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## Effect of thermosonication on pathogenic bacteria, quality attributes and stability of soursop nectar during cold storage

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### ABSTRACT

In this work, the effect of the thermosonication (TS) on the inactivation of *Escherichia coli* and *Staphylococcus aureus* in soursop nectar was evaluated, as well as the quality and stability of the nectar during its storage at 4°C. A reduction of 4.5–5 log CFU/mL was achieved with the TS treatment (24 kHz, average temperature of 50–51°C, acoustic energy density of 1.3–1.4 W/mL for 10 min) in both pathogens. Likewise, thermosonicated nectar retained 85% of ascorbic acid, polyphenol oxidase inactivation and microbiological and physicochemical stability during 30 days at 4°C. Sensorially, the thermosonicated nectar was predominantly preferred over a commercial one. TS is a viable alternative to extend the shelf life of minimally processed soursop nectar.

### Efecto de la termosonicación sobre bacterias patógenas, atributos de calidad y estabilidad de néctar de guanábana durante su almacenamiento en refrigeración

#### RESUMEN

En este trabajo se evaluó el efecto de la termosonicación sobre la inactivación de *Escherichia coli* y *Staphylococcus aureus* en néctar de guanábana; así como la calidad y estabilidad del néctar durante su almacenamiento a 4°C. Se logró una reducción de 4,5–5,0 log CFU/mL con el tratamiento de termosonicación (24 kHz, temperatura promedio de 50–51°C, densidad de energía acústica de 1,3–1,4 W/mL, durante 10 min) en ambos patógenos. Del mismo modo, el néctar termosonicado conservó el 85% de ácido ascórbico, la inactivación de polifenol oxidasa y la estabilidad microbiológica y fisicoquímica durante 30 días a 4°C. Sensorialmente, el néctar termosonicado fue predominantemente preferido sobre un néctar comercial. La termosonicación es una alternativa viable para extender la vida útil del néctar de guanábana mínimamente procesado.

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Thermosonication; soursop nectar; *Escherichia coli*; *Staphylococcus aureus*; quality; stability

### PALABRAS CLAVE

termosonicación; néctar de guanábana; *Escherichia coli*; *Staphylococcus aureus*; calidad; estabilidad

## 1. Introduction

Thermosonication (TS) is a technology that combining cavitation (ultrasound) with heat produces an additive effect that greatly increases bacterial and enzymatic inactivation compared to thermal pasteurization (Cruz, Vieira, Fonseca, & Silva, 2011). According to the FDA (2004), any technology that is applied for the preservation of juices and nectars must be able to reduce at least 5 logs (vegetative cells) of the target microorganism.

Some of the microorganisms studied when applying TS in fruit juices include *Escherichia coli* O157H:7 (Muñoz et al., 2012), *Salmonella* Enteritidis (Kiang, Bhat, Rosma, & Cheng, 2013) and *Staphylococcus aureus* (Walkling-Ribeiro et al., 2009) among others. Most of the authors reported that they complied with the FDA stipulation, attributing this behavior to the intracellular cavitation, which produces disruption of the structure and function of the cell wall. Additionally, it has been reported that TS applied to grape juice (Aadil et al., 2015), purple cactus pear juice (Cruz-Cansino et al., 2015) and carrot juice (Jabbar et al., 2015; Martínez-Flores, Garnica-Romo, & Bermúdez-Aguirre, 2015)

allowed to maintain high levels of bioactive compounds. In our laboratory, we have reported that TS treatment of soursop nectar at 54°C decreased the polyphenol oxidase (PPO) activity among 91% and 99% (Anaya-Esparza et al., 2017b).

Although it has been shown that TS at the time of application is effective for the treatment of fruit-based juices and nectars, there are few studies on the conservation of their quality during storage. Therefore, the objective of this study was to evaluate the lethal effect of TS on two microorganisms considered as pathogenic bacteria by surface-response methodology, as well as the quality and stability of the thermosonicated soursop nectar during storage in refrigeration.

## 2. Materials and methods

The development of the research was carried out in two stages. The initial stage was conducted to evaluate the lethal effect of TS in different conditions on *E. coli* ATCC 8739 and *S. aureus* ATCC 33862, while the final stage consisted of monitoring the quality and stability of the thermosonicated

nectar during storage at 4°C. Bacterial strains were purchased from Microbiologics® (Minnesota, USA) and rehydrated according to the instructions of the manufacturer.

