCC®49025™) was obtained from the American Type Culture Collection (ATCC). The culture was grown for 3 days at 45°C on potato dextrose agar (PDA) adjusted to pH 4.0 with 10% (w/v) 0.1 g/mL of tartaric acid as a source of inoculum for sporulation (Evelyn and Silva, 2016a).

2.2 Spore production

The sporulation of AAT was done according to Silva *et al.* (2012) instruction. 20 plates of PDA (adjusted to a pH of 5.6) were inoculated with the fresh culture from the initial culture. The plates were incubated for 21 days at 45°C, or until at least 80% of the cells were sporulated as seen through a phase contrast microscope. The final pellet was resuspended in 10 mL of sterile sodium phosphate buffer (pH 7.2) and stored at 4°C until it was required (Silva *et al.,* 2012; Evelyn and Silva, 2016b; Uchida and Silva, 2017).

2.3 Tropical juice preparation and inoculation with Alicyclobacillus acidoterrestris spores

Tropical juices were formulated from mangoes and pineapples. Mangoes (cv. Chokonan) and pineapples (cv. Josaphine) were purchased from a local supermarket. Juice samples for both pure and mixed juices were prepared. The mangoes and pineapples were thoroughly washed under running water, sliced, and cut into small pieces. Pineapples were pressed in a slow juicer (Panasonic, Malaysia) and filtered using a nylon filter to obtain clarified pineapple juices. The mango juices were extracted using a commercial blender (Panasonic, Malaysia). Samples of juice were prepared as 100% pineapple, 100% mango, 70% pineapple-30% mango, and 50% pineapple- 50% mango.

Immediately before thermal treatment, a 10% (by volume) spore solution of *A. acidoterrestris* was added to the tropical juice to yield an initial count of 10^6 spores CFU/mL (Hartyáni *et al*., 2013). A vacuum pouch measuring 6 × 8 cm (Quiware Malaysia) was used to pack the inoculated juice made of polyethylene with an outer layer of nylon that can withstand boiling water temperature.

2.4 Spores enumeration

Spore concentration (N) in the juice before and after treatment was enumerated, similar to Silva and Uchida (2017).

2.5 Experimental design

Thermal processing was conducted by submitting a retort pouch containing juice samples in a water bath (Protech, Malaysia) to three different temperatures (85° C, 90°C and 95°C). Five different thermal processing periods were selected for each temperature (85℃/5 to 40 mins, 90℃/2 to 25 mins, and 95℃/0.17 to 5 mins based on Ceviz *et al*. (2009) with an additional time of 2 mins of come up time to make sure the samples heated at actual temperature (come up time was tested by putting thermocouple at the centre of the pouch at different temperature). The samples were removed from the water bath after treatment and immediately cooled down.

The pH values of juices were measured using a pH meter (Mettler Toledo Seveneasy, USA) and total soluble solids were estimated as °Bx with a digital refractometer (Krüss, Germany) before and after thermal treatment. After thermal processing, the number of surviving spores was determined as described in section 2.4.

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2.6 Modelling the kinetics of Alicyclobacillus acidoterrestris inactivation

Minitab® version 17 (Version 17, Minitab Statistical Software, United States) was used to model the kinetics of AAT inactivation by using two types of mathematical models, which were first-order kinetics (Equation 1) and the Weibull model (Equation 2) to represent microbial log survivors in foods after thermal processing.

The first-order model's mechanism is defined by the equation below:

$$\log \frac{N}{N_0} = -\frac{t}{D_{PT}} \quad (1)$$

Where log N/N_0 = number of log reduction in spores during the processing time, t, N_0 = Initial or untreated cell population in the food (CFU/g or CFU/mL), N = number of survivors after being subjected to treatment for a specific time t (min) and $D_{P,T}$- value = the time (min) at a pressure and temperature needed to minimize microbial population by 90%.

On the other hand, z-values (the rise in temperature necessary to reduce the D-value by 1 log) of AAT spores were calculated as the negative reciprocal slope of the linear regressions obtained by plotting logarithms of D-values versus their corresponding temperatures.

Weibull's model used a heterogeneity principle in the resistance distributed among individual cells within a population (Pin and Baranyi, 2006). The mechanism of the Weibull model can be described as:

$$\log \frac{N}{N_0} = -bt^n \quad (2)$$

Where log N/N_0 = number of log reduction in spores during the processing time, t, b = scale factor and n = survival curve shape factor

The Weibull model's critical parameters are b and n. Scale factor *b* is a rate parameter related to the rate at which the microorganism is inactivated, and *n* is described as the degree of curvilinearity, with n<1 (concave upward) and n> 1(convex or concave downwards). When n=1, the Weibull model is said to be identical to the first-order model.

