20

Effect of thermosonication on quality parameters and production of probiotic juices

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ABSTRACT

Thermosonication is an emerging technology that combines the use of ultrasound and moderate temperature and it is being considered as a potential technology for reducing microorganisms in fruit juices without causing the degradation of bioactive compounds that occurs with pasteurization. Acerola (*Malpighia emarginata*) and guava (*Psidium guajava*) juices were submitted to thermosonication in an ultrasonic processor to evaluate the influence of the power intensity and temperature on the microbiological inactivation, total phenolic compounds, antioxidant activity, ascorbic acid, carotenoids, and color. The effect of thermosonication on the production of these fermented probiotic juices using *Lactobacillus rhamnosus* ATCC 7469 was also evaluated. The production and the refrigerated storage of fermented probiotic juices at 37 ºC for 24 hours were analyzed by thermosonication at 60% ultrasound power intensity and temperature at 65ºC. Thermosonication was as effective as pasteurization in microbial inactivation, with the advantage of keeping the bioactive compounds more available which favored the growth of the probiotic.

Palavras-chaves: Bioactive Compounds, Microbial Inactivation, Probiotic Juices, Cell Viability, Ultrasound.

INTRODUÇÃO

Brazil is the third-largest fruit producer in the world with 35% for exportation (Embrapa 2020). Fruit juices contain essential nutritional components, such as carbohydrates, proteins, minerals, vitamins, and carotenoids for the equilibrium of the human body (Khandpur and Gogate, 2016; Dias et al. 2015). Guava (*Psidium guajava* L) and acerola (*Malpighia punicifolia* L) are tropical fruits, which have excellent consumer acceptance and command a significant participation in the Brazilian market (Embrapa, 2020; IBGE 2019; Campoli et al. 2018).

Fruit juices are subject to deterioration associated with the presence of **microorganisms** such as yeasts; fungi and acid-tolerant bacteria (Elvira 2014). Microbiological inactivation guarantees a safe food product for the consumer, being an indispensable part of food processing (Roobab et al. 2018). Traditional methods of microbiological inactivation, however, affect the nutritional composition and bioactive compounds (Abid et al. 2014). Thermosonication is an emerging technology that combines the use of ultrasound and moderate temperature. It is been considered as a potential technology for reducing microorganisms in fruit juices (Cervantes-Elizarrarás et al. 2017; Cruz-Cansino et al. 2015) without causing the degradation of bioactive compounds that occurs with conventional heat treatments (Aguilar et al. 2017; Zafra-Rojas et al. 2013).

The consumption of plant probiotics, such as fruits and their products, has emerged because there is a demand for low-fat or lactose-free foods (Fonteles et al. 2013). There has been an increasing number of studies of non-dairy probiotic drinks, mainly made from a vegetable matrix, such as fruit juices. This growth is explained by such reasons as the increase in the number of people allergic to milk proteins, or who have high cholesterol levels, and lactose intolerance. As well, there are market segments that consume only products of plant origin (Andrade et al. 2019).

Probiotic fruit juices have a lower sugar content since lactic acid bacteria consume sugar during fermentation (White and Hekmat, 2018). The production of probiotic fruit juices is strongly influenced by parameters such as pH, nutritional composition, cell viability, and survival in refrigerated storage (Fonteles et al. 2013). Some studies have shown that fruit juices can serve as a medium for Lactobacillus rhamnosus ATCC 7469 (Nematollahi et al. 2016; Farias et al. 2016; Santos et al. 2017; Andrade et al. 2019).

OBJETIVO

The aim of this work was to investigate the effects of thermosonication on microbiological inactivation, bioactive compounds, and color of acerola and guava juices, evaluating

the behavior of these different thermosonicated vegetable matrices and on the probiotic fermented juices using *Lactobacillus rhamnosus* ATCC 7469.

MÉTODOS

Juice preparation

Malpighia emarginata (acerola) and *Psidium guajava* (guava) juices were prepared from non-pasteurized frozen pulp without the addition of preservatives or water, produced by Fresh Fruit company (Recife, Brazil). The juices were prepared by diluting the pulp in distilled water (1:1 ratio) and then processed, as described below (Section 2.2). Before thermosonication or pasteurization, the pH of the juices was adjusted with 2 mol L^{-1} NaOH (Dinâmica, São Paulo, SP, Brazil) using a potentiometer (Tecnal model Tec-3MP).

Thermosonication and pasteurization

Thermosonication treatment was conducted with 500 W ultrasound equipment with a 1.3 cm probe diameter and a constant frequency of 19 kHz (Unique, DES500, Brazil). An ultra-thermostatized bath (SOLAB® SL-152/157, Brazil) was used to maintain the juice processing temperature. The juice samples (150 mL) were placed in a 250 mL thermostated vessel (8.5 cm diameter) and an ultrasonic probe was immersed to 2.5 cm below the surface of the juice. All trials lasted 10 minutes and started when the pre-set temperature was reached by the system, being recorded by a digital thermometer for calibration (Abid et al. 2013; Saeeduddin et al. 2015). The temperature of the jacket water was maintained according to the experimental design described below. The pasteurization treatment of the juices was conducted at 67 °C for 35 minutes, followed by thermal shock in an ice-water bath for 5 minutes (Fellows 2009; Madigan et al. 2010; Farias et al. 2016; Santos et al. 2017; Andrade et al. 2019).

Experimental design

The effects of temperature and ultrasound power intensity were studied on micro-biological inactivation, bioactive compounds and color using a composite central complete factorial design 2^2, with the central points (level 0) and axial points (levels $\pm \alpha$), according to Tables 1 and 2. The range of variation between the lower and upper limits of each variable was established based on the literature (Dias et al. 2015). Analysis of statistical data was performed using Statistica 7.0. Tukey test was used to evaluate the statistical significance of tests for acerola and guava juices.

Microbiological inactivation, quality parameters and color analyses

Microbiological inactivation, total phenolic compounds content, antioxidant capacity, ascorbic acid content, carotenoids and color of the treated samples were investigated. The analyzes were performed in triplicate. The determination of color in quintuplicate.

The evaluation of viability for microbiological inactivation (Inat) in juices was determined by the spread plate method. Aliquots of 0.1 mL of the samples were plated, in triplicate, in nutrient MRS broth (Merck, KGaA Germany) containing agar 2% w/V (KASVI, São Paulo, Brazil). The plates were incubated upside-down at 37 ºC for 24 h. Plates containing between 30 and 300 colonies were measured and recorded as colony forming units (CFU) per mL of solution. Control (without treatment), pasteurized, and sonicated samples of the two juices were also evaluated.

The content of total phenolic compounds (TPC) was determined according to the methodology of Singleton et al. (1999). The results were expressed as mg of gallic acid equivalent (GAE) per 100 mL of sample. The antioxidant activity (AC) was determined by the analysis of the free radical sequestration capacity, using the reagent 1'-1'Diphenyl-2'picrylhydrazyl (DPPH) (Brand-Williams et al. 1995). Values were expressed as percent inhibition of DPPH. Ascorbic acid content was determined according to the methodology of Strohecker and Henning (1967). The values were expressed as mg of AA per 100 g of juice. The total carotenoid content (C) was measured according to Rodriguez-Amaya (1999), expressed as µg of carotenoids per gram of juice. The color of the samples (TCD) was evaluated by means of the three parameters reading system, CIELAB, obtained using a previously calibrated colorimeter (Minolta, CR400, Japan).

Microorganism and inoculum

Lactobacillus rhamnosus ATCC 7469 was purchased from The American Type Culture Collection (ATCC, Manassas, VA, USA). The strain was stored at -20°C (Model DC49A, Electrolux, Recife, PE, Brazil) in 10% (v/v) glycerol (Farias et al. 2016). For inoculum preparation, the stock culture was subcultured in 25 mL of MRS (De MAN, ROGOSA, and SHARPE) broth (Merck, Darmstadt, Germany) and was incubated (Model SP-101, Splabor, Presidente Prudente, SP, Brazil) at 37°C for 24 h. The 10% (v/v) bacterial suspensions, in MRS broth, were inoculated into the fruit juices (item 2.4).