### 2.1. Samples and treatments

Nectars were prepared according to the requirements of the *Codex Alimentarius* for fruit juices and nectars (CODEX STAN 247-2005). The pulp was diluted with purified water (350 g/L); subsequently, the mixture was homogenized and adjusted with sucrose (50 g/L). Nectar samples were treated by discontinuous TS (batch process) as schematized (Figure 1) (Anaya-Esparza et al., 2017a), using an ultrasound (Hielsher UP400S, Teltow, Germany) at 400 W, with constant frequency of  $24 \pm 1$  kHz, coupled with a shaking water bath (Thermo Scientific 2870, Ohio, USA) to maintain a constant temperature. A fractionated factorial design  $3^{3-1}$  was used, considering as factors at different levels: acoustic energy density (AED) (1.1, 1.2 and 1.4 W/mL), average temperature between initial and final temperature (34, 44 and 54°C) and time (2, 6 and 10 min) to obtain a total of nine treatments (Table 1). For each treatment, 200 mL of soursop nectar was placed in a 250-mL beaker.

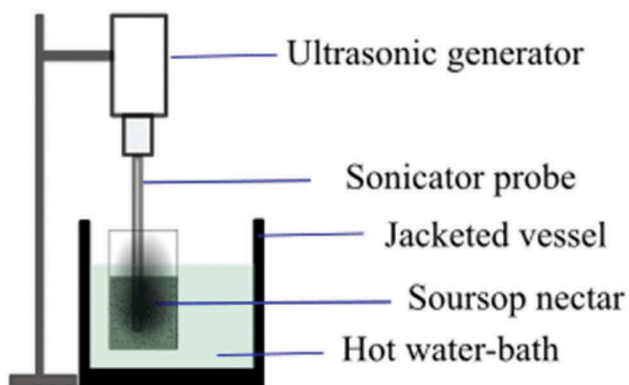


Figure 1. Schematic diagram of thermosonication treatment to laboratory scale used in this study.

Figura 1. Diagrama esquemático del tratamiento de termosonicación a escala de laboratorio usado en este estudio.

### 2.2. Lethal and sublethal damage of TS on *E. coli* and *S. aureus*

Cell suspension and inoculation of *E. coli* and *S. aureus* in the nectars were performed, as recommended by Briñez, Roig-Sagués, Hernández and Guamis (2006). Previously, cryobeads were prepared inoculating strain culture separately (*E. coli* or *S. aureus*) in tryptone soy broth (DIBICO Mexico City, Mexico) and stored at  $-20^{\circ}\text{C}$  to provide stock cultures for the assays. Before each experiment, one cryobead (2 mL) was inoculated into 10 mL of tryptone soy broth and incubated at  $37^{\circ}\text{C}$  for  $24 \pm 2$  h. After incubation, the broth was spread using a disposable loop in a Petri dish with tryptone soy agar (TSA) (DIBICO Mexico City, Mexico) incubated at  $37^{\circ}\text{C}$  for  $24 \pm 2$  h. Subsequently, cell suspension was prepared adding enough inoculum of bacteria (*E. coli* or *S. aureus*) in 11 mL of sterile peptone water (0.1%). The final concentration ( $9.0 \pm 0.1$  log CFU/mL) of cell suspension was determined using a spectrophotometer (Jenway 6705 spectrophotometer, Felsted, UK) when absorbance was  $2.0 \pm 0.5$  at 405 nm. The nectar was inoculated with cell suspension (10 mL/L) and homogenized. Lethal and sublethal injury in bacterial strains were assessed by serial dilution using pour plate methods. Ten milliliters of each treatment were placed into 90 mL of sterile peptone water and homogenized. Serial dilutions (up to  $10^{-7}$ ) were made in sterile peptone water (9 mL) with samples (1 mL) taken before TS treatment; then, 1 mL of diluted aliquots was plated in TSA by pour-plated method and incubated at  $37^{\circ}\text{C}$  for 24 h. Procedure described above comes next TS and was repeated for each treatment (TS1–TS9). Results were expressed as log CFU/mL. Lethality was calculated as the difference between the logarithms of colony counts in TSA before (approximately  $7.1 \pm 0.2$  log CFU/mL) and after TS treatment (log  $N_0$ –log  $N$ ). Also, to detect the bacterial cell injury, dilutions of TS samples were pour-plated in TSA added with 2% sodium chloride and incubated at  $37^{\circ}\text{C}$  for 24 h (García, Gómez, Condón, Raso, & Pagán, 2003; Maldonado, Aguilar, Carvajal, González, & Klotz, 2011). Sublethal damage was calculated per the difference obtained from the counts between the cultures in TSA (before treatment) and TSA + NaCl for each treatment (TS1–TS9) and expressed as percentage according to the following equation (García et al., 2005):

Table 1. Experimental matrix and treatments (TS1–TS9) and lethal and sublethal effects of thermosonication on *Escherichia coli* and *Staphylococcus aureus* inoculated in soursop nectar.