2.7 Model evaluation

The fitting accuracy of models was observed based on accuracy factor (A_f) and mean squared error (MSE) values calculated using Equations 3 and 4, respectively. The smallest MSE and A_f close to 1 show that the model is well fitted.

$$A_f = 10^{\sum \frac{\left|\log \frac{predicted}{observed}\right|}{n}} \quad (3)$$

$$MSE = \frac{\sum (Predicted - Observed)^2}{n} \quad (4)$$

Where n = number of observation

2.8 Statistical analysis

Data for inactivation, pH, and °Bx were analyzed using Minitab® version 17 (Version 17, Minitab Statistical Software, United States). After processing, Tukey's HSD post hoc test was used to compare the findings for different treatments. The criterion for statistical significance was set at $p<0.05$. Results analysis for all the parameters analyzed was compared with the control sample (unprocessed) and labeled with different alphabets in ascending order, which showed a less significant difference of $p<0.05$.

3. Results and discussion

3.1 Effect of thermal treatment on pH and total soluble solids

Tables 2 and 3 demonstrate the effects of thermal treatment at specified treatment time and temperature on the total soluble solid (°Bx) and the pH of tropical juice with different formulations. The pH value for 100% pineapple juice (P) starts to show a significant reduction ($P<0.05$) after 5 mins of treatment at 85°C. However, during thermal treatment at 90°C, there were no significant changes observed with increasing treatment time. No significant changes were observed for the 70% pineapple-30% mango juice blend (70P30M) with increasing treatment time at 85°C and 90°C. However, as the temperature rose to 95°C, a significant pH reduction ($P<0.05$) was noticeable after 10 s. Meanwhile, the pH value of 50% pineapple-50% mango juice blend (50P50M) and 100% mango juice (M) start to show a significant reduction ($P<0.05$) after 10 mins.

Total soluble solids for P, 50P50M, and 70P30M start to show a significant reduction ($P<0.05$) after 5 mins of treatment at 85°C. At thermal treatment at 95°C, there were no significant changes in total soluble solids observed with increasing treatment time for 50P50M and 70P30M. No significant changes also were observed in total soluble solids for M with increasing treatment time at 85°C. As the temperature rises to 90°C, a significant reduction ($P<0.05$) in total soluble solids for M was noticed after 2 mins. No significant changes were noticed after 10 mins. Significant reductions in pH and total soluble solids of juice samples were noticed in all formulations which were likely caused by the biochemical reaction in juices during heating which involves the conversion of sugar in mango (14 g/100 g) and pineapple (10 g/100 g) to acid (Khare *et al.*, 2012). A similar reduction in pH and the total soluble solids was also observed by Jasmi *et al.* (2019) in juices from two

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Table 2. Total soluble solids (°Bx) of tropical fruit juices before and after thermal treatment.

Temperature	Time (min)	P	50P50M	70P30M	M
Room temperature	Control	10.73±0.12^{a}	13.57±0.06^{a}	12.03±0.06^{a}	15.07±0.12^{a}
85°C	5	10.10±0.36^{bc}	12.33±0.29^{c}	11.27±0.31^{b}	14.83±0.06^{a}
	10	10.47±0.25^{abc}	12.87±0.06^{bc}	10.97±0.21^{b}	14.83±0.06^{a}
	20	9.97±0.29^{c}	12.73±0.12^{b}	10.83±0.06^{b}	15.03±0.15^{a}
	30	10.40±0.10^{abc}	12.83±0.21^{bc}	11.10±0.20^{b}	15.03±0.23^{a}
	40	10.67±0.06^{ab}	12.60±0.10^{b}	11.23±0.29^{b}	15.17±0.15^{a}
90°C	2	8.83±0.06^{a}	12.87±0.15^{ab}	11.03±0.06^{b}	11.03±0.06^{b}
	5	9.70±0.17^{b}	13.00±0.20^{bc}	11.27±0.23^{b}	11.27±0.23^{b}
	10	9.83±0.25^{b}	13.13±0.12^{bc}	12.07±0.06^{a}	12.07±0.06^{a}
	15	9.97±0.12^{b}	13.20±0.10^{bc}	12.27±0.25^{bca}	12.27±0.25^{a}
	25	10.50±0.17^{c}	13.37±0.12^{c}	12.37±0.15^{ab}	12.37±0.15^{a}
95°C	⅙	10.60±0.27^{a}	13.40±0.17^{a}	11.07±0.15^{b}	14.97±0.15^{a}
	½	10.57±0.12^{a}	13.37±0.15^{a}	11.13±0.06^{b}	14.97±0.06^{a}
	1	10.47±0.29^{b}	13.27±0.31^{a}	11.03±0.21^{b}	14.90±0.10^{a}
	2	9.03±0.25^{b}	13.07±0.06^{a}	11.13±0.12^{b}	14.93±0.06^{a}
	5	9.20±0.20^{b}	13.40±0.27^{a}	11.00±0.27^{b}	14.87±0.06^{a}

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different (P<0.05). P: Pineapple juice, 50P50M: 50% pineapple juice/50% mango juice blend, 70P30M: 70% pineapple juice/30% mango juice blend, M: mango juice.

Table 3. pH of tropical juice before and after thermal treatment.