Kinetic study of the production of probiotic juices of acerola and guava

The bacterial cells (Section 2.5) were aseptically added to the pasteurized or thermosonicated juices and were incubated at 37°C (Model SP-101, Splabor, Presidente Prudente, SP,

Brazil). Six different fermentations were carried out during 24 h. Two of these were acerola juices with pH adjusted to 4, pasteurized (PA4), or termossonicate (TA4). Concerning guava juices, four conditions were established: PG4 (pasteurized guava and pH adjusted to 4), TG4 (thermosonicated guava and pH adjusted to 4), PG6 (pasteurized guava and pH adjusted to 6) and TG6 (thermosonicated guava and pH adjusted to 6). Samples were collected every six hours. All fermentations were performed in duplicate. Viability, pH, and glucose, fructose, and lactic acid concentrations were determined. The fermented juices were dispensed into sterile tubes, stoppered and stored at 4 ºC in a refrigerator. Viability of *L. rhamnosus* ATCC 7469 was determined at the initial time and at 28 days of refrigerated storage.

Determination of the viability of L. rhamnosus ATCC 7469

Serial dilutions of the probiotic acerola and guava juices in a 0.9 % w/v saline solution were carried out for viable cell count using a spread plate method in MRS broth (Merck, KGaA Germany) containing agar 2 % w/V (KASVI, São Paulo, Brazil). The plates were incubated upside down at 37°C for 48 h. Viability was measured as CFU/mL of suspension.

Determination of pH, glucose, fructose, and lactic acid

The pH of the juices was measured in a potentiometer (Tecnal model Tec-3MP). High-performance liquid chromatography (HPLC) was used to determine glucose, fructose, and lactic acid concentrations (Farias et al. 2016). A Shimadzu system (Model Prominence LC-20AD, Kyoto, Japan) equipped with a Shimadzu diode array detector (Model SPDM20A) for lactic acid or refractive index detector (Model RID-10A) for glucose and fructose was used. A 300×7.8mm ionic exchange column (Aminex® HPX-87H, Bio-Rad, Hercules, CA, USA), with a 9 µm particle size, was used at 28 °C (lactic acid) or 40 °C (glucose and fructose). The mobile phase was a solution of sulfuric acid (5 mM) prepared in ultrapure water (Model MS 2000, Gehaka, São Paulo, SP, Brazil) at a flow rate of 0.6 mL/min in isocratic elution. The samples were filtered with a 0.22 µm membrane (Durapore, Merck) and diluted in the mobile phase at a 1:10 ratio. Diferent volumes (10, 20, 30, 40, and 50 µL) of standards of lactic acid, glucose, and fructose (Sigma Aldrich) at a concentration of 1 g/L were put through the HPLC to obtain each linear calibration curve; the correlation coe□cients of all calibration curves were ≥0.999. The retention times of the standards and their peak area were used to identify and quantify the compounds. The data were obtained using the software LabSolutions LC solutions that came with the instrument.

Statistical analysis

The mean values of variables were obtained from two experimental replicates. Data were analyzed using a one-way analysis of variance (ANOVA) followed by the Tukey test using Statistica 7.0 at a 5 % of significance level.

RESULTADOS

Effects of thermosonication on microbiological inactivation and quality parameters

Polynomial models of the correlation between dependent and independent variables were tested but they did not present significant regression. Thus, the Tukey test was chosen to evaluate the statistical significance of 11 runs for acerola and guava juices. The results of the microbiological inactivation (Inat) for the both juices show that the set of tests performed presented a high reduction when compared to the control sample (without treatment). There was a reduction from uncountable to 2 logs.

In the set of assays, for both juices, test 11 presented the most promising result (Table 1 and 2), with an Inat increase of 17% and 28% for acerola and guava juices, respectively, when compared to the pasteurized sample. It was observed that for acerola juice, the conditions 60% (65 °C - test 11) and 90% (59 ° C - test 4) presented the lowest values of cell viability, with no significant difference between these conditions. Similar behavior was observed for guava juice. However, when the thermosonicated samples from test 11 were compared with the pasteurized samples from both juices, a significant difference between values was observed, evidencing that this test is most promising.

The results of the TPC analysis for the thermosonicated acerola juice (Table 1) showed a reduction of this content from 3% to 48%, except for tests 4, 8 and 11 which showed an increase, without registering a statistical difference on the Tukey test. Experiment 11 presented the highest value of TPC, with an increase of 5.2% and 8.6% when compared to the control sample (fresh juice) and pasteurized sample, respectively. For guava juice (Table 2), the results showed a reduction between 2 to 15% in the content of TPC. From the tests carried out, only assay 10 failed to present a reduction when compared to the control sample (*without treatment*). The pasteurized sample had a reduction of around 21% when compared to the control sample. Among the two treatments for each juice, acerola and guava, there was no significant difference.

Table 1. Total phenolic compounds (TPC), antioxidant capacity (AC), Ascorbic acid content (Ascorbic acid), carotenoid content (C) and total color difference (TCD) for acerola juice.

Assay	IP (%)	T (°C)	TPC (mg GAE/100mL)	AC (%Inhibition DPPH)	Ascorbic acid (mg AA/100 g)	C (µg/g)	TCD
Control			499.68 ± 2.05	70.68 ± 0.00	598.31 ± 3.16	3.30 ± 0.00	-
1	30	31	457.97 ± 8.63	70.24 ± 0.00	551.43 ± 0.00	3.36 ± 0.00	2.24
2	90	31	367.89 ± 4.84	67.69 ± 0.00	544.73 ± 3.16	3.50 ± 0.00	2.39
3	30	59	373.63 ± 0.00	48.66 ± 0.01	560.36 ± 3.16	3.43 ± 0.00	2.66
4	90	59	522.84 ± 1.12	75.98 ± 0.02	609.47 ± 0.00	3.39 ± 0.00	2.35
5	60	45	257.84 ± 3.72	42.35 ± 0.00	589.38 ± 0.00	3.71 ± 0.00	2.33
6	60	45	246.53 ± 2.23	43.16 ± 0.02	598.31 ± 3.16	3.90 ± 0.00	1.70
7	60	45	236.00 ± 5.95	43.08 ± 0.01	609.47 ± 3.16	3.90 ± 0.00	2.02
8	20	45	509.16 ± 3.72	52.67 ± 0.02	605.01 ± 3.16	3.01 ± 0.00	3.29
9	100	45	382.32 ± 1.54	52.83 ± 0.04	618.40 ± 0.00	3.41 ± 0.00	2.80
10	60	25	499.95 ± 4.14	60.53 ± 0.03	616.17 ± 3.16	3.23 ± 0.00	2.51
11	60	65	525.47 ± 5.96	75.43 ± 0.01	640.73 ± 0.00	2.86 ± 0.00	2.46
P			483.90±11.91	64,58 ± 0.01	526.87 ± 0.00	3.94 ± 0.00	1.95

IP: intensity of the ultrasound power dissipated by the probe; P: Pasteurized

Table 2. Total phenolic compounds (TPC), antioxidant capacity (AC), Ascorbic acid content (Ascorbic acid), carotenoid content (C) and total color difference (TCD) for guava juice.

Assay	IP (%)	T (°C)	TPC (mg GAE/100mL)	AC (%Inhibition DPPH)	Ascorbic acid (mg AA/100 g)	C (µg/g)	TCD
Control			46.06 ± 1.23	66.06 ± 0.00	10.72 ± 0.00	33.38± 0.01	---
1	30	31	45.88 ± 0.60	55.48 ± 0.00	5.13 ± 0.32	21.64± 0.00	6.16
2	90	31	40.93 ± 1.19	55.22 ± 0.00	6.25 ± 0.00	21.48± 0.00	4.55
3	30	59	43.04 ± 0.74	56.27 ± 0.00	8.93 ± 0.00	26.83± 0.00	1.69
4	90	59	41.25 ± 0.34	54.18 ± 0.00	9.82 ± 0.00	26.19± 0.00	1.97
5	60	45	45.35 ± 0.56	52.09 ± 0.01	8.93 ± 0.00	27.45± 0.00	2.05
6	60	45	44.77 ± 0.37	53.26 ± 0.00	8.93 ± 0.00	28.06± 0.00	3.78
7	60	45	44.14 ± 0.15	51.87 ± 0.01	8.93 ± 0.00	39.42± 0.00	1.83
8	20	45	39.77 ± 0.07	53.72 ± 0.00	5.36 ± 0.00	21.81± 0.00	5.53
9	100	45	38.80 ± 0.11	50.72 ± 0.00	4.47 ± 0.00	26.14± 0.01	9.69
10	60	25	46.72 ± 0.74	62.21 ± 0.01	8.93 ± 0.00	30.86± 0.00	5,69
11	60	65	40.91 ± 0.26	63.64 ± 0.00	8.04 ± 0.00	30.00± 0.01	6.53
P			36.48 ± 0.34	59.66 ± 0.00	6.25 ± 0.00	31.70 ± 0.01	5.60

IP: intensity of the ultrasound power dissipated by the probe; P: Pasteurized

The results of the antioxidant capacity (AC) analysis for the acerola juice (Table 1) showed a reduction in the antioxidant activity of the experiments performed and of the pasteurized sample, except for tests 4 and 11 that presented AC values around 6% higher than the control (*without treatment*). There was no significant difference between these two treatments. For the thermosonicated guava juice (Table 2), a reduction in AC content was observed in all trials. The lowest reduction was observed in test 11, around 3% when compared to the control sample (*without treatment*). The pasteurized sample had a reduction of 9% when compared to the control sample. There was no significant difference between the juices; however, in absolute values, experiment 11 shows values higher for the thermosonicated over the pasteurized. The thermosonicated acerola juice presented ascorbic acid content values higher than the control sample. Experiment 11 presented the highest ascorbic acid increase, around

7% when compared to the control sample. The pasteurized sample showed a 12% reduction of this compound when compared to the control sample (without treatment) (Table 1).

The ascorbic acid content of guava juice (Table 2) showed a reduction in all the experiments, with values higher than those observed for acerola juice (Table 1), between 18 and 59%, when compared to the control sample (*without treatment*), except for experiment 4 that presented a significant reduction. The pasteurized sample presented a reduction of 45% when compared to the control sample.

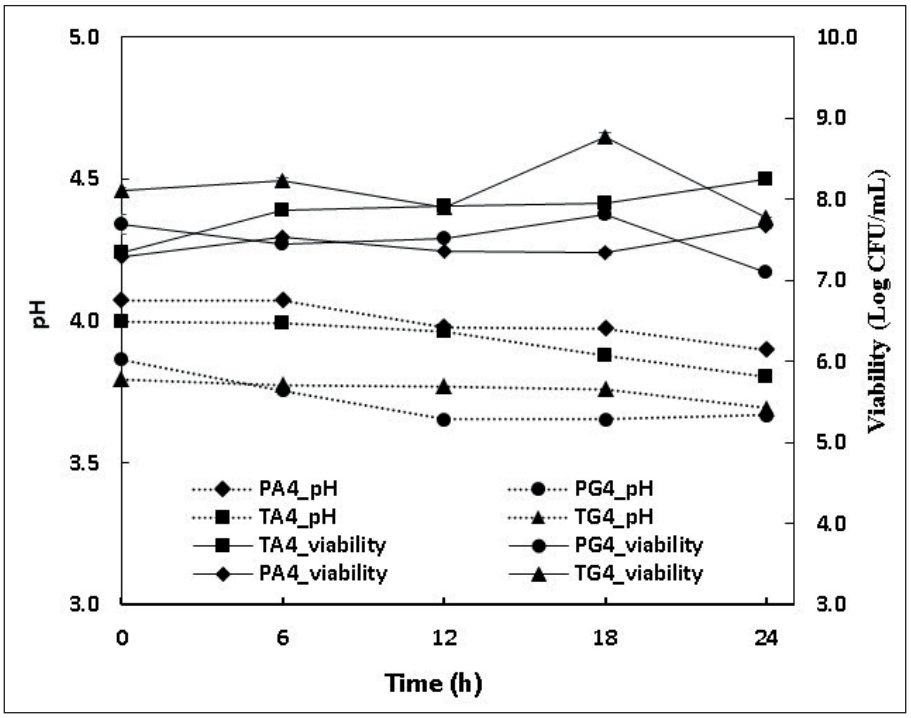
The thermosonicated acerola juice presented increased carotenoid content (C), around 15% when compared to the control (*without treatment*). The pasteurized sample had an increase of carotenoids around 16% when compared to the control sample (Table 1). The thermosonicated guava juice presented a reduction in all the trials, with the highest values observed in tests 1, 2 and 8; around 33% and decreases between 21 and 6% for others. The pasteurized sample presented a reduction of 5% when compared to the control sample. Tests 10 and 11 presented the least reductions, with values close to the pasteurized sample (Table 2). Guava juice presented higher levels of carotenoids than those of acerola juice. The ascorbic acid content in the juices had the opposite behavior with respect to carotenoid content. In this case, the guava had ten times higher levels of carotenoids than acerola. In the set of guava juice experiments, however, tests 10 and 11 presented higher values than the others.

As for the total color variation, for acerola juice, values of the TCD between 1.70 to 3.29 were observed for the sonicated sample; and 1.95 for the pasteurized sample (Table 1). For guava juice, values of the TCD were between 1.69 to 9.69, and 5.60 for the pasteurized sample (Table 2).

Production of fermented probiotic juices varying fruit, pulp treatment and pH

Initially, four different fermentation conditions were established with the acerola and guava juices with initial pH adjusted to 4: pasteurized acerola (PA4), thermosonicated acerola (TA4), pasteurized guava (PG4) and thermosonicated guava (TG4). The initial pH was adjusted to 4 so that it was closer to the natural pH of the juices of these fruits. This adjustment enabled evaluation of the robustness of the *Lactobacillus rhamnosus* ATCC 7469 and made it possible to compare the results with those reported by Nematollahi et al. (2016). These authors adjusted the pH of cornelian cherry juice to 3.5 in the production of unfermented probiotic juices, using *Lactobacillus* strains, including *L. rhamnosus* ATCC 7469. Figure 1 shows the viability of *L. rhamnosus* ATCC 7469 and the pH during the fermentation.