Tabla 1. Matriz experimental y tratamientos (TS1–TS9) y efectos letal y subletal de la termosonicación sobre *Escherichia coli* y *Staphylococcus aureus* inoculadas en néctar de guanábana.

Treatments	Experimental conditions	Lethality <i>E. coli</i> (log CFU/mL)	Sublethal damage <i>E. coli</i> (%)	Lethality <i>S. aureus</i> (log CFU/mL)	Sublethal damage <i>S. aureus</i> (%)
TS1	1.1 W/mL, 2 min, 34°C	$3.44 \pm 0.36^a$	99.99	$3.14 \pm 0.11^a$	99.98
TS2	1.4 W/mL, 6 min, 34°C	$3.58 \pm 0.48^a$	99.99	$3.31 \pm 0.21^a$	99.99
TS3	1.2 W/mL, 10 min, 34°C	$3.89 \pm 0.26^a$	99.99	$3.43 \pm 0.23^{ab}$	99.99
TS4	1.4 W/mL, 2 min, 44°C	$3.79 \pm 0.07^a$	99.99	$3.31 \pm 0.10^a$	99.99
TS5	1.2 W/mL, 6 min, 44°C	$3.90 \pm 0.27^a$	99.99	$3.50 \pm 0.01^{ab}$	99.99
TS6	1.4 W/mL, 10 min, 44°C	$4.02 \pm 0.09^a$	100	$4.03 \pm 0.18^c$	100
TS7	1.2 W/mL, 2 min, 54°C	$3.68 \pm 0.21^a$	99.99	$3.78 \pm 0.20^{bc}$	100
TS8	1.4 W/mL; 6 min, 54°C	$4.81 \pm 0.15^b$	100	$4.50 \pm 0.11^d$	100
TS9	1.4 W/mL; 10 min, 54°C	$5.16 \pm 0.03^b$	100	$5.18 \pm 0.05^e$	100

Values are the average  $\pm$  standard deviation ( $n = 6$ ). Different letters in each column indicate significant statistical differences between treatments ( $\alpha = 0.05$ ). Los valores son la media  $\pm$  desviación estándar ( $n = 6$ ). Distintas letras en cada columna indican diferencias estadísticamente significativas entre tratamientos ( $\alpha = 0,05$ ).

$$\text{Sublethal injury (\%)} = \frac{(\log \text{CFU/mL in TSA}) - (\log \text{CFU/mL in TSA} + 2\% \text{ NaCl})}{\log \text{CFU/mL in TSA}} \times 100. \quad (1)$$

Procedure described above was followed with *E. coli* and *S. aureus* one by one.

### 2.3. Evaluation of quality and stability of soursoop nectar during storage in refrigeration

From the data of the first stage (analyzed with response surface methodology [RSM]) and after obtaining the best experimental conditions of TS ( $24 \pm 1$  kHz, 1.4 W/mL, 51°C and 10 min), the nectar was treated again under these TS conditions (TSN). A control-unpasteurized nectar (UPN) was used. The TSN nectars after being treated were cooled to  $30 \pm 2^\circ\text{C}$  in a cold-water bath and, subsequently, nectars were stored at 4°C prior to analyses. Samples were analyzed at 0, 5, 15, 30 and 45 days, considering the beginning of the preparation as day 0.

#### 2.3.1. Microbiological stability

Microbial counting for aerobic mesophilic bacteria (AMB), yeast and molds was performed according to the Bacteriological Analytical Manual (FDA, 2001a; 2001b; respectively). One milliliter of the homogenized sample was 10-fold serially diluted using sterile peptone water, and 0.1 mL of the appropriate dilutions was pour-plated into appropriate selective media. Samples (1 mL) were taken immediately after processing and during shelf-life analysis of nectars. AMB count was determined using plate count agar, cultures were incubated at 35°C for 48 h, for yeast and molds, potato dextrose agar was used; dishes were incubated at 25°C for 5 days. Samples were also pour-plate in violet red bile glucose for Coliform bacteria count and incubated at 35°C for 24 h (Cruz et al., 2007; FDA, 2001c). Results were expressed as log colony-forming units per milliliter of nectar (log CFU/mL). Culture media were prepared according to the instructions of the manufacturer (DIBICO Mexico City, Mexico).