Temperature	Time (min)	P	50P50M	70P30M	M
Room temperature	Control	3.70±0.01^{a}	3.65±0.01^{a}	3.64±0.01^{a}	3.58±0.00^{a}
85°C	5	3.65±0.01^{b}	3.63±0.01^{ab}	3.64±0.01^{a}	3.58±0.00^{a}
	10	3.65±0.00^{bc}	3.63±0.01^{b}	3.64±0.01^{a}	3.56±0.01^{ab}
	20	3.64±0.01^{cd}	3.63±0.01^{b}	3.63±0.01^{a}	3.57±0.01^{b}
	30	3.64±0.01^{cd}	3.63±0.01^{b}	3.63±0.01^{a}	3.57±0.01^{b}
	40	3.63±0.01^{d}	3.63±0.00^{b}	3.63±0.01^{a}	3.65±0.01^{b}
90°C	2	3.54±0.01^{b}	3.64±0.01^{ab}	3.63±0.01^{a}	3.56±0.01^{b}
	5	3.54±0.01^{b}	3.63±0.01^{bc}	3.63±0.01^{a}	3.56±0.01^{ab}
	10	3.54±0.01^{b}	3.63±0.01^{bc}	3.63±0.01^{a}	3.56±0.01^{ab}
	15	3.53±0.00^{b}	3.63±0.01^{bc}	3.63±0.01^{a}	3.56±0.01^{ab}
	25	3.52±0.01^{b}	3.62±0.01^{c}	3.62±0.01^{a}	3.55±0.01^{b}
95°C	⅙	3.56±0.00^{b}	3.54±0.01^{b}	3.52±0.00^{b}	3.47±0.01^{bc}
	½	3.55±0.01^{b}	3.53±0.01^{b}	3.53±0.01^{b}	3.48±0.00^{b}
	1	3.54±0.01^{c}	3.53±0.01^{b}	3.54±0.01^{b}	3.46±0.01^{cd}
	2	3.53±0.01^{c}	3.52±0.01^{b}	3.53±0.01^{b}	3.45±0.01^{de}
	5	3.53±0.01^{c}	3.53±0.01^{b}	3.53±0.01^{b}	3.45±0.01^{e}

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different (P<0.05). P: Pineapple juice, 50P50M: 50% pineapple juice/50% mango juice blend, 70P30M: 70% pineapple juice/30% mango juice blend, M: mango juice.

sugarcane variants with sugar content (12.85 g/100 g) after thermal treatment at 90°C.

3.2 Effect pH, total soluble solids, different temperatures, and formulation for Alicyclobacillus acidoterrestris spore's inactivation in tropical juice

Figure 1 shows the survival of AAT spores (CFU/mL) at different temperatures and formulation after 5 mins of treatment. Heat shock treatment at 80°C/ 10 mins before thermal treatment maintains the viability of 10^{6} CFU/mL AAT spores while inactivating other vegetative cells (Sinigaglia *et al*., 2003). Thus, it was regarded as an unprocessed or controlled sample with 6 log CFU/mL of AAT spores. As the temperature rises to 85°C and 90°C, 1 log reduction of the spores was

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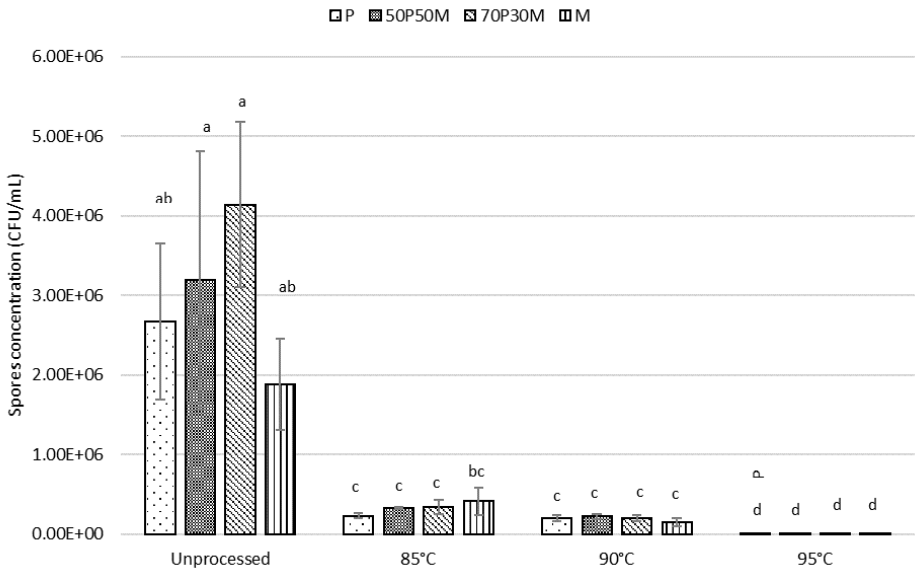

Figure 1. Effect of different temperatures and formulations on *A. acidoterrestris* spore concentration (CFU/mL) after 5 mins treatment in Mango (M), Pineapple (P), 70% Pineapple-30% Mango (70P30M), and 50% Pineapple-50% Mango (50P50M). Data are indicated as mean±SD of triplicates. Error bars indicate standard errors. Bars with different notation are statistically significantly different ($p<0.05$).

observed after treatment for 5 mins indicating that AAT spores are gradually inactivated. Spore concentration for each juice showed a sharp decrease when the temperature reached 95°C, which resulted in a significant reduction ($P<0.05$) of 1 to 2 log CFU/mL.

The effects of thermal processing on the inhibition of AAT spores in tropical fruit juice with a different formulation were investigated under various temperature and time conditions. The endospore concentration and time were found to have a linear-log relationship for AAT spores that followed first-order kinetics when heated at 80°C and 90°C, as displayed in Figures 2, 3, 4 and 5(A). According to some other authors, there was no "shoulder" in thermal inactivation curves (Pontius *et al.*, 1998; Silva *et al.*, 1999). The thermal inactivation curve could be considered linear if the R^2 value was greater than 0.90 (Byrne *et al.*, 2006). The spores were inactivated at a constant rate while the microbial population was heated to a particular temperature. A nonlinearity is observed in the AAT spore's survival curves with the low R^2 (0.5208 - 0.6163) in all formulated juice treated at 95°C. Thus, the D-values and z-values were deemed to be inaccurately estimated for AAT spores heated at 95°C. Instead of inaccuracy, the D_{95} value for tropical juice in this study is less than a min. The D_{95} values of AAT spores vary from 0.06 to 5.3 mins in different studies, according to a review article by Merle and Montville (2014).

D-values have been observed to decrease with increased temperature. The D-value of AAT spores heated at 90°C was noticed to have about double the reduction than the D-value of AAT spores heated at 85°C. These values were found to be substantially different ($P<0.05$) from one another. The D-values measured for similar temperatures, on the other hand, varied between strains and studies (Table 1). McKnight

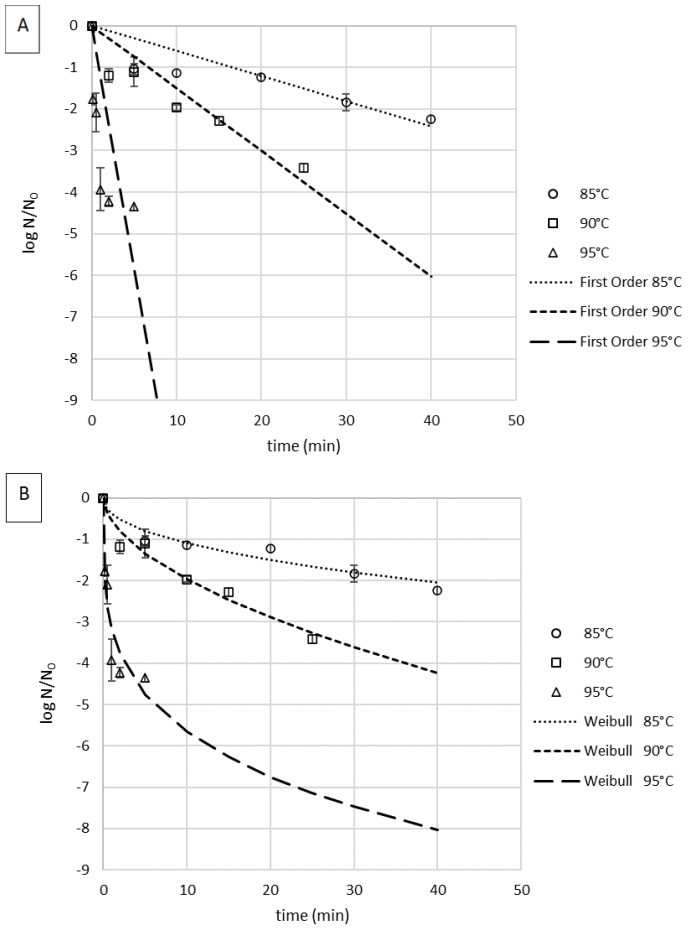

Figure 2. Log survivors of AAT spores in pineapple juice. The first-order model (A) fitted to thermal inactivation. The Weibull Model (B) fitted to thermal inactivation. Data are indicated as mean±SD of triplicates. Error bars indicate standard errors. Bars with different notation are statistically significantly different ($p<0.05$).

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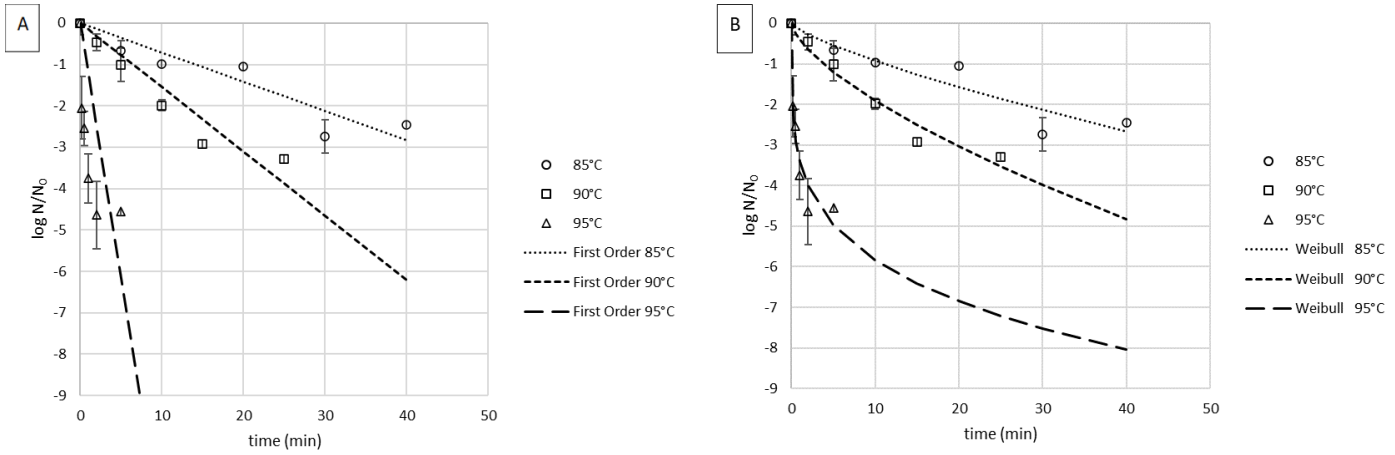

Figure 3. Log survivors of AAT spores in mango juice. The first-order model (A) fitted to thermal inactivation. The Weibull Model (B) fitted to thermal inactivation. Data are indicated as mean±SD of triplicates. Error bars indicate standard errors. Bars with different notation are statistically significantly different ($p<0.05$).

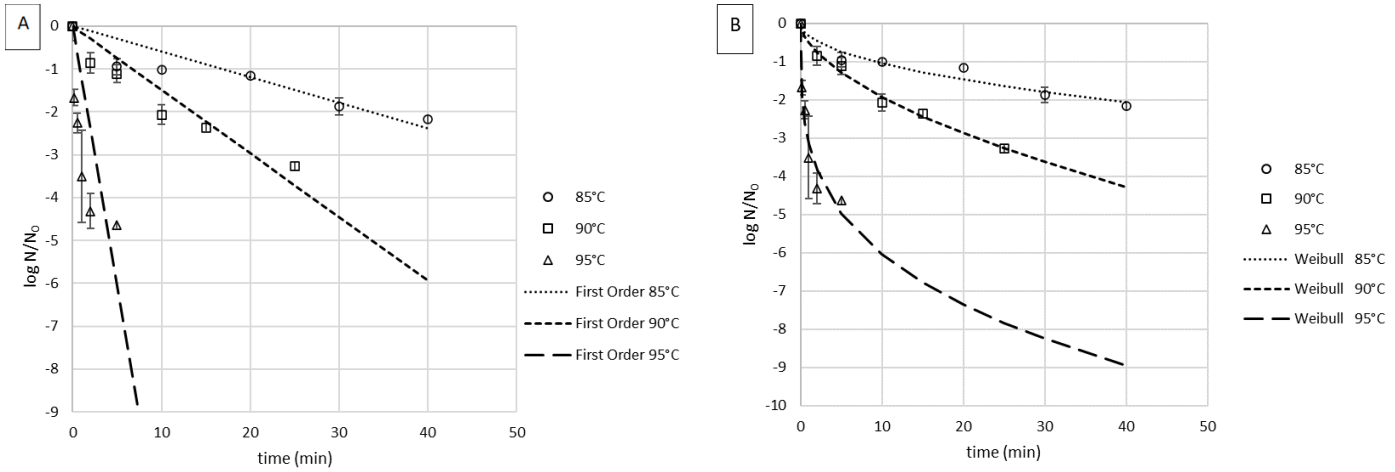

Figure 4. Log survivors of AAT spores 50% pineapple juice / 50% mango juice blend. The first-order model (A) fitted to thermal inactivation. The Weibull Model (B) fitted to thermal inactivation. Data are indicated as mean±SD of triplicates. Error bars indicate standard errors. Bars with different notation are statistically significantly different ($p<0.05$).

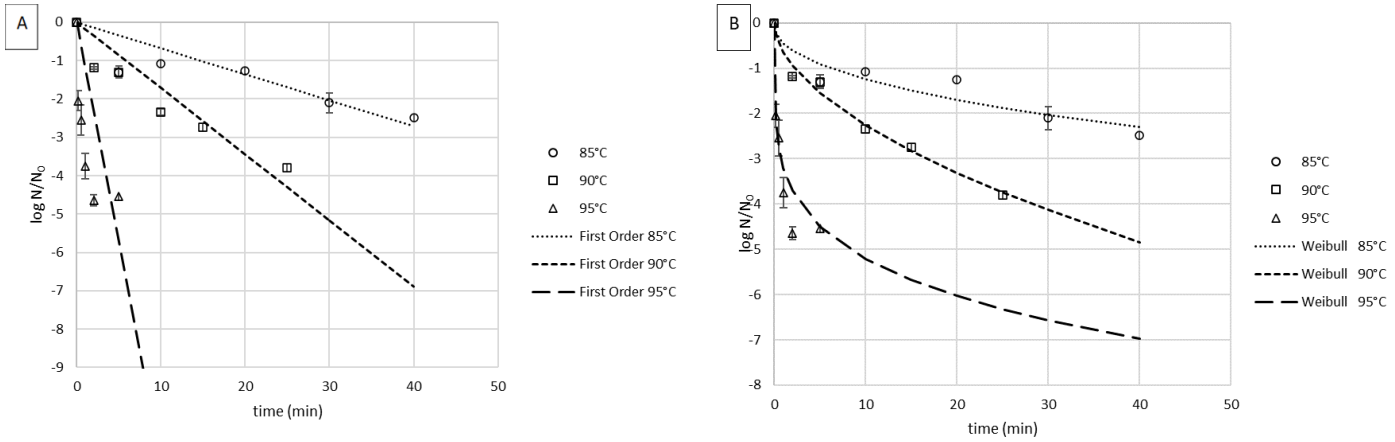

Figure 5. Log survivors of AAT spores 70% pineapple juice / 30% mango juice blend. The first-order model (A) fitted to thermal inactivation. The Weibull Model (B) fitted to thermal inactivation. Data are indicated as mean±SD of triplicates. Error bars indicate standard errors. Bars with different notation are statistically significantly different ($p<0.05$).

et al. (2010) obtained almost similar D_{90} values to this study (Table 4) with a difference of less than 2 mins conducted with AAT spore in passion fruit media (3.5 pH and 13°Bx): D_{90} = 4.9 mins Furthermore, this study's reported D_{85} value was lower (Table 4) than most reported D_{85} values for other fruit juices displayed in Table 1 indicating lower thermal resistance of AAT spores in pineapple and mango juice.

From observation, the D-value seems to be decreasing with increasing value of total soluble solids and decreasing value of pH in tropical juice after thermal treatment at 85°C and 90°C. The statistical analyses revealed that the impact of pH and total soluble solids on AAT spores' inactivation was not significant for all

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Table 4. D-values of *Alicyclobacillus acidoterrestris* spores heated in tropical juice of different pH, total soluble solid and temperature.

Formula	pH	°Brix	D values (min)			z-value (˚C)
			85˚C	90˚C	95˚C	
P	3.70±0.01	10.73±0.12	16.59±1.35^{a}	6.65±0.43^{b}	0.85±0.15^{c}	7.77±0.42^{a}
50P50M	3.65±0.01	13.57±0.06	16.76±1.22^{a}	6.75±0.42^{b}	0.82±0.13^{c}	7.63±0.14^{a}
70P30M	3.64±0.01	12.03±0.06	14.74±1.15^{a}	5.81±0.36^{b}	0.87±0.15^{c}	8.25±0.16^{a}
M	3.58±0.00	15.07±0.12	14.12±1.14^{a}	6.45±0.39^{b}	0.81±0.14^{c}	8.04±0.29^{a}

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different ($P<0.05$). P: Pineapple juice, 50P50M: 50% pineapple juice/50% mango juice blend, 70P30M: 70% pineapple juice/30% mango juice blend, M: mango juice.

formulations ($P>0.05$). Splittstoesser *et al.* (1998) found that an increase in the soluble solids up to a certain limit had no effect on the thermal resistance of AAT spores. Ceviz *et al.* (2009) reported that they did not observe any difference stemming from soluble solids changing from 10°Bx to 20°Bx in thermal resistance. However, high levels of soluble solid values have been linked to an increase in thermal resistance. A higher D-value of AAT spores in the sample with high soluble solids content than that in samples with a low soluble solids content confirms the protective effect of sugars (Chmal-Fudali *et al.*, 2011).

In this study, the blend juices' pH was slightly different (pH 3.58 to 3.70). Murakami *et al.* (1998) observed no significant reduction in AAT spore thermal resistance in buffer solutions of various pH. In another study performed by Ceviz *et al.* (2009) on the inactivation of AAT spores suspended in apple and orange juices with solids content changing from 10 ºBx to 20ºBx at pH 3.5 and 4.0, neither the conversion of solids nor different juice pH levels led to reduction of D-values. Variation and dissimilarity in D-values may be attributable to modified thermal processing temperature and different species as well as strains of microorganisms, according to the evidence provided in this study and reported in the literature.

However, applying the same environment with a different pH significantly affected the D and z-values ($P<0.05$). This may be due to the differences in the process of sporulation and activation of vegetative cells in comparison to other species (Chmal-Fudali *et al.*, 2011). D-values with different AAT strains vary when tested in the same fruit juices with similar acidity levels and concentrations of dissolved sugars. In the specific case of orange juice, Silva *et al.* (1999) reported that D_{85}-values in orange juice (pH 3.5, 10°Bx) for AAT NCIMB 13137 of 93.5 mins. Meanwhile, AAT DSM2498 strains in orange juice (pH 3.15, 9.0°Bx) reported a D_{85}-value of 65.6 mins (Ceviz *et al.*, 2009).

Different species, or even strains of the same species, can have different heat resistance. Under the same conditions, Eiroa *et al.* (1999) discovered spores of four AAT strains with different D values. Orange juice with slightly different pH and soluble solids content inoculated with AAT strain DSM 2498 by Eiroa *et al*. (1999) and Ceviz *et al*. (1999) gave D_{85}-value of 50 mins and 93.5 mins, respectively. Other researchers also reported various ranges of D-values for different kinds of fruit juices (Table 1). They reported that the D-values might be attributed to differences in strains, sporulation temperature, differences in nutrient composition and pH of the heating media, water activity, presence or absence of divalent cations and antimicrobial compounds. (Parish, 2005). *Alicyclobacillus* sp. heat resistance is also influenced by sporulation temperature. Endospores of *A. acidocaldarius* that sporulated and grew at 65°C had a far higher heat resistance than those that sporulated and grew at 45°C because they had adapted to the lower protoplast water content (Palop *et al.*, 2000).

There was no significant difference in z-values among spores in juices of different pH and concentrations, soluble solids content. The z-value at different juice blends, pH, and °Bx found in this study was between 7.6 to 8.2°C. The z-value is a useful tool when attempting to change commercial processing conditions to reduce the time required to achieve product safety and stability or reduce the temperature to enhance product quality. The z-value is used to determine the new target processing temperature when the processing time is reduced as a lower temperature is desired to improve the sensory characteristics of products without compromising safety and stability (Parish, 2005).

3.3 Modelling the kinetics of thermal inactivation of Alicyclobacillus acidoterrestris spores in tropical fruit juice

The effects of thermal processing on the inhibition of AAT spores in various tropical juice formulations were investigated under different temperature and duration conditions. The first-order model's use allowed the estimation of D- and z-values for the predicted value of log survivors of AAT spores under different temperatures and treatment times.

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At three different processing temperatures, the log spore survivors of AAT behave as a function of time (85℃, 90℃ and 95℃) in pineapple, mango, 50P50M, and 70P30M juice are illustrated in Figures 2, 3, 4 and 5, respectively. Temperature and the duration of the thermal treatment significantly affect the inhibition of AAT spores. A 10 s treatment at 95°C resulted in 2.05 and 2.7 log reductions for 70P30M and mango juice, respectively. While at 85°C, more than 30 mins was required to achieve the same degree of inactivation. Temperature rise has been shown to have a major impact on the speed of inhibition, with inhibition occurring more rapidly under 100°C media in particular (Ceviz *et al.*, 2009).

Pineapple juice with the highest pH value and lowest total solubility solid (pH 3.7 and 10.73°Bx) recorded the highest log reduction (3.93) for 1 min at 95℃ compared to other formulations. The 50P50M blend (pH 3.65 and 13.57°Bx) achieved the highest log reduction after treatment at 95℃ in 5 mins and the lowest log reduction at 85℃ in 40 mins with the values of 4.63 and 2.17, respectively. Thermal treatment at 85℃ shows increased log reduction ranging from 0.67 to 2.49 for all formulated juice with increasing duration of treatment from 5 mins to 40 mins. Meanwhile, thermal treatment at 95°C shows increased log reduction ranging from 1.67 to 4.63 for all formulated treatments only in 5 min duration started with 10 s.

Within the temperature range of 85℃ to 90℃, both linear and Weibull models provided reasonably good fits to the inactivation data as displayed in Table 5. However, the nonlinearity of survival curves observed in the AAT spore's survival curves in all formulated juice treated at 95℃ obtained for first-order kinetics. The latter inferred that the first-order kinetic model is not a relevant model for thermal treatment at 95°C. The non-linear Weibull model was a good model to describe the survivors of AAT spores heated at 95°C (0.88 - 0.93 A_f, 0.7243 - 0.8129 R^2, 0.15 - 0.048 MSE) in comparison to (1.96 - 2.34 A_f, 0.5208 - 0.6163 R^2, 3.36 - 4.40 MSE) in first-order kinetic model.

The first-order linear kinetic model has been widely used to explain the thermal inactivation of bacterial spores in isothermal process conditions. However, documentation of nonlinearity in microbial survival curves, including bacterial spores, is growing. Thus, making the first-order mortality kinetics becomes inappropriate (Peleg and Cole, 1998). Based on the Weibull model's good fit to spore survivor curves and high correlation between the predicted and the observed values of spore survival ratios, the Weibull model has been proven to be a well-fitted mathematical model describing inactivation of AAT spores in all tropical juices tested (Table 5). The most widely accepted explanation is that the microbial population is divided into subpopulations, each with its own inactivation kinetics. The survival curve results from several inactivation patterns, giving rise to nonlinear survival curves (van Boekel, 2002). Head *et al.* (2009) reported that the Weibull model successfully fitted all survivor curves obtained for *Geobacillus stearothermophilus* spores treated with superheated steam (130°C - 175°C).

Bozkurt *et al*. (2016) reported that the Weibull model consistently produced the best fit for all the survival curves of *Sporolactobacillus nakayamae* spores after thermally treated at 70, 75, and 80℃ showing a monotonic downward concave (shoulder) behaviour with $n > 1$. The b and n values are two parameters in Weibull's model that explain inactivation kinetics. b parameters are known as a scale factor that relates to the

Table 5. First-order and Weibull model parameters for thermal inactivation of *A. acidoterrestris* spores in tropical juice.

Type of juices	Temperature (°C)	First-order model				Weibull model				
		D-value (min)	R^2	A_f	MSE	b	n	R^2	A_f	MSE
Pineapple	85	16.59±1.35	0.8622	1.41	0.22	0.39±0.13	0.45±0.10	0.9192	1.12	0.12
	90	6.65±0.43	0.9227	1.51	0.28	0.56±0.09	0.55±0.06	0.9511	1.19	0.09
	95	0.85±0.15	0.5212	2.06	3.71	3.18±0.17	0.25±0.05	0.7794	0.88	0.39
Mango	85	14.12±1.14	0.8673	1.16	0.30	0.16±0.10	0.76±0.19	0.9886	1.06	0.31
	90	6.45±0.39	0.9199	1.07	0.26	0.41±0.10	0.67±0.08	0.9199	0.92	0.15
	95	0.81±0.14	0.5208	2.33	4.40	3.42±0.19	0.23±0.05	0.7551	0.90	0.48
70P30M	85	14.74±1.15	0.8298	1.57	0.26	0.45±0.13	0.44±0.09	0.9154	1.20	0.12
	90	5.81±0.36	0.9277	1.48	0.34	0.64±0.08	0.55±0.04	0.9505	1.12	0.06
	95	0.87±0.15	0.5208	2.34	3.52	3.21±0.10	0.21±0.03	0.7243	0.93	0.15
50P50M	85	16.76±1.22	0.8895	1.35	0.17	0.33±0.10	0.50±0.09	0.935	1.10	0.08
	90	6.75±0.42	0.9352	1.40	0.25	0.51±0.08	0.58±0.06	0.9581	1.05	0.07
	95	0.82±0.13	0.6163	1.96	3.36	3.15±0.17	0.28±0.05	0.8129	0.90	0.38

R^2: correlation coefficient of the regression line, A_f: Accuracy factor, MSE: mean square error, 70P30M: 70% pineapple juice/30% mango juice blend, 50P50M: 50% pineapple juice/50% mango juice blend.

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spore inactivation rate. The D-value was inversely related to the non-linear Weibull model scale factor b, which increased with an increase in temperature. Mango juice with the lowest b value among all formulated juice increased from 0.16±0.10 to 3.42±0.19 as the highest b value among all formulated juice when processing temperature increased from 85℃ to 95℃.

The shape factor of the survival curves and the deviation from linearity are defined by the n parameters. Table 5 shows that all of the n values are less than one, indicating that the spore survivor curves were concave upward, as seen in Figures 2, 3, 4 and 5(B), confirming their nonlinearity. This indicates a greater rate of decline at first, followed by a slower rate of destruction later on, which is characteristic of spore populations with mixed resistance to thermal destruction or the stress adaptability of a sub-population of spores. (Peleg and Cole, 1998; van Boekel, 2002). The combined effect of pH and temperature on the thermal resistance of *Bacillus cereus* spores was studied by Fernandez *et al.* (2002), who found that the b value of the Weibull model was independent of external factors (pH and temperature). They also argued that the shape factor only showed the kinetic pattern of the mechanism controlling the process under investigation and should be independent of external factors.

Despite the fact that the Weibull model is empirical, a correlation can be drawn between the physiological effects of thermal treatment on microorganisms and the Weibull model. For example, when n<1, the remaining organisms could adapt to the applied stress. It is more difficult to inactivate them, whereas when n>1, the remaining organisms become increasingly more damaged (Head *et al.*, 2009).

4. Conclusion

Inactivation of AAT spores in tropical juice was strongly affected by processing temperature, with little effect on pH and soluble solids due to limited pH and Bx range. D-values of AAT spores heated at 85°C doubled at 90°C ($P<0.05$). The first-order kinetic model of AAT spore survival curves discovered nonlinearity in D-values for 95°C-heated spores. The Weibull model describes AAT spore thermal inactivation in pineapple-mango juice blends well. The mean squared error (MSE) and accuracy factor (A_f) were lower in the Weibull second-order model than in the first-order model.

Conflict of interest

The authors declare no conflict of interest.

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