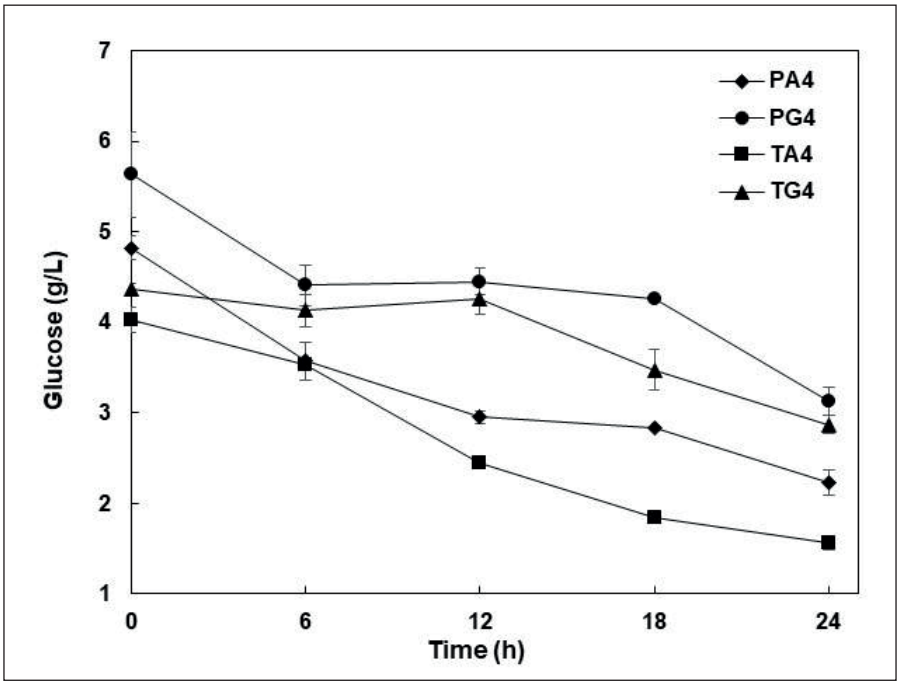
Figure 1. shows the viability and the pH during fermentation of acerola and guava juices (initial pH adjusted to 4)



There was a decrease in pH between 3 and 5%. Maximum viability was observed in the fermentation using the thermosonicated juice, both for acerola and guava. Higher growth was observed in the fermentation of the guava pulps. The stationary phase seems to have occurred between 18 and 24 hours in the fermentation of guava juice. There was a decrease in viability at 24 hours, indicating that the microorganism was in the death phase. On the other hand, in fermentation of acerola juices, the microorganism was probably still in the exponential phase at 24 hours.

Maximum volumetric productivity in cells (Px) was calculated from the difference between the maximum viability value subtracted from the value at the initial time. The result was divided by the time of maximum viability. In fermentation of thermosonicated juices, the maximum volumetric productivities (0.04 and 0.037 Log CFU / mL.h for TA4 and TG4, respectively) were four times higher than those achieved in the fermentation of pasteurized juices (0.014 and 0.007 Log CFU / mL.h for PA4 and PG4, respectively), for both pulps. There was a significant difference between the values obtained in the four fermentation conditions. The initial glucose concentrations (Figure 2) in the juices of each pulp (considering both treatments) were higher in the fermentations of pasteurized juices.

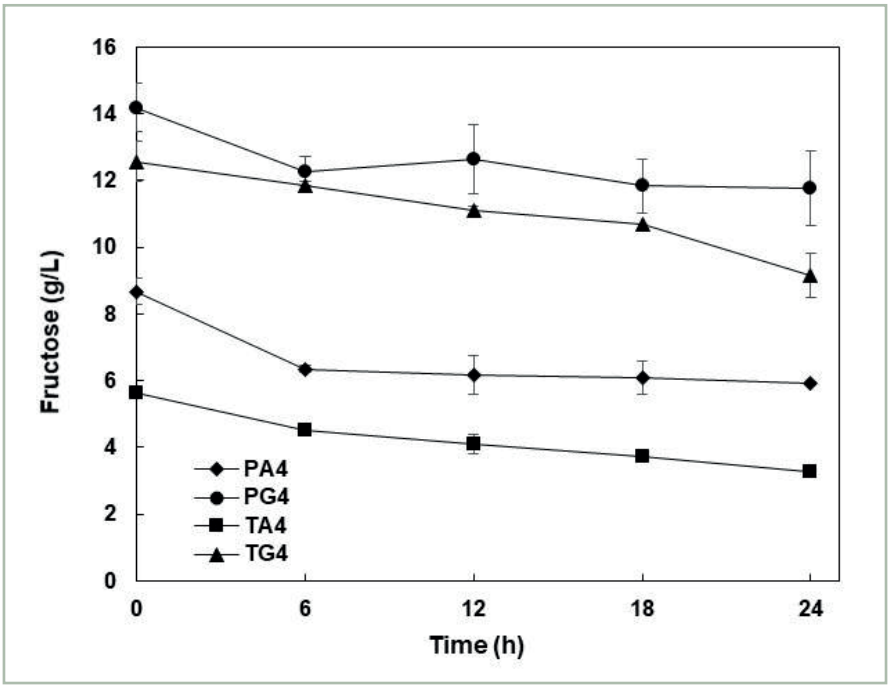
Figure 2. Glucose concentration during fermentation of acerola and guava juices (initial pH adjusted to 4).



Although the initial glucose concentration in the pasteurized guava juice was 22% higher than in the thermosonicated juice, the 24-hour concentrations were similar, with a difference of 8%. This was because glucose consumption was faster in the first six hours in the fermentation of pasteurized juice. Concerning the fermentations of acerola juices, there was higher consumption of glucose between six and 24 hours for the thermosonicated juice, which favored a difference in the final concentrations obtained in these fermentations. The final glucose concentration in the fermentation of thermosonicated acerola juice, therefore, was 30% lower than in the fermentation of the pasteurized acerola juice.

As observed for the glucose concentration, the initial fructose concentrations (Figure 3) in pasteurized juices were higher than in the thermosonicated juices. Fructose consumption was more pronounced in the first six hours in the fermentation of pasteurized juices.

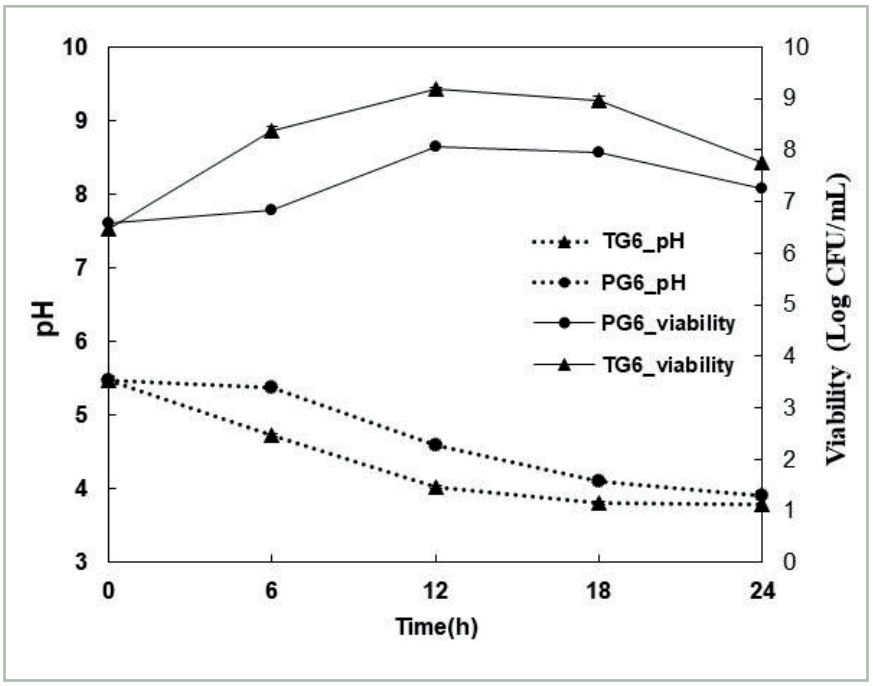
Figure 3. Fructose concentration during fermentation of acerola and guava juices (initial pH adjusted to 4)



The highest consumption of glucose and fructose was found in the fermentation of the thermosonicated acerola juice. On the other hand, in the fermentation of guava juice, the highest consumption of glucose was obtained with pasteurized juice, and the highest consumption of fructose in the fermentation of thermosonicated juice.

The kinetic study of fermentations with the initial pH adjusted to 6 was performed only with guava juices; adjustment of pH to this value in the acerola juice would have changed the color of the juice to “greenish-brown”. This pH value was chosen based on studies with *L. rhamnosus* ATCC 7469, where the production of probiotic juices was carried out at pH adjusted to close to 6 (Chang and Liew, 2013, Farias et al. 2016, Santos et al. 2017, Andrade et al. 2019). Figure 4 shows the pH and viability during the fermentation of guava juices with the initial pH adjusted to 6.

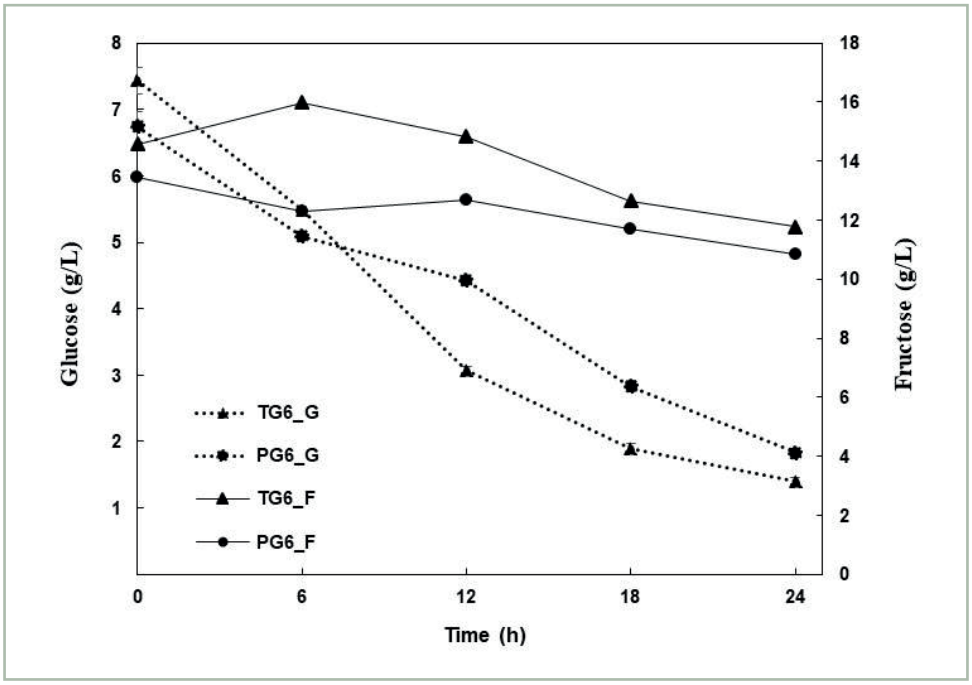
Figure 4. Viability and pH during fermentation of guava juices (initial pH adjusted to 6).



The decrease in pH accompanied the increase in viability in both fermentation conditions (TG6 pH). This reduction, however, occurred only in the first 18 hours. On the other hand, in the fermentation of pasteurized guava juice (PG6 pH), the pH reduction occurred up to 24 hours. Between 18 and 24 hours, the stationary phase could be observed. The maximum volumetric productivity (Log CFU / mL.h) of the fermentation of thermosonicated juice was 45% higher than that of pasteurized juice.

The decrease in glucose concentration was similar in both fermentations of guava juices (Figure 5). Glucose consumption was 73% in the fermentation of the thermosonicated juice and 81% in the fermentation of pasteurized juice. Fructose consumption was observed after 12 hours, in both fermentations (19 %), with values lower than those obtained for glucose.

Figure 5. Glucose and fructose concentrations during fermentation of guava juice (initial pH adjusted to 6).



Comparison of fermentations varying the initial pH of the medium of fruit juices

A higher decrease in pH was obtained in the fermentation of the samples at pH equal to 6 (Table 3). Although the pH reduction was 30% in the PG6 and TG6 fermentations, the production of lactic acid was different under these conditions. Therefore, the reduction in pH can be considered to be only an indication of acid production. Thus, it is essential to quantify lactic acid by high-performance liquid chromatography (HPLC), using a diode array detector, as was done in this work and not just by titratable acidity. When the pH reduction was equal to or less than 5%, there was no detectable lactic acid production.

Table 3. Comparison of kinetic parameters of fermentations varying the fruit, the initial pH, and the type of treatment of the pulp.

Fermentation	pH decrease (%)	Px (Log CFU/mL.h)	Pp (g/L.h)	Glucose (Consumption in %)	Fructose (Consumption in %)
PA4	4 ± 0.02	0.014 ± 0.000	Nd	40 ± 0.11	33 ± 0.31
TA4	5 ± 0.01	0.040 ± 0.012	Nd	50 ± 0.06	50 ± 0.13
PG4	5 ± 0.02	0.007 ± 0.001	Nd	50 ± 0.20	14 ± 0.83
TG4	2.6 ± 0.02	0.037 ± 0.003	Nd	25 ± 0.15	31 ± 0.30
PG6	30 ± 0.01	0.124 ± 0.010	0.1125 ± 0.0029	73 ± 0.08	19 ± 0.18
TG6	30 ± 0.02	0.227 ± 0.000	0.1792 ± 0.0054	81 ± 0.09	19 ± 0.23

PA4: Pasteurized Acerola in pH4; TA4: Thermosonicated Acerola in pH4; PG4: Pasteurized Guava in pH4; TG4: Thermosonicated Guava in pH4; PG6: Pasteurized Guava in pH6; TG6: Thermosonicated Guava in pH6; Nd: Not detected. Px: maximum volumetric productivity of cells; Pp: maximum volumetric productivity of the product.

The maximum volumetric productivities in cells (P_x) were higher in fermentations with the initial pH adjusted to 6 and almost double when the juice was thermosonicated (TG6 with PG6). Similar behavior was observed for maximum volumetric productivity in the product (P_P).

There was no production of lactic acid in fermentations with the pH adjusted to 4. The highest consumption of glucose also occurred in juice fermentation with the initial pH adjusted to 6. Fructose consumption did not follow this behavior.

According to the results of P_X and P_P, there is evidence that thermosonication treatment is associated with a higher bioavailability of bioactive compounds (Saeeduddin et al. 2015) and probably favoring the growth of *Lactobacillus rhamnosus* ATCC 7469 and the production of lactic acid.

Farias et al. (2016) obtained a 25% decrease in pH during the fermentation of passion fruit from Caatinga juice by *L. rhamnosus* ATCC 7469. The juice was pasteurized and the initial pH was adjusted to 6. The maximum volumetric productivity in cells (P_X) and in the product (P_P) was 0.08 g/L.h and 0.22 g/L.h, respectively. The consumption of total reducing sugars was almost 100% in 24 hours.

Survival of Lact*obacillus rhamnosus ATCC* 7469 in the fermented probiotic guava juices with initial pH adjusted to 6 during refrigerated storage and simulated gastrointestinal conditions

Survival was higher than 80% for both treatments, during the 28 days of refrigerated storage. Concerning thermosonicated juices, the survival was 7.5% higher than that obtained for pasteurized juices. Viability was higher or equal to 8 Log CFU/mL in both juices. Similar survival was reported by Farias et al. (2016) for the same strain and different pulp. These authors added sucrose before the refrigerated storage. However, the presence of sucrose did not influence survival.

The survival of *L. rhamnosus* ATCC 7469 in unfermented cherry juice was zero in less than 2 days when the pH was not adjusted (Nematollahi et al. 2016). On the other hand, when the pH was adjusted to 3.5, survival only reached zero between 20 and 30 days of refrigerated storage. These authors attribute this reduction to the presence of phenolic compounds when the pH was adjusted to 3.5. In the present study, despite the pH of fermented guava juice being close to 4, there was almost 80% survival with 28 days. The vegetable matrix was different, however, and the content of phenolic compounds in guava juice is low, favoring greater survival, as was also observed in the study by Andrade et al. (2019).

Probiotic survival in the simulation of gastrointestinal conditions during the refrigerated storage of 28 days was around 46% for pasteurized guava juice at zero time; and it was zero at 28 days. For thermosonicated juice, the survival was zero regardless of the time. Farias et al (2016) found survival of *L. rhamnosus* ATCC 7469 only when using pH 2.7, using passion fruit from Caatinga juice with the addition of sucrose. On the other hand, Andrade et al. (2019) found gastrointestinal survival of 40 and 30%, at the beginning and at 28 days of storage, respectively, using pasteurized and fermented guava juice, with the addition

of inulin and stevia. It is worth mentioning that in the present work, the juices used did not contain additives.

DISCUSSÃO

According to Cervantes-Elizarrarás et al. (2017), thermosonication was effective in the microbiological inactivation of mulberry juice. The values found showed total inactivation for yeasts, lactic acid bacteria (BAL) and Enterobacteriaceae when compared with control and thermosonicated samples. Similar results were observed in apple, pineapple and grape juices (Abid et al. 2014; Tiwari et al. 2008; Tiwari et al. 2009).

Inactivation of microorganisms responsible for foodborne disease is the main objective of food processing. Inactivation of microorganisms was observed when thermosonication was performed at 65 °C for 10 minutes (20 kHz ultrasonic processor) compared to the pasteurized sample at 95 °C for 2 minutes (Saeeduddin et al. 2015). Similar results were found in pineapple, red blueberry and mandarin juices (Bermúdez-Aguirre et al. 2012).

A study on the effect of pathogenic bacteria on soursop nectar showed that there was a reduction in the microbiological viability of the analyzed samples after thermosonication when compared to the untreated sample. It was observed that one condition (test number 9), showed lethality reduction greater than the 5 logs, FDA (Food and Drug Administration) stipulated value (Anaya-Esparza et al. 2017). Orange juice sonicated at mild temperature and different times (1, 10, 20 and 30 minutes) showed a significant reduction of the microbiological load. It was observed that the reduction increased with increasing temperature and that the samples treated for 30 minutes showed total decontamination. The observed effects are attributed to the combined physical and chemical mechanisms that occur during cavitation (Guerrouj et al. 2016).

D'Amico et al. (2006) reported on the use of ultrasound with or without moderate heat (thermosonication). They noted that the reduction of cell viability of pathogenic bacteria in milk and apple cider occurred under both conditions. However, effective reduction and in accordance with the requirements of current food law was achieved by the use of thermosonication. In the case of apple cider, the inactivation was observed for *E. coli* O157: H7, with a reduction of 5 logs. This study, thermosonicated acerola and guava juices presented a reduction in microbiological inactivation of the initial microbial load from uncountable to 1.70 and 1.60 logs (acerola and guava, respectively).

A thermosonicated carrot juice study showed a value of 1.85 ± 0.02 for total counts of bacteria in log CFU/mL; under conditions of time and temperature processing between 5 to 6.5 minutes and 52 to 60 °C, respectively (Adiamo et al. 2018). The results of treatment

11 (IP 60% and T of 65 °C) for Inat of both juices, acerola, and guava heat-treated presented similar values.

In the study by Adiamo et al. (2018), an ultrasound bath was used, with variations of time and temperature. In our study, an ultrasound probe was used with temperature and power variations. The results observed for microbiological inactivation in both studies, however, suggested that the efficacy of this inactivation by thermosonication is similar. This fact supports the undeniable action of acoustic cavitation, among other effects of this processing.

The phenolic compounds comprise a group of natural antioxidants responsible for beneficial effects on human health, being a primary factor in the fight against free radicals that promote chronic-degenerative diseases such as cancer, cardiovascular, metabolic syndrome, among others (Dias et al. 2015; Aadil et al. 2013).

Santhirasegaram et al. (2013) observed an increase between 30 and 35% in sonicated mango juice when compared to a control sample. This increase can be attributed to the reaction between the aromatic ring of the polyphenols and the free radicals generated in the sonication, which contributes to the increase of the antioxidant capacity (Ashokkumar et al. 2008). Sonication removes oxygen dissolved in the juice, contributing to greater bioavailability of the phenolic compounds (Masuzawa et al. 2000). This may be associated with the formation of free radicals, which may have affected the total phenolic compounds of the juice, since -OH radicals formed during the application of ultrasound may affect bioactive compounds such as phenolics (Wan et al. 2005).

The behavior of the bioactive compounds, when submitted to different processes, is related to the food matrix (fruits and their products) (Campoli et al. 2018), synergic effect among independent variables, acoustic cavitation effects with oxygen elimination, the release of compounds from the intracellular environment and formation of free radicals. These changes (increase or decrease) in TPC depend on the composition of the different types of phenolic acids present in the juice and on the occurrence of isomerization reactions (Saikia et al. 2015; Anaya-Esparza et al. 2017).

The antioxidant activity of plants and their products is related to the content of antioxidant compounds they present, which have different mechanisms of action with synergistic interactions (Perez-Jimenez et al. 2008). Thermosonicated purple cactus juice presented higher antioxidant capacity by the sequestration of the DPPH free radical when compared to control and pasteurized (Cruz-Cansino et al. 2015). Alteration of antioxidant activity in fruit juices have been reported with retention of 90 to 100% (Abid et al. 2014). The stability of AC in sonicated juices may be associated with the elimination of the dissolved oxygen present in the medium during cavitation (Anaya-Esparza et al. 2017; Cruz-Cansino et al. 2015).

Wang et al. (2019a) found more activity in the bioactive compounds of sonicated strawberry juice samples than in the control sample. Observations by scanning electron microscopy (SEM) have demonstrated that this behavior is associated with changes that acoustic cavitation causes in cells, such as damage to the cell wall causing ruptures that release intracellular content, also giving rise to microchannels in the plant cell structure. Alterations in the microstructure of the food matrix and bioavailability of the bioactive compounds can be directly related.

Changes in bioactive compounds of different plant matrices present different responses after thermosonication processing. Acerola juice is a source of ascorbic acid, a water-soluble bioactive compound; while guava juice is rich in carotenoids, a liposoluble bioactive compound. This may be the reason for the different responses observed in this study. A study carried out on thermosonication of tangerine and grapefruit juices suggested synergism in their action mechanisms, such as free radical formation, oxygen elimination, and intracellular membrane rupture (Aguilar et al. 2017, Khandpur et al. 2015).

Ascorbic acid is used as an indicator of nutritional value and as an index for estimating quality deterioration in processed products (Lima et al. 2010). It is a compound of potent antioxidant activity, involved in the prevention of chronic-degenerative diseases (Dias et al. 2015). The process of aerobic oxidation of ascorbic acid is a reaction whose mechanism of action involves factors such as temperature and presence of oxygen. In fruit juices, the presence of oxygen along the processing chain contributes to the degradation of the ascorbic acid content of the same. The use of sonication in fruit juices favors the release of the oxygen present in these juices and contributes to the preservation of the nutritional quality of these juices. The deaeration of fruit juices is shown as an important procedure in their processing step to prevent or minimize the degradation of ascorbic acid (Abid et al. 2014, Aguilar et al. 2017).

Carotenoids are pigments present in plants and play important roles in human metabolisms such as immunology, regulation of the cellular mechanism and as antioxidants (Santhirasegaram et al. 2013). During food processing, the generation of free radicals is a fact. These radicals react with lipids, proteins, and sugars. The kinetics of the reaction is determined by the velocity of the process and can present different rates for different reaction mechanisms, involving competition between hydroxyl radicals, and oxydryl and its substrates. Moreover, when oxidation involves temperature increase, the free radicals produced tend to react primarily with the solvents from the medium rather than the lipids (Pingret et al. 2013).

Carotenoids are liposoluble compounds. In this study, a discrete increase of carotenoids in the acerola juice was noted, with a carotenoid content of 3.30 µg/g for the control sample. The thermosonicated guava juice, with a carotenoid content of 33.38 µg/g (control sample), presented a significant decrease in carotenoid content. Thus, the behavior observed in the

sonication treatments of the two juices suggests that there were divergent mechanisms at work and the possible contribution of several factors, such as temperature, the presence of oxygen, and different nutritional composition of each processed food matrix. Lee et al. (2005) observed that the carotenoid content in orange juice was reduced by around 10 % after mano-thermosonication (use of ultrasound associated with the use of heat and pressure). However, in a stock study, the sonicated samples presented superior stability in carotenoid content when compared to the control samples which presented a rapid degradation of the compound.

In this study, the contribution of the carotenoid content of the thermosonicated acerola suggested that carotenoids contribute synergistically to the activity of bioactive compounds, since vegetables have been identified as sources of this compound, for example buriti (*Mauritia vinifera*) and carrots (*Daucus carota*), which present approximately 360 µg/g and 20 µg/g β-carotene, respectively (Rodriguez-Amaya et al. 2008). Carotenoid content was observed to be similar to that observed in acerola juice, with non-significant differences between the thermosonication and pasteurization treatments (Adiamo et al. 2018).

Changes in the color parameters of the thermosonicated fruit juices suggest activity of pigment release mechanisms located inside the cells (Aadil et al. 2013; Bhat et al. 2011), associated with the conditions used in the thermosonication, such as time, temperature, food matrix and the different reactions that occurred in the treatment (Anaya-Esparza et al. 2017). The study of color variation in foods is important because it affects consumers' acceptability and willingness to buy, being a visual indicator of judgment (Dias et al. 2015). Conventional heat treatments such as pasteurization cause darkening of the juice, while thermosonication improves brightness (Abdullah and Chin 2014), as observed in our study. Thermosonicated apple and guava juices presented TCD considered to be perceptible and related to the fact that cavitation caused the release of pigments such as carotenoids (Saad et al. 2013).

Farias et al. (2016) observed the growth of *Lactobacillus rhamnosus* ATCC 7469 in fermented juice of passion fruit from Caatinga (*Passiflora cincinnata* Mast.). There was a reduction in pH from 6 to 4.5 and growth of 2 logs, reaching a maximum value of 10 Log CFU/mL. The production of lactic acid, in this case, was almost 6 g/L. Fonteles et al. (2013) found cell viability around 9 Log CFU/mL, final pH 3.9, and lactic acid concentration 1.23 g/L in the fermentation of sonicated melon juice using *Lactobacillus casei* NRRL B-442. According to Khorasani and Shojaosadati (2017), resistance to low pH values is one of the main parameters for the probiotic food industry. These results indicate that *Lactobacillus rhamnosus* ATCC 7469 adapts to an acidic medium. Andrade et al. (2019) observed survival of *L. rhamnosus* ATCC 7469 at around 100% with 28 days of refrigerated storage using pasteurized and fermented guava juice, with the addition of inulin and stevia.

CONCLUSÃO

The microbiological inactivation, bioactive compounds and color evaluated in all the trials showed that test 11 (60% - 65 °C) was considered the most promising for both juices. The fruit juices obtained had a nutritional value closer to fresh juice when compared to those treated by pasteurization. Acerola and guava juices showed behaviors that demonstrated a direct relation with the food matrix and its intrinsic characteristics. The study demonstrated that thermosonication is an alternative and a potentially successful technique for the processing of acerola and guava juices of nutritional value, superior to the pasteurized samples of both juices. We can conclude that thermosonication is an alternative to pasteurization: when the initial pH was 4 there was a higher increase in viability than that observed for pasteurized juices. The production of fermented probiotic acerola juices was not possible with the initial pH adjusted to 6. This indicates that there is a limit to pH adjustment for fermentation of acidic fruit juices, such as acerola. The refrigerated storage of probiotic fermented guava juices showed stability regardless of the treatment used. Thermosonication was as effective as pasteurization, with the advantage of keeping bioactive compounds more stable and preserved, in addition to causing microbial inactivation.

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REFERÊNCIAS

1. Aadil, R.M., Zeng, X.A., Han, Z., Sun, D.W. (2013). Effect of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*, 141, 3201-3206.

2. Abdullah, N., Chin, N.L. (2014). Application of Thermosonication Treatment in Processing and Production of High Quality and Safe-to-Drink Fruit Juices. *Agriculture and Agricultural Science Procedia*, 2, 320 – 327.

3. Abid, M., Jabbar, S., Hua, B., Hashim, M.M., Wua, T., Lei, S., Khan, M.A., Zeng, X. (2014). Thermosonication as a potential quality enhancement technique of apple juice. *Ultrasonic Sonochemistry*, 21, 984–990.

4. Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Hu, B., Lei, S., Zhang, X., Zeng, X. (2013). Effect of ultrasound on different quality parameters of apple juice. *Ultrasonics Sonochemistry*, 20, 1182-1187.

5. Adiamo, O.Q., Ghafoor, K., Al-Juhaimi, F., Babiker, E.E., Ahmed, I.A.M. (2018). Thermosonication process for optimal functional properties in carrot juice containing orange peel and pulp extracts. *Food Chenistry*, 245, 79-88.

6. Aguilar, K., Garvín, A., Ibarz, A., Augusto. P.E.D. (2017). Ascorbic acid stability in fruit juices during thermosonication. *Ultrasonics Sonochemistry***,** 37. 375–381.

7. Anaya-Esparza, L.M., Méndez-Robles, M.D., Sayago-Ayerdi, S.G., García-Magaña, M.L., Ramírez-Mares, M.V., Sánchez-Burgos, J.A., Montalvo-González, E. (2017). Effect of thermosonication on pathogenic bacteria, quality attributes and stability of soursop nectar during cold storage. *Cyta – Journal of* food, 15(4), 592-600.

8. Andrade, R., Santos, E., Azoubel, P., Ribeiro, E. (2019). Increased survival of Lactobacillus rhamnosus ATCC 7469 in guava juices with simulated gastrointestinal conditions during refrigerated storage. *Food Bioscience*, 32, 1-8,

9. Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilkhu, K., Versteeg, C. (2008). Modification of food ingredients by ultrasound to improve functionality: a preliminary study on a model system. *Innovation in Food Science and Emerg. Technology***,** 9, 155-160.

10. Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2012). Inactivation of Saccharomyces cerevisiae in pineapple, grape and cranberry juices under pulsed and continuous thermo-sonication treatments**.** *Journal of Food Engineering*, 108, 383-392.

11. Bhat, R., Kamaruddin, N.S.B.C., Min-Tze, L., Karim, A.A. (2011). Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrasonics Sonochemistry*, 18, 1295–1300.

12. Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use od free radical method to evaluate antioxidant activity. *Food Science and Technology*, 289(1), 25-30.

13. Campoli, S.S.; Rojas, M.L., Amaral, J.E.P.G., Canniatti-Brazaca, S., Augusto, P.E.D. (2018). Ultrasound processing of guava juice: Effect on structure, physical properties and lycopene in vitro accessibility. *Food Chemistry*, 268, 594-601.

14. Cervantes-Elizarrarás. A., Piloni-Martini. J., Ramírez-Moreno. E., Alanís-García. E., Güemes--Vera, N., Zafra-Rojas. Q.Y., Cruz-Cansino, N.S. (2017). Enzymatic inactivation and antioxidant properties of lackberry juice after thermoultrasound: Optimization using response surface methodology. *Ultrasonics Sonochemistry*, 34, 371–379.

15. Chang, C.P., Liew, S.L. Growth medium optimization for biomass production of a probiotic bacterium, *Lactobacillus rhamnosus* ATCC 7469. (2013). *Journal of Food Biochemistry*, 37, 536-543, 2013.

16. Cruz-Cansino, N.S., Ramirez-Moreno, E., Leon-Rivera, J.E., Delgado-Olivares, L., Alanis-Garcia, E., Ariza-Ortega, J.A., Manriquez-Torres, J.J., Jaramillo-Bustos, D.P. (2015). Shelf life, physicochemical, microbiological and antioxidant properties of purple cactus pear (*Opuntia ficus indica*) juice after thermoultrasound treatment. *Ultrasonics Sonochemistry***,** 27, 277–286.

17. D'Amico, D.J., Silk, T.M., Wu, J., Guo, M. (2006). Inactivation of Microorganisms in Milk and Apple Cider Treated with Ultrasound. *Journal of Food Protection*, 69(3), 556–563.

18. Dias, D.R.C., Barros, Z.M.P., Carvalho, C.B.O., Honorato, F.A., Guerra, N.B., Azoubel, P.M. (2015). Effect of sonication on soursop juice quality. *LWT - Food Science and Technology*, 62(1), 883-889.

19. Elvira, L., Durán, C.M., Urréjola, J., Espinosa, F.R.M. (2014). Detection of microbial contamination in fruit juices using noninvasive. *Ultrasound Food Control***,** 40, 145-150.

20. Embrapa – Empresa Brasileira de Pesquisa Agropecuária. (2020). *Science to transform life Agricultural research results and positive impacts on the economy, the environment and on consumers' tables.* Available in: https://www.embrapa.br/en/grandes-contribuicoes-para-a-agricultura-brasileira/frutas-e-hortalicas. Access in 09 June 2020.

21. Farias, N., Soares, M., Gouveia, E. (2016). Enhancement of the viability of Lactobacillus rhamnosus ATCC 7469 in passion fruit juice: Application of a central composite rotatable design. *LWT - Food Science and Technology*, 71, 149-154.

22. Fellows, P.J. (2009). *Food processing technology: principles and practice*, Third Edition. Boca Raton: CRC Press, 895pp.

23. Fonteles, T.V., Costa, M.G.M., de Jesus, A.L.T., Fontes, C.P.M.L., Fernandes, F.A.N., Rodrigues, S. (2013). Stability and Quality Parameters of Probiotic Cantaloupe. Melon Juice Produced with Sonicated Juice. *Food and Bioprocess Technology,* 6, 2860–2869. DOI 10.1007/s11947-012-0962-y.

24. Guerrouj, K., Sánchez-Rubio, M., Taboada-Rodríguez, A., Cava-Roda, R.M., Marín-Iniesta, F. (2016). Sonication at mild temperatures enhancesbioactive compounds and microbiological qualityof orange juice. *Food and Bioproducts Processing*, 99, 20-28.

25. IBGE – Instituto Brasileiro de Geografia e Estatística. (2019). *Tabela 1613 - Área destinada à colheita, área colhida, quantidade produzida, rendimento médio e valor da produção das lavouras permanentes (goiaba).* Available in: PAM – SIDRA: https://sidra.ibge.gov.br/tabela/1613#resultado. Access in 18 March 2019.

26. Khandpur, P., & Gogate, P.R. (2015). Effect of novel ultrasound based processing on the nutrition quality of different fruit and vegetables juices. *Ultrasonic Sonochemistry*, 27, 125–136.

27. Khorasani, A.C., Shojaosadati, S.A. (2017). Improvement of Probiotic Survival in Fruit juice and under gastrointestinal conditions using Pectin-Nanochitin-Nanolignocellulose as a Novel Prebiotic Gastrointestinal-Resistant Matrix. *Applied food biotechnology***,** 4(3), 179-191.

28. Lee, J. J., Crosby, K. M., Pike, L. M., Yoo, K. S., Leskovar, D. I. (2005). Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (*Capsicum* spp.). Scientia Horticulturae, 106(3), 341-352.

29. Lima, J.R., Elizondo, N.J., Bohuon. P.P. (2010). Kinetics of ascorbic acid degradation and colour change in ground cashew apples treated at high temperatures (100 – 180 °C). *International Journal of Food Science Technology*, 45, 1724–1731.

30. Madigan, M.T., Martinko, J.M., Dunlap, P.V., Clark, D.P. (2010). *Microbiologia de Brock*. 12ª. Edição. Porto Alegre: Artmed, 1160p.

31. Masuzawa, N., Ohdaira, E., Ide, M. (2000). Effects of ultrasonic irradiation on phenolic compounds in wine, *Japanese Journal of Applied Physics*, 39, 2978–2979.

32. Nematollahi, A., Sohrabvandi, S., Mortazavian, A.M., Jazaeri, S. (2016). Viability of probiotic bactéria and some chemicalsensory characteristics in cornelian cherry juice during cold storage. *Electronic Journal of Biotechnology*, 21, 49-53.

33. Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M.E., Serrano, J., Goñi, I., Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, 41, 274–285.

34. Pingret, D., Fabiano-Tixier, A., Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, 31, 593-606.

35. Rodrigues-Amaya, D. *A guide to carotenoids analysis in food*. Washington: Internacional Life Sciences Institute Press, 1999. 64p.

36. Rodrigues-Amaya, D.B., Kimura, M., Amaya-Farfan, J. *Fontes brasileiras de carotenoides: tabela brasileira de composição de carotenoides em alimentos*. Brasilia, MMA/SBF, 2008, 100p.

37. Roobab, U., Aadil, R.M., Madni, G.M., Bekhit, A.E. (2018). The Impact of Nonthermal Technologies on the Microbiological Quality of Juices: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 17, 437-457.

38. Saad, S.M., Elaleem, I.M.A., Foda, F.F.A., Eissa, H.A., Abdelmoniem, G.M., Ibrahim, W.A. (2013). Effects of thermosonication on apple and guava juices quality. *Journal of Applied Sciences Research*, 9(8), 5323-5336.

39. Saeeduddin, M., Abid, M., Jabbar, A., Wu, T., Hashim, M.M., Awad, F.N., Hu, B., Lei, S., Zeng, X. (2015). Quality assessment of pear juice under ultrasound and comercial pasteurization processing conditions. *LWT Food Science and Technology*, 64, 452-458.

40. Saikia, S., Mahnot, N.K., Mahanta, C.L. (2015). A comparative study on the effect of conventional thermal pasteurisation, microwave and ultrasound treatments on the antioxidant activity of five fruit juices. *Food Science and Technology International*, 22(4), 288–301.

41. Santhirasegaram, V., Razali, Z., Somasundram, C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonics Sonochemistry*, 20, 1276–1282.

42. Santos, E., Andrade, R., Gouveia, E. (2017). Utilization of the pectin and pulp of the passion fruit from Caatinga as probiotic food carriers. *Food Bioscience*, 20, 56-61.

43. Singleton, V.L., Orthofer, R., Lamuela, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymoly*, 299, 52-178.

44. Strohecker, R., & Henning, H. *Analisis de vitaminas: métodos comprobados*. Madrid: Paz Montalvo, 1967, 28p.

45. Tiwari, B.K., O´ Donnell, C.P., Cullen, P. J. (2009). Effect of non-thermal processing technologies on the anthocyanin content of fruit juices. *Trends Food Science Technology*, 20, 137–145.

46. Tiwari, B.K., O´ Donnell, C.P., Muthukumarappan, K., Cullen, P. J. (2008). Effect of ultrasound processing on quality of fruit juices. *Stewart-Post Harvest Review*, 4(5), 1-6.

47. Wan, J., Mawson, R., Ashokkumar, M., Ronacher, K., Coventry, M.J., Roginski, H. (2005). Emerging processing technologies for functional foods. *Australian Journal of Dairy Technology*, 60, 167–169.

48. Wang, J., Ye, J., Vanga, S.K., Raghavan, V. (2019a). Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food control*, 96, 128-136.

49. White, J., & Hekmat, S. (2018). Development of Probiotic Fruit Juices Using Lactobacillus rhamnosus GR-1 Fortified with Short Chain and Long Chain Inulin Fiber. *Fermentation*, 4(27), 1-12.

50. Zafra-Rojas, Q.Y., Cruz-Cansino, N., Ramírez-Moreno, E., Delgado-Olivares, L., Villanueva--Sánchez, J., Alanís-García, E. (2013). Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrasonics Sonochemistry*, 20, 1283–1288.