#### 2.3.2. PPO activity and residual enzymatic activity

Enzymatic extraction of PPO was done according to the method described by Bora, Holschuh and da Silva (2004). Results were expressed as specific activity of PPO (SAPPO, U/mg protein). Total protein concentration (mg) was determined by the Bradford method (Bradford, 1976). Residual enzymatic activity of PPO (PPO<sub>RA</sub>) was calculated and expressed as percentage (Fonteles et al., 2012).

#### 2.3.3. pH, titratable acidity, total soluble solids, nonenzymatic browning index and color

The pH (Method 981.12), titratable acidity (TA) (942.15) and total soluble solids (TSS) (932.12) were determined according to the methods of AOAC (2005). The nonenzymatic browning index (NEBI) was measured spectrophotometrically at 420 nm following the methodology proposed by Meydav, Saguy and Kopelman (1977). The color was quantified using a colorimeter (Konica Minolta, CR300, Osaka, Japan).

#### 2.3.4. Ascorbic acid

The ascorbic acid (AA) content was measured spectrophotometrically following the method of Rahman, Mizanur and Khan (2007). Results were expressed as gram of AA per liter of nectar (g AA/L).

#### 2.3.5. Total soluble polyphenols

For the aqueous–organic extraction, we follow the methodology proposed by Pérez-Jiménez et al. (2008). The total soluble polyphenols (TSP) content was quantified in the extracts by the Follin–Ciocalteu method at 750 nm (Montreau, 1972). The concentration was obtained by performing a calibration curve with gallic acid (standard). The results were reported as milligrams of gallic acid equivalents per 100 mL of nectar (mg GAE/100 mL).

#### 2.3.6. Antioxidant capacity

From the aqueous–organic extracts of the soluble polyphenols, the antioxidant capacity (AC) was quantified by the ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) test (Re et al., 1999), DPPH (2,2-diphenyl-1-picrylhydrazyl) test (Prior, Wu, & Schaich, 2005) and the ferric-reducing antioxidant power (FRAP) test following the methodology of Benzie and Strain (1996). The results were expressed as millimoles of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per milliliter of nectar (mmol Trolox equivalents/mL).

### 2.4. Sensory evaluation

The evaluated nectars were the TSN after 30 days of storage at 4°C, UPN (freshly prepared) and a commercial soursoop nectar (CN). Acceptance tests (color, taste, odor and overall impression) were performed with a panel of 100 untrained judges (Pedrero & Pangborn, 1997).

### 2.5. Statistical analysis

In the first stage, RSM was used. In the second stage, the experiment was conducted in a factorial completely randomized design  $3 \times 5$ . The analysis was performed using an analysis of variance ( $p < 0.05$ ) with STATISTICA v.10 (StatSoft, Tulsa, Oklahoma, USA) software. Differences between means were compared using the Tukey test ( $\alpha = 0.05$ ). Results were expressed as the mean  $\pm$  SD of values obtained in triplicate from two different experiments.

## 3. Results and discussions

### 3.1. Lethal and sublethal effects of TS on *E. coli* and *S. aureus*

Table 1 shows that all treatments exhibit a reduction of the plate count after TS (initial cell concentration was approximately 7.1 log CFU/mL for both microorganisms); the TS9 treatment was the only one that presented lethality above the stipulated ( $\geq 5$  logs) by the FDA (2004). Likewise, all

treatments showed more than 99% sublethal damage, for both microorganisms. Similar results have been reported by Muñoz et al. (2012) at conditions of 24 kHz, 50°C for 5 and 2.9 min in orange and apple juices, they achieved a reduction of 5.1 and 4.9 logs, respectively, using *E. coli* as indicator. Walkling-Ribeiro et al. (2009), in orange juice (30 kHz, 30 min and 55°C), attained a reduction of 5.5 logs in *S. aureus*. Yusaf (2014) mentioned that some microorganisms might be more susceptible than others to TS treatment. Also, shape or size of the microorganisms may affect the treatment efficiency, probably due to an increase of surface area. In our study, similar values in lethality between *E. coli* and *S. aureus* at the same TS treatment were detected. Results agree with Scherba, Weigel and Brien (1991) who reported no differences on reduction range between gram-negative and gram-positive organisms when ultrasound was applied. Evelyn and Silva (2016) have suggested that bacterial cells generally become more sensitive when they undergo TS, mainly due to the absorption of energy by the membranes providing a cumulative effect on the basic functions of the microorganism causing a weakening and/or disruption of the cell membrane, leading to cell lysis.

According to the response surface, the better treatment conditions that guarantee FDA compliance with soursop nectar are 1.37 W/mL at 51°C for 9.2 min with a lethality of 5.16 log CFU/mL for *E. coli*, while for *S. aureus*, the conditions are 1.4 W/mL at 50°C for 10 min with a lethality of 5.18 log CFU/mL. The regression model and the lethality on both microorganisms in soursop nectar can be predicted using the following polynomial equations (Equation (2):  $R^2 = 0.98$ ; Equation (3):  $R^2 = 0.96$ , both with 95% confidence level):

$$\begin{aligned} E. coli \text{ lethality (log CFU/mL)} = & 51.92 - 44.62AED \\ & + 4.58AED^2 - 2.16T + 0.02T^2 + 0.34t - 0.01t^2 \\ & + 1.67AED \times T - 0.01AED \times T^2 \end{aligned} \quad (2)$$

$$\begin{aligned} S. aureus \text{ lethality (log CFU/mL)} = & 39.39 - 52.62AED \\ & + 20.37AED^2 - 0.24T + 0.0037T^2 - 0.09t^2 + 0.04AED \\ & \times T \end{aligned} \quad (3)$$

where AED is the acoustic energy density (W/mL),  $T$  is the temperature (°C) and  $t$  is the treatment time (min).

The Pareto charts for *E. coli* (Figure 2(a)) and *S. aureus* (Figure 2(b)) show the effect of independent variables on lethality of bacteria at a confidence level of 95%, AED, time, temperature and interaction between AED and temperature for both microorganisms. Evelyn and Silva (2016) observed that an increase of AED during TS treatment produced a lethality near sixfold compared to the thermal process at the same temperature. From the TS9 treatment, a microbial survival curve was elaborated for each pathogen and it was compared with the curve elaborated with the polynomial equation obtained in the response surface analysis for each microorganism, the comparisons are visualized (Figure 2(c)) by plotting the observed data points and the predicted inactivation curves of the bacterium on the same graph. As can be seen, the actual log reductions are so different of the predicted ones for both bacteria. However, the tendency of the real and predicted survival curves is the same; moreover, the final log value (at 10 min) is the same for both curves and both microorganisms. These results can be explained by the fact that

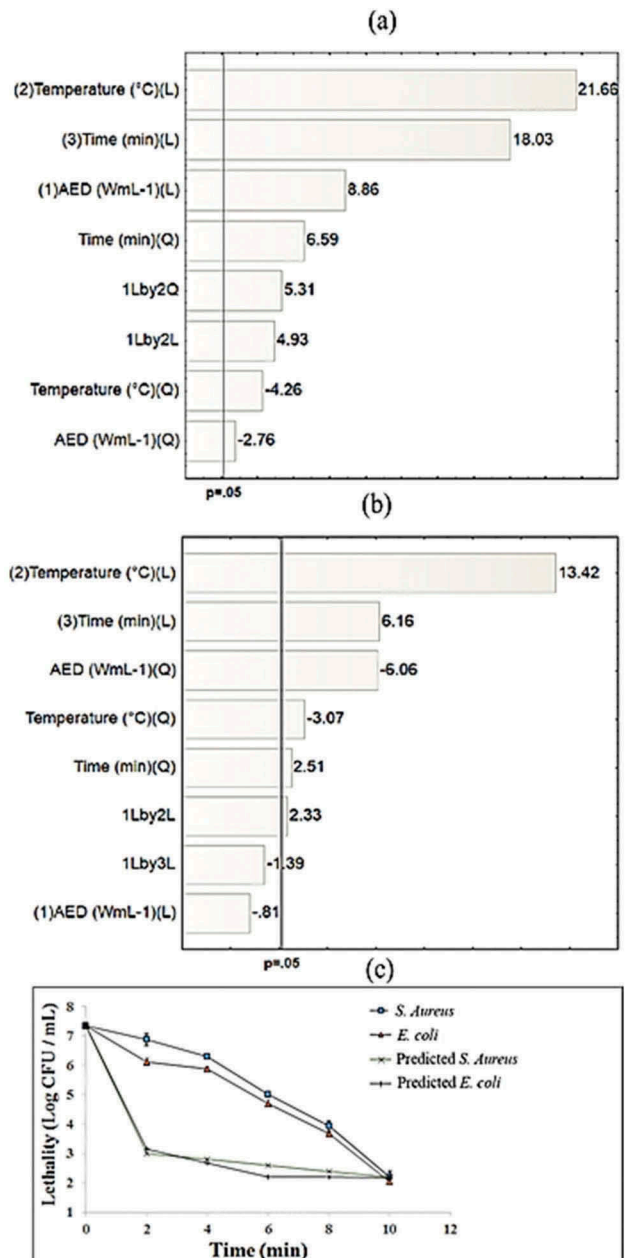


Figure 2. Pareto chart of the effect of the thermosonication on *E. coli* (a), *S. aureus* (b) and predicted/evaluated survival curve (c).

Figura 2. Diagrama de Pareto de efecto del termosonicado sobre *E. coli* (a) y *S. aureus* (b) y curva de supervivencia (c) predicha y evaluada.

real food systems may influence the thermal resistance of microorganisms (Knorr et al., 2011), which the mathematical model does not consider, as described by Ávila-Sosa, Gastélum, López-Malo and Palou (2010).

### 3.2. Microbiological stability

Table 2 shows the microbiological counts during the shelf life of soursop nectars. Untreated soursop nectar (UPN) exhibits growth of AMB (1.97 log CFU/mL), Enterobacteria (1.10 log CFU/mL), molds (1.10 log CFU/mL) and yeast (1.65 log CFU/mL) from the day 0, in comparison with thermosonicated sample; this treatment did not show growth of microorganisms at the same time. The UPN from the day 5 of storage presented bubbling, which indicates that

**Table 2.** Microbiological counts in unpasteurized (UPN) and thermosonicated (TSN) soursop nectars, during storage at 4°C.**Tabla 2.** Conteos microbiológicos en néctares de guanábana sin pasteurizar (UPN) y termosonicado (TSN), durante su almacenamiento a 4°C.

Microorganism	Treatments	Days of storage (log CFU/mL)				
		0	5	15	30	45
Aerobic mesophilic bacteria	UPN	1.97 ± 0.11 <sup>a</sup>	4.06 ± 0.02 <sup>BA</sup>	4.34 ± 0.01 <sup>CA</sup>	–	–
	*TSN	ND	ND	ND	1.60 ± 0.15 <sup>aB</sup>	2.16 ± 0.75 <sup>b</sup>
Coliform bacteria	UPN	1.10 ± 0.17 <sup>a</sup>	2.79 ± 0.09 <sup>b</sup>	3.11 ± 0.02 <sup>c</sup>	–	–
	*TSN	ND	ND	ND	ND	1.20 ± 0.17
Molds	UPN	1.10 ± 0.17 <sup>a</sup>	1.69 ± 0.21 <sup>b</sup>	2.50 ± 0.04 <sup>c</sup>	–	–
	*TSN	ND	ND	ND	ND	ND
Yeast	UPN	1.65 ± 0.15 <sup>a</sup>	3.90 ± 0.01 <sup>b</sup>	4.18 ± 0.07 <sup>CA</sup>	–	–
	*TSN	ND	ND	ND	1.10 ± 0.44 <sup>aB</sup>	3.56 ± 0.04 <sup>b</sup>

Values are the average ( $n = 6$ ) ± standard deviation (SD). Lowercase letters indicate significant statistical differences ( $p < 0.05$ ) between days of storage. Capital letters indicate significant statistical differences ( $p < 0.05$ ) between treatments. \*Experimental conditions TSN = 1.4 W/mL at 51°C for 10 min. ND: Not detectable. Blank space = Samples were not evaluated due to visible spoilage.

Los valores son el promedio ( $n = 6$ ) ± desviación estándar (SD). Letras minúsculas indican diferencias estadísticamente significativas ( $p < 0,05$ ) entre días de almacenamiento. Letras mayúsculas indican diferencias estadísticamente significativas ( $p < 0,05$ ) entre tratamientos. \*Condiciones experimentales TSN = 1,4 W/mL a 51°C por 10 min; ND = No detectable; Espacio en blanco = Las muestras no fueron evaluadas debido a visible deterioro.

fermentation has occurred. Imade, Ikenebomeh, Obayagbona and Igiehon (2013) have mentioned that there is a high content of endogenous yeasts that are responsible for the natural fermentation of soursop juice. UPN was not evaluated after 15 days due to visible spoilage and no further microbiological analyses were conducted as recommended by Martínez-Flores et al. (2015).

The TSN after 30 days of storage at 4°C kept the microbiological counts sufficiently low to consider that the thermosonicated nectar has good microbiological quality because of accomplished criteria of the Codex Alimentarius (CAC/GL 21-1997), with 1.60 and 1.10 log CFU/mL for AMB and yeasts, respectively, without the presence of molds or coliforms. According to Wordon, Mortimer and McMaster (2012) and Herceg et al. (2013), microbial cells are more sensitive to TS compared to traditional thermal pasteurization, due to the combined effects of heat with ultrasound, which causes weakening, pore formation and/or rupture of the cell membrane, hindering their normal development. Although the inactivation effect of TS was more effective than traditional pasteurization, all microorganisms were not inhibited because there was fermentation after 45 days of storage, the count of AMB and yeast increased and coliforms were detected (Chapman, Ferguson, Consalo, & Bliss, 2013); however, 30 days of shelf life under refrigerated storage (4°C) temperature is enough time to sell a nectar that has been minimally processed.

### 3.3. PPO activity and residual enzymatic activity

A significant effect ( $p < 0.05$ ) was observed for the type of treatment in SAPPO (Table 3). The UPN presented a SAPPO between 6.10 and 6.79 U/mg protein during storage time. On the other hand, TS reduced significantly ( $p < 0.05$ ) SAPPO to 99% of the initial activity (0.16 U/mg protein) on the day of treatment application (Anaya-Esparza et al., 2017b), after that no PPO activity was detected during all the time of storage. The effect of TS on PPO activity can be attributed to the denaturation of the protein and the change in its structural conformation by the additive effect between cavitation and heat; the cumulative effect can be so effective that a total enzymatic inactivation is achieved, as was described by Sulaiman, Soo, Farid and Silva (2015). Thus, it was demonstrated that the TS conditions evaluated in this experiment are effective in avoiding the enzymatic darkening in soursop nectar during its storage.

### 3.4. pH, TA, TSS, NEBI and color

Table 4 shows that changes in pH, TA and TSS were dependent on fermentation of nectars (Table 2). TA increased when UPN nectar was fermented, while pH and TSS decreased ( $p < 0.05$ ); however, it is important to mention that in the TSN, TA was stable for 30 days of storage due to its greater microbiological stability (Shaheer et al., 2014).

The NEBI is considered a quality indicator in the processing of fruit drinks, as it provides data on loss of antioxidants via

**Table 3.** Polyphenol oxidase (PPO) activity in unpasteurized (UPN) and thermosonicated (TSN) soursop nectars, during storage at 4°C.**Tabla 3.** Actividad de la polifenoloxidasa en néctar de guanábana sin pasteurizar (UPN) y termosonicado (TSN), durante su almacenamiento a 4°C.

Parameter	Treatments	Days of storage				
		0	5	15	30	45
Specific PPO activity (U/mg protein)	UPN	6.10 ± 0.59 <sup>aA</sup>	6.79 ± 0.55 <sup>A</sup>	6.26 ± 0.50 <sup>A</sup>	–	–
	*TSN	0.16 ± 0.02 <sup>b</sup>	ND	ND	ND	ND

Values are the averages of triplicate determinations from two different experiments ( $n = 6$ ) ± standard deviation (SD). Different lowercase letters indicate significant differences between treatments (day 0) and uppercase letters indicate significant differences by storage time ( $p < 0.05$ ). \*Experimental conditions TSN = 1.4 W/mL at 51°C for 10 min. Blank space = Samples were discarded due to visible spoilage. ND = No PPO activity was detected.

Los valores son promedio de tres determinaciones de dos diferentes experimentos ( $n = 6$ ) ± desviación estándar (SD). Letras minúsculas distintas indica diferencias significativas entre tratamientos (día 0) y letras mayúsculas indican diferencias significativas por el tiempo de almacenamiento ( $p < 0,05$ ).

\*Condiciones experimentales TSN = 1,4 W/mL a 51°C por 10 min. Espacio en blanco = Las muestras se descartaron debido a visible deterioro. ND = No se detectó actividad enzimática de la PPO.

**Table 4.** pH, titratable acidity, total soluble solids, nonenzymatic browning index (NEBI) and color attributes ( $L$ ,  $a^*$ ,  $b^*$  and  $\Delta E$ ) of unpasteurized (UPN) and thermosonicated (TSN) soursop nectars, during storage at 4°C.**Tabla 4.** pH, acidez titulable, sólidos solubles totales, índice de oscurecimiento no enzimático (NEBI) y atributos de color ( $L$ ,  $a^*$ ,  $b^*$  y  $\Delta E$ ) del néctar de guanábana sin pasteurizar (UPN), y termosonicado (TSN), durante su almacenamiento a 4°C.

Parameter	Treatments	Days of storage				
		0	5	15	30	45
pH	UPN	3.75 ± 0.02 <sup>aA</sup>	3.65 ± 0.01 <sup>bB</sup>	3.43 ± 0.03 <sup>cC</sup>	–	–
	*TSN	3.73 ± 0.01 <sup>aA</sup>	3.74 ± 0.01 <sup>aA</sup>	3.74 ± 0.01 <sup>aA</sup>	3.73 ± 0.01 <sup>aA</sup>	3.71 ± 0.01 <sup>b</sup>
Titratable acidity (g MAE/L)	UPN	2.0 ± 0.01 <sup>cA</sup>	2.4 ± 0.01 <sup>bA</sup>	3.3 ± 0.01 <sup>aA</sup>	–	–
	*TSN	2.2 ± 0.02 <sup>bA</sup>	2.2 ± 0.01 <sup>bA</sup>	2.2 ± 0.03 <sup>bB</sup>	2.4 ± 0.01 <sup>bB</sup>	2.6 ± 0.01 <sup>a</sup>
Total soluble solids (°Brix)	UPN	14.43 ± 0.15 <sup>aA</sup>	14.20 ± 0.10 <sup>aA</sup>	13.83 ± 0.05 <sup>bB</sup>	–	–
	*TSN	14.36 ± 0.05 <sup>aA</sup>	14.35 ± 0.05 <sup>aA</sup>	14.35 ± 0.05 <sup>aA</sup>	14.30 ± 0.04 <sup>aA</sup>	14.18 ± 0.04 <sup>ba</sup>
NEBI	UPN	0.022 ± 0.01 <sup>cA</sup>	0.094 ± 0.01 <sup>bB</sup>	0.108 ± 0.03 <sup>aA</sup>	–	–
	*TSN	0.008 ± 0.02 <sup>aA</sup>	0.017 ± 0.01 <sup>aA</sup>	0.018 ± 0.01 <sup>aC</sup>	0.019 ± 0.01 <sup>aB</sup>	0.022 ± 0.01 <sup>a</sup>
Luminosity ( $L^*$ )	UPN	47.26 ± 0.75 <sup>aA</sup>	35.56 ± 0.14 <sup>bB</sup>	30.66 ± 0.49 <sup>cC</sup>	–	–
	*TSN	48.04 ± 0.71 <sup>aA</sup>	48.62 ± 0.77 <sup>aA</sup>	48.23 ± 0.62 <sup>aA</sup>	47.71 ± 0.55 <sup>aA</sup>	41.25 ± 1.13 <sup>b</sup>
$a^*$	UPN	−0.68 ± 0.08 <sup>aA</sup>	−0.79 ± 0.03 <sup>aA</sup>	−3.14 ± 0.03 <sup>bC</sup>	–	–
	*TSN	−1.52 ± 0.14 <sup>aC</sup>	−1.47 ± 0.12 <sup>aB</sup>	−1.55 ± 0.06 <sup>aB</sup>	−1.45 ± 0.11 <sup>aA</sup>	−1.65 ± 0.18 <sup>b</sup>
$b^*$	UPN	1.31 ± 0.38 <sup>cA</sup>	2.23 ± 0.05 <sup>bA</sup>	4.19 ± 0.09 <sup>aA</sup>	–	–
	*TSN	0.59 ± 0.30 <sup>bB</sup>	1.11 ± 0.43 <sup>bB</sup>	1.07 ± 0.08 <sup>bB</sup>	1.47 ± 0.10 <sup>aB</sup>	1.84 ± 0.27 <sup>a</sup>
$\Delta E$	UPN	0	11.72	17.01	–	–
	*TSN	1.35	1.58	2.21	2.61	2.98

Values are the average ( $n = 6$ ) ± standard deviation (SD). Lowercase letters indicate significant statistical differences ( $p < 0.05$ ) between days of storage. Capital letters indicate significant statistical differences ( $p < 0.05$ ) between treatments. MAE: Malic acid equivalents. \*Experimental conditions TSN = 1.4 W/mL at 51°C for 10 min. Blank space = Samples were discarded due to visible spoilage.

Los valores son el promedio ( $n = 6$ ) ± desