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Microbiological feasibility of microwave processing of coconut water

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ABSTRACT

Thermal processing of coconut water is designed to ensure safety and stability and microwave may be used as an alternative process. This study evaluated the feasibility of microwave processing of acidified green coconut water (pH 4.30–4.50) concerning inactivation of *Bacillus coagulans* spores with specific power between 115 and 135 W/mL. Four variables were evaluated (temperature, microwave power, amount, and composition of added acids) according to a central composite design with four replicates at the center point. After holding periods of 5, 10, 15 and 20 min, the reduction in spore population was determined. The only significant variable for spore inactivation was the temperature; consequently, the survival curves were described by Weibull model, which was adjusted in a single-step procedure that integrated the time-temperature profile of 112 experiments. The α value (time of the first decimal reduction) at reference temperature of 90 °C was 33.8 s and the z value (temperature required for a 10-fold change in α) was 5.06 °C. The reduction in the population of *B. coagulans* spores and the kinetics parameters suggest that microwave may be used as a viable alternative for coconut water processing.

1. Introduction

Green coconut water (*Cocos nucrifera* L.) is consumed worldwide because of its rehydration quality and pleasant flavor. It is a moderate acid beverage with pH values that vary between 5.1 and 6.1, according to the maturity of the fruit (Prades, Dornier, Diop, & Pain, 2012).

Inside the green coconut, the beverage is sterile, but its chemical composition can support microbial growth after extraction and contamination. Furthermore, enzymes naturally present in the fruit, such as polyphenol oxidase (PPO) and peroxidase (POD), causes loss of sensorial quality of coconut water after it is extracted and exposed to air. Thus, it is important to reduce microbial contamination to ensure the microbiological safety of coconut water (Matsui, Gut, Oliveira, & Tadini, 2008; Salazar-González, Martín-González, & Sosa-Morales, 2012). Besides, it is important for the industry to reduce the costs associated with the transportation of coconuts and to enhance the shelf-life of the product. For this purpose, coconut water is commercialized in bottles, after being acidified and thermally processed (Prades et al., 2012; Food and Agriculture Organization of United Nation - FAO, 2007).

According to the US Food and Drug Administration (FDA), the thermal processing for acidified foods should ensure a minimum reduction (5 log) of microorganisms of public health significance and microorganisms capable of reproducing in the food until consumption (Food and Drug Administration - FDA, 2004). *Bacillus coagulans* is a spore former heat resistant microorganism that can grow at pH values lower than 4.5 and it is commonly involved in the spoilage of acidic foods (Stumbo, 1970; Buchanan et al., 1974). Therefore, in order to validate the thermal processing of coconut water, spores of *B. coagulans* can be used as a bioindicator of commercial sterility (Brinley et al., 2007).

The traditional technologies used for thermal processing of foods, such as pasteurization, should ensure the safety of the product. Pasteurization is carried out at temperatures under 100 °C for coconut water, normally at 75–95 °C (Adubofuor, Amoah, & Bonsu, 2016;

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Penha, Cabral, & Matta, 2018). However, heating may cause chemical alterations and nutritional losses; therefore, alternative processes for food preservation have been studied. Microwave technology produces heat through electromagnetic irradiation and can reduce the thermal processing time (Buffler, 1993). The heating process of food using microwave energy depends on the frequency of the equipment, on ionic composition, moisture content and dielectric properties of the food (Heddleson & Doores, 1994; Tajchakavit, Ramaswamy, & Fustier, 1998).

Studies have demonstrated that the mechanism of microbial lethality or enzymatic inactivation from microwaves occurs due to heating (Cañumir, Celis, Brujin & Vidal, 2002; Siguemoto, Pereira, & Gut, 2018). However, some studies propose the existence of a non-thermal specific effect that enhances inactivation. For instance, Tajchakavit; Ramaswamy & Fustier (1998) reported that microwave processing was more efficient in inactivating spoilage microorganisms in apple juice than conventional heating. A similar effect was shown with kiwifruit puree processed by microwaves in which the inactivation of *Listeria monocytogenes* was higher than when submitted to traditional heating (Benlloch-Tinoco, Pina-Pérez, Martínez-Navarrete, & Rodrigo, 2014). Additionally, the inactivation of *Escherichia coli* O157:H7 and *L. monocytogenes* in apple juice by microwaves was more efficient than the conventional heat treatment (Siguemoto, Gut, Martinez, & Rodrigo, 2018). Nevertheless, a recent review on the topic discussed how the use of unreliable methods and assumptions compromises the assessment of non-thermal effects, which are subtle and of difficult detection (Kubo et al., 2020).

Thermal processing of foods using microwaves has shown potential for sterilization, but more studies with diverse food matrices are needed for process design and optimization. Therefore, we aimed to assess the feasibility of employing microwave processing to commercially sterilize green coconut water by using *B. coagulans* as an indicator microorganism.

2. Materials and methods

2.1. Preparation of spore suspension

B. coagulans CCGB (LFB-FIOCRUZ) 1433 was cultivated on nutrient broth (3 g L^{-1} meat extract BBL-BD, Sparks, United States of America - USA; 5 g L^{-1} peptone - Oxoid, Basingstoke, United Kingdom) supplemented with 0.3% yeast extract (Oxoid), for 48 h at 33 °C. Sporulation was carried out transferring an aliquot of 1 mL from a 48 h culture to Roux bottles with nutrient agar (nutrient broth, added of 15 g L^{-1} bacterial agar BBL-BD). After 4 days at 33 °C, the agar was flooded with sterile distilled water and spores were collected and centrifuged at 16,000×*g* (Sigma 6–16K, Osterode am Harz, Germany) for 10 min at 4 °C and the supernatant was discarded. The procedure of washing the spores with sterile distilled water and centrifugation was repeated three times. The last time, the pellet was suspended in sterile distilled water and stored at 4 °C.

2.2. Enumeration of spore population

Spore suspension was heated in water bath at 80 °C for 15 min in order to kill vegetative cells. After this treatment, spore concentration was determined transferring 1 mL of suspension to sterile peptone water 0.1% (Oxoid), followed by plating 0.1 mL of selected dilutions onto nutrient agar and incubating the plates for 48 h at 33 °C.

2.3. Experimental design and statistical analysis

A central composite design with four replicates at the center point was carried out to investigate the significance of four variables (temperature, amount of acids added to coconut water, mixture of citric acid and ascorbic acid, and specific microwave power) on the survival of *B. coagulans* spores (Table 1). The selected temperature range was based on Matsui et al. (2008) and preliminary runs, while power levels were adjusted to achieve these processing temperatures. Temperatures higher than 95 °C were not possible because the reactor was not pressurized. The amount and ratio of added acids were selected according to recommendation of the current Brazilian legislation that requires a pH between 4.3 and 4.5 for pasteurized coconut water (Brazil, 2009).

The statistical evaluation of the effects of the four variables, the response surface methodology, and the analysis of variance (ANOVA) were done using Minitab® 16 software (Minitab Inc., State College, USA), at a significance level of 5%.

2.4. Sample preparation

Green coconuts were purchased from a local supplier in the city of Sao Paulo, Brazil, and 100 mL of the internal liquid were extracted and mixed, comprising the main sample. Aliquots were acidified with the mixture of citric acid and ascorbic acid (Table 1) and inoculated with the suspension of *B. coagulans* spores to give an initial concentration of 10^7 CFU mL^{-1}.

2.5. Microwave processing of coconut water

The pasteurizing equipment is shown in Fig. 1A. The multimodal microwave cavity was built in aluminum with dimensions (110 mm × 130 mm × 115 mm) to allow a proper placement of the reactor flask inside it. The cavity was connected at one side to a 3 kW variable-power generator, Richardson model SM 1050D (Richardson Electronics Ltd., Sao Paulo, Brazil), operating at 2.45 GHz and protected by a circulator. A power meter (model 8481A, Agilent, California, USA) was used, connected to two directional couplers and two power sensors (model EPM-E44198B, Agilent). This system is capable of measuring both microwave transmitted and reflected powers in which the difference is the effective power applied to the sample. At the other side of cavity there is a moving short used to adjust the point of maximum power absorption.

The reactor, in which samples were held, was a 250 mL Pyrex round-bottomed flask (Fig. 1C), which was connected to three add-on features in order to control the temperature, refluxing, mixing, and sampling. The temperature was controlled using a polypropylene coiled tube inserted into the reactor, in which cooled kerosene at 0 °C flowed, controlled by a needle valve (Fig. 1B). Kerosene was chosen as cooling fluid due to its low microwave absorption. A stirrer with PTFE propeller and glass shaft was used to homogenize the sample in the flask during processing. Since microwave absorption can be heterogeneous, temperature homogenization of a liquid food is fundamental to obtain reliable results (Kubo et al., 2020).

Temperature and power were measured after the start of the generator and then every 30 s. After achieving the target processing temperature, the cooled kerosene flow was started to control the sample temperature. Preliminary tests were carried out to establish the proper kerosene flow for each power and temperature setting. After the come-up time, 5 mL samples were collected at 5, 10, 15 and 20 min of processing. The samples were immediately cooled in water-ice bath for 1 min and spores that survived the treatment were enumerated, with results expressed as CFU mL^{-1} of coconut water.

Table 1
Central Composite Design (CCD) for four independent variables.

Variables	Coded Levels				
	−2	−1	0	+1	+2
A - Temperature (°C)	83	86	89	92	95
B - amount of acids (mg/100 mL)	73	74	75	76	77
C - Ratio of acids (%citric/% ascorbic)	67/33	71/29	75/25	78/22	80/20
D - Specific power (W/mL)	115	120	125	130	135

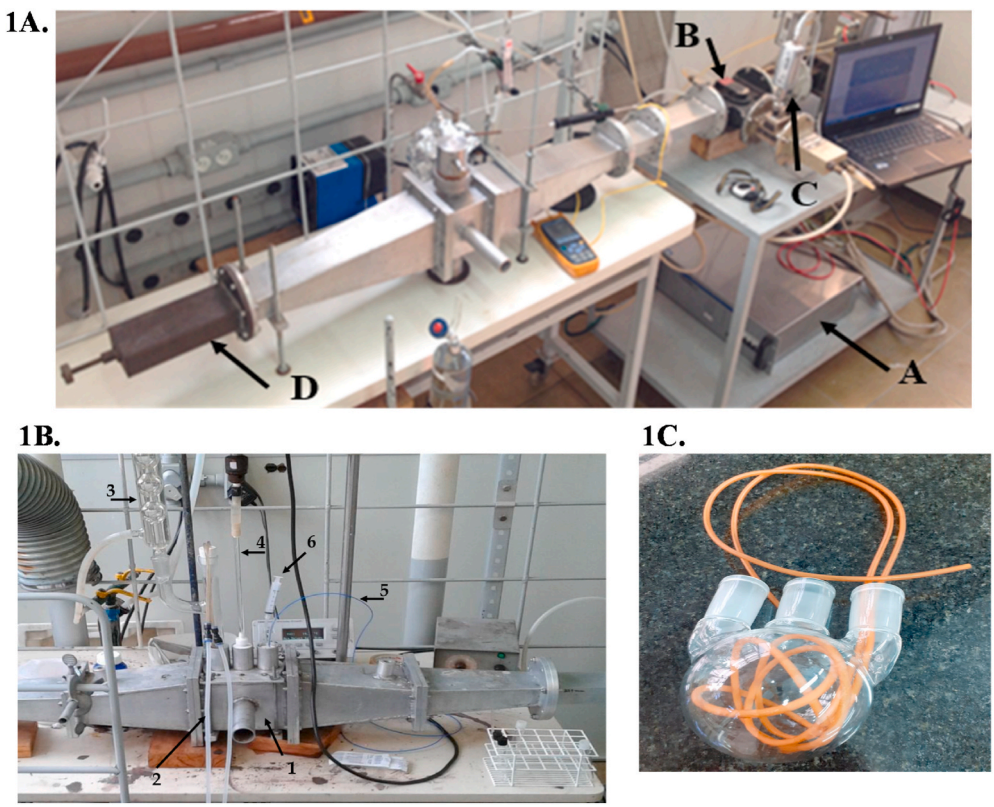

Fig. 1. Details of microwave equipment and accessories for processing coconut water. **1A.** A. microwave generator 3 kW 2.45 GHz, B. circulator, C. directional coupler and power sensor, D. moving short. **1B.** 1. reaction cavity, 2. polypropylene serpentine for circulating cooling liquid, 3. total reflux column, 4. mechanical stirrer, 5. fiber optic thermometer, and 6. sampling. **1C.** Reactor, 250 mL Pyrex round-bottomed flask.

2.6. Modelling of Microwave Inactivation Kinetics

The thermal inactivation of *B. coagulans* spores was assumed to follow the Weibull model (Eq. (1)):

$$\log(S) = -\left(\frac{t}{\alpha}\right)^{\beta} \tag{1}$$

where α is the scale factor (time required for the first decimal reduction, or first D-value), β is the shape factor and S is the ratio (N_t/N_0) between the number of surviving spores after processing time t and the initial spore count (Siguemoto, Gut, et al., 2018; Mafart, Couvert, Gaillard, & Leguerinel, 2002).

The temperature dependence of the scale parameter (α) was modelled by a logarithmic relationship based on a reference temperature of $T_{ref} = 90$ °C (Eq. (2)):

$$\alpha = \alpha_{ref}\ 10^{-\frac{T-T_{ref}}{z}} \tag{2}$$

where z' is the temperature increase required to reduce the scale factor (α) by 10-fold (equivalent to the z-value from the classic first order model), T is temperature (°C) and α_{ref} is the reference scale factor (Siguemoto, Gut, et al., 2018; Daryaei & Balasubramaniam, 2013).

A S-curve model was used to describe the influence of the temperature on the shape parameter (β) (Eq. (3)):

$$\beta = 1 - \frac{1}{1 + e^{a-b\ T}} \tag{3}$$

where, a and b are empirical parameters of the equation.

Each test based on the experimental design in Table 1 delivered four time-temperature conditions since samples were collected at 5, 10, 15 and 20 min of processing, after the come-up time. The first 16 experiments in Table 1 represent the factorial design 2^4; tests 17 to 20 are the central point, and tests 21 to 28 are the axial points. That gives a total of $4 \times 28 = 112$ experimental determinations. Since results presented in the next section show that temperature was the only statistically significant variable, these 112 determinations could be grouped to fit the Weibull model.

Typically, for isothermal experiments, the Weibull model is adjusted using a two-step approach. First, parameters α and β are adjusted for each processing temperature using Eq. (1), then these two parameters are correlated with temperature using Eqs. (2) and (3). However, the come-up time in the experiments was between 6 and 8 min and the assumption of isothermal treatment can introduce an error in the model fitting. Consequently, a single-step approach to adjust the Weibull model based on non-isothermal treatments was used (Cavalcante, Funcia, & Gut, 2021).

In this approach, each experiment has its own recorded time-temperature profile $T(t)$ and the replicates are treated individually. Instant cooling was assumed after the sample was collected from the reactor flask because of the small amount of liquid and immediate immersion is ice-water bath. The Weibull lethality rate was integrated along the temperature profile according to Eq. (4), where $\Delta t = 30$ s was the time step and α and β come from Eqs. (2) and (3) using the instant temperature, respectively:

$$\log(S) = -\sum_{k=0}^{t/\Delta t} \frac{\beta}{\alpha}\left(-\log(S)_{k-1}\right)^{\frac{\beta-1}{\beta}}\Delta t \tag{4}$$

For the calculation of $\log(S)$, initial deductions were needed for the four model parameters: α_{ref}, z', a and b, which were obtained using the two-step approach assuming isothermal processing. The non-linear Generalized Reduced Gradient algorithm in the Excel solver (Microsoft, Redmond, USA) was used to minimize the mean squared error in the prediction of $\log(S)$, coupled with the numerical integration of the n

= 112 treatments:

$$\text{minimize } MSE = \frac{1}{n}\sum_{j=1}^{n}\left[\log(S)_{experimental,j} - \log(S)_{predicted,j}\right]^2 \quad (5)$$

Coefficient of determination (R^2), mean squared error (*MSE*) and parity charts of predicted *versus* experimental survival ratios were used to evaluate the goodness of the model fit.

3. Results

Table 2 shows the results of log(S) for the 28 experiments in the central composite design at holding times of 5 min (log $S1$), 10 min (log $S2$), 15 min (log $S3$) and 20 min (log $S4$). Among the four variables tested on the inactivation of the spores, only the temperature was statistically significant ($p < 0.05$) (Table 3); consequently, the kinetic model was adjusted only having temperature and time as variables.

The minimization of the mean squared error for the Weibull model prediction provided the adjusted parameters shown in Table 4 with *MSE* = 0.49 and R^2 = 0.823. Fig. 2 illustrates the model fitting method described in section 2.6 - Modelling of Microwave Inactivation Kinetics. A linear temperature increase can be seen in the 6.5 min come-up time, which is coherent with microwave heating. The adjusted Weibull model provides $\log(S) = -0.53$ using Eq. (4) at the end of the come-up time (vertical dashed line), which indicates that the heating period indeed should not be neglected in the model fitting. The following model prediction for the four holding times are close to the measurements of surviving spores.

The parity chart for the prediction of log(S) is presented in Fig. 3A and it can be seen that the 112 points are well dispersed around the 45° line given an uncertainty of one log unit. Moreover, points are well distributed between 1 and 7 log reductions. Fig. 3B brings the inactivation curve at the reference temperature of 90 °C, as given by the adjusted model, along with the experimental points considering their corresponding calculated F-values at the reference temperature (equivalent time for isothermal processing in Eq. (6), which is based on Eq. (1)):

$$F_{ref} = \alpha_{ref}\left[-\log(S)_{experimental}\right]^{\frac{1}{\beta}} \quad (6)$$

The Weibull model showed good fit to the experimental data as it can be observed by the R^2 value and by the dispersion of points in Fig. 3. The adjusted inactivation curves of the Weibull model at 83, 86, 89, 92 and 95 °C, are displayed on Fig. 4A. This model is valid for the processing conditions of acidified coconut water presented in Table 1.

The scale parameter (α) represents the time for the first decimal reduction and a logarithmic relationship was observed (Fig. 4B) with a large dependence (low z-value). The shape parameter (β) decreased with the temperature as shown in Fig. 4C. Values of β lower than 1 indicate a varying thermal resistance among spore population.

4. Discussion

Although the spore inactivation was temperature dependent, the effect of microwave power was not significant ($p < 0.05$). In other words, increasing power from 115 to 135 W/mL did not contribute to the reduction of *B. coagulans* spores in green coconut water. The electromagnetic effect cannot be detected separately, as previously observed in the study of Pina-Pérez, Benlloch-Tinoco, Rodrigo, and Martinez (2014). These authors showed a reduction in the population of *Cronobacter sakazakii* in reconstituted powder infant formula milk heated by microwaves, but they were not able to demonstrate if the reduction was due to heating alone or to heating plus the electromagnetic effect.

On the other hand, Rougier, Prorot, Chazal, Leveque, and Leprat (2014) indicated that the damage to the cell membrane of *Escherichia coli* is power dependent (400–2000 W), suggesting the existence of an electromagnetic field effect. Herein the effect of electromagnetic field on spore reduction for power ranging from 115 to 135 W/mL could not be detected. It would be interesting to test a larger power range in order to evaluate the effect of the electromagnetic field.

Although microwave systems have been studied for aseptic processing of foods, for example, orange juice (Fratianni, Cinquanta, & Panfili, 2010), sweet potato puree (Brinley et al., 2007), milk (Lin & Ramaswamy, 2011) and asparagus pickled (Lau & Tang, 2002), the

Table 2
Results of *B. coagulans* inactivation (log N_t/N_0) in the Central Composite Design for the four holding times: 5 min (log $S1$), 10 min (log $S2$), 15 min (log $S3$) and 20 min (log $S4$).

Tests	Temperature (°C)	Amount of acids (mg/100 mL)	Ratio of acids (cit./asc.)	Specific power (W/mL)	log $S1$	log $S2$	log $S3$	log $S4$
1	86	74	2.5	120	−0.9	−2.0	−2.8	−3.7
2	86	74	2.5	130	−0.5	−1.1	−1.8	−2.7
3	86	74	3.5	120	−1.1	−2.4	−2.9	−3.2
4	86	74	3.5	130	−1.1	−2.3	−3.0	−3.8
5	86	76	2.5	120	−0.7	−1.9	−2.6	−3.2
6	86	76	2.5	130	−1.9	−3.2	−3.4	−3.8
7	86	76	3.5	120	−0.9	−1.9	−2.5	−3.0
8	86	76	3.5	130	−0.5	−1.2	−2.2	−2.7
9	92	74	2.5	120	−3.4	−4.6	−4.8	−5.3
10	92	74	2.5	130	−3.8	−4.5	−4.7	−4.7
11	92	74	3.5	120	−4.9	−5.9	−6.4	−6.3
12	92	74	3.5	130	−2.0	−3.9	−4.7	−4.4
13	92	76	2.5	120	−5.2	−6.3	−6.2	−6.5
14	92	76	2.5	130	−3.4	−3.6	−3.7	−3.8
15	92	76	3.5	120	−4.4	−6.1	−6.1	−6.3
16	92	76	3.5	130	−4.6	−5.7	−5.9	−7.0
17	89	75	3.0	125	−2.1	−2.9	−3.0	−3.3
18	89	75	3.0	125	−1.8	−3.4	−3.9	−4.1
19	89	75	3.0	125	−2.2	−2.8	−3.0	−3.1
20	89	75	3.0	125	−2.5	−3.0	−3.2	−3.1
21	83	75	3.0	125	−0.2	−0.5	−0.9	−1.1
22	95	75	3.0	125	−5.6	−6.9	−7.1	−7.6
23	89	73	3.0	125	−1.8	−3.3	−4.1	−4.6
24	89	77	3.0	125	−1.6	−2.9	−3.8	−3.8
25	89	75	2.0	125	−2.3	−3.9	−4.6	−4.6
26	89	75	4.0	125	−1.4	−2.5	−2.9	−3.2
27	89	75	3.0	115	−1.8	−3.3	−4.2	−4.3
28	89	75	3.0	135	−3.6	−4.3	−4.7	−5.3

Table 3
Estimated effects (linear, quadratic and interaction), and p values for ($\log N_t/N_0$) for the four holding times: 5 min ($\log S1$), 10 min ($\log S2$), 15 min ($\log S3$) and 20 min ($\log S4$) using a central composite design for evaluation of the effects of temperature (A), amount of acid (B), ratio of acid (C) and specific power (D) on *B. coagulans* inactivation.

Factor	Estimated Effects							
	$\log S1$	p value[a]	$\log S2$	p value[a]	$\log S3$	p value[a]	$\log S4$	p value[a]
intercept	**−2.160**	**0.000**	**−3.019**	**0.000**	**−3.271**	**0.000**	**−3.426**	**0.000**
A[b]	**−1.456**	**0.000**	**−1.563**	**0.000**	**−1.404**	**0.000**	**−1.305**	**0.000**
B[b]	−0.145	0.391	−0.111	0.527	−0.031	0.848	−0.018	0.923
C[b]	0.091	0.585	0.013	0.941	−0.020	0.901	−0.006	0.974
D[b]	0.016	0.925	0.154	0.385	0.154	0.350	0.106	0.570
AA[c]	−0.215	0.211	−0.185	0.301	−0.174	0.295	−0.253	0.185
BB[c]	0.081	0.628	−0.040	0.819	−0.163	0.325	−0.213	0.259
CC[c]	0.044	0.790	−0.058	0.742	−0.116	0.481	−0.124	0.503
DD[c]	−0.166	0,329	−0.204	0.254	−0.280	0.102	−0.354	0.072
AB[d]	−0.199	0.337	−0.153	0.480	−0.079	0.692	−0.235	0.309
AC[d]	−0.033	0.870	−0.201	0.356	−0.240	0.240	−0.286	0.219
AD[d]	0.271	0.197	0.308	0.166	0.260	0.205	0.272	0.241
BC[d]	0.079	0.696	0.138	0.522	0.131	0.515	−0.031	0.888
BD[d]	−0.117	0.568	−0.038	0.861	−0.034	0.865	−0.084	0.711
CD[d]	0.151	0.464	0.053	0.805	−0.042	0.833	−0.173	0.449

[a] $p < 0.05$ indicates that the term is statistically significant (highlighted in bold).
[b] Linear term.
[c] Quadratic term.
[d] Interaction term.

Table 4
Model parameters for inactivation of *B. coagulans* spores in coconut water by microwave.

Parameter	Value
α_{ref} (s)	33.8
z (°C)	5.06
a (–)	5.91
b ($°C^{-1}$)	0.0692

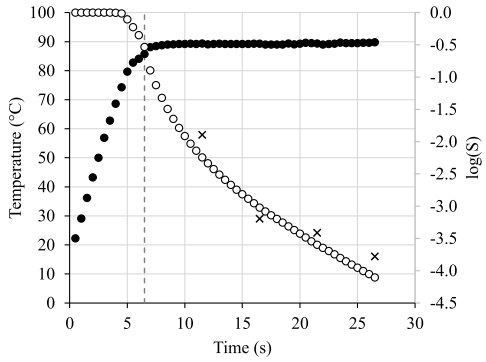

Fig. 2. Example of model fitting using non-isothermal data (Test 6 in Table 2) showing acquired temperature profile of the coconut water sample (●), end of come-up time (- - -), calculated profile of $\log(S)$ using the adjusted Weibull model (x̂), and measured values of $\log(S)$ (×).

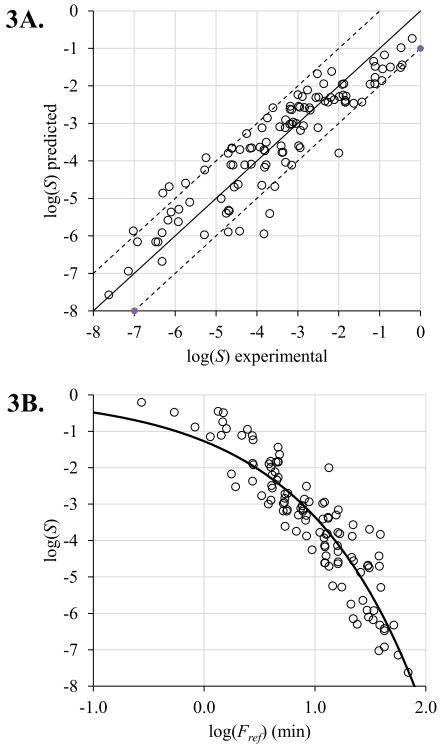

Fig. 3. Model adjustment results. **3A.** Parity chart of inactivation of *B. coagulans* spores between experimental and predicted model data. **3B.** Inactivation curve at T_{ref} = 90 °C with experimental data against equivalent processing time F_{ref}.

feasibility of its use in other food matrices, such as coconut water, has not been proven. In this study, the results showed that microwaves can inactivate spores of *B. coagulans* present in coconut water to safe levels. From the Weibull model, a reduction of 5.0 log was obtained with a holding time of 14.4 min at 92 °C or 6.6 min at 95 °C (Fig. 4A).

The heat resistance of spores in foods can be reduced by combining heat with a pH reduction of the product (Palop, Raso, Pagán, Condón, & Sala, 1999). Palop, Marco, Raso, Sala, and Condón (1997) reported that citric acid was more effective with mildly heated *B. coagulans* spores whereas lactic acid was more effective with more severe heating. In our study, the pH of coconut water was reduced to 4.3–4.5 to conform with the current Brazilian legislation, so this may have contributed to reducing the spore heat resistance. However, the different relationship between citric and ascorbic acid on spore resistance was not observed ($p > 0.05$).

The result of spore inactivation in this study corroborates the findings from the literature. The classical first order inactivation kinetics is typically used in the literature to fit survival curves of thermal treated spores and to determinate the D-value (time required to reduce spore

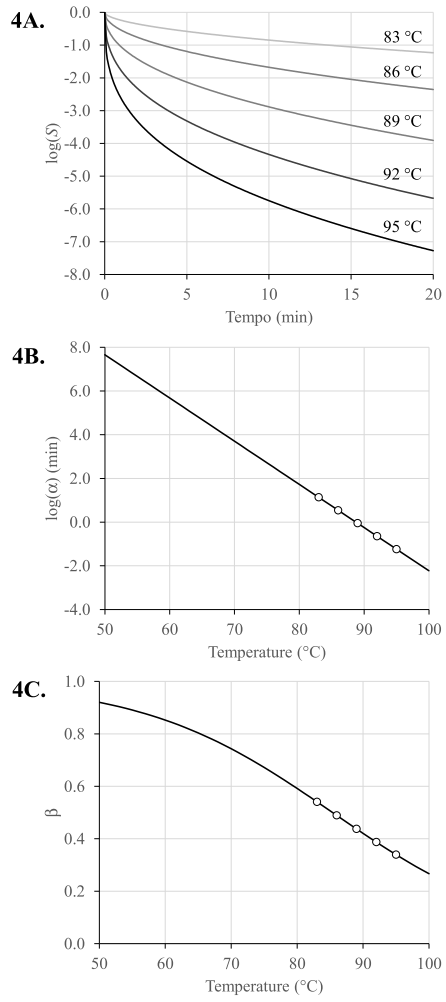

Fig. 4. Adjusted Weibull model. **4A.** Inactivation curves of *B. coagulans* spores in coconut water after microwave processing at the indicated temperatures. **4B.** Relationship between α and temperature. **4C.** Relationship between β and temperature. Points (x̄) indicate processing temperatures of 83, 86, 89, 92 and 95 °C.

population by 1.0 log). The *D*-value of inactivation of *B. coagulans* spores in tomato juice by thermal processing at 100 °C was 1.66 min (Brasil, 2009). Other *D*-values for *B. coagulans* spores have been reported by Palop et al. (1999) with 1.7 and 0.88 min in medium at pH 4 and heating at 105.1 °C and 107.9 °C, respectively. Additionally, Wang et al. (2009) determined a *D*-value of 4.17 min at 80 °C and 5.00 min at 70 °C in buffer solution under 600 MPa pressure. Our results showed a non-linear function for the inactivation of *B. coagulans* spores exposed to microwaves at isothermal conditions and the Weibull model was well fitted. This behavior was also observed by other authors and the scale factor was determinative instead of the D-value. In tomato juice the scale factor (α) for *B. coagulans* spores was 1.52 min at 100 °C (Daryaei & Balasubramaniam, 2013), while in nutrient broth it was 1.71 min at the same temperature (Haberbeck, Riehl, Salomão, & Aragão, 2012). These values were much higher than those found in this work (0.36 s at 100 °C from Fig. 4A), suggesting that the first decimal reduction of *B. coagulans* spores was faster for microwave treatment of coconut water.

The concavity of the inactivation curves of *B. coagulans* spores, given by the β parameter, depends on the process temperature and this result indicates that the spores have different heat resistances. Fig. 4C shows that β decreases almost linearly with temperature, and values are lower than 1, which indicates that the most sensitive spores of the population are first inactivated, and the more resistant ones remain. This result was similar to that reported by Siguemoto, Gut, et al. (2018) for *Listeria monocytogenes* in apple juice exposed to conventional heating. However, the authors did not observe a clear dependence of β with the temperature for *L. monocytogenes* and *E. coli* in microwave-heated apple juice.

The z' value is the temperature change necessary to cause a 10-fold variation in α (time for the first reduction of 90% of the population at a given temperature). In this study z' was 5.06 °C, which is lower than values found by other authors in the literature. In tomato juice, processed at constant pressure, the z value for *B. coagulans* spores was 33 °C, determined by plotting log *D versus* process temperature (Daryaei & Balasubramaniam, 2013). However, z' values ranging from 6.7 to 39.1 °C were reported for heat inactivation of *B. coagulans* spores (Okazaki & Suzuki, 2006). Siguemoto, Gut, et al. (2018) reported z' value for *E. coli* of 4.56 °C and 6.17 °C for *L. monocytogenes* in apple juice processed by conventional heating at a range of 55–70 °C. These results also suggest that microwave enhance bacterial inactivation in comparison with conventional heating.

5. Conclusions

Microwave processing was effective at inactivating *B. coagulans* spores in acidified green coconut water. Applying temperature of 95 °C for 6.61 min, with specific power between 115 and 135 W/mL, amount of acids between 73 and 77 mg/100 mL and a ratio of citric and ascorbic acids between 2.0 and 4.0 was enough to achieve a reduction of 5 log CFU mL^{-1} in spore population, which is considered effective to avoid microbial spoilage. This study suggested that the temperature was the most important parameter for the process, but other studies are necessary in order to better assess the effect of microwave power and higher temperatures. Our findings demonstrate that microwave is a technology with potential for commercial sterilization process of acidified coconut water in order to achieve desired microbial inactivation; however the effect on enzyme inactivation and sensorial characteristic must also be evaluated.

CRediT authorship contribution statement

Raquel O.M. Pinto: Data curation, Formal analysis, Writing – original draft, and, Writing – review & editing. **Renata B. do Nascimento:** Methodology, and Data curation. All authors have read and agreed to the published version of the manuscript. **Luiz Alberto Jermolovicius:** Conceptualization, and, Methodology. **Cynthia Jurkiewicz:** Supervision, Formal analysis, and, Writing – Reviewing and Editing. **Jorge A.W. Gut:** Conceptualization, Methodology, Formal analysis, and, Writing – review & editing. **Uelinton Manoel Pinto:** Writing – Reviewing and Editing. **Mariza Landgraf:** Conceptualization, Supervision, Funding acquisition, and, Writing – Reviewing and Editing.

Declaration of competing interest

The authors declare no conflict of interest.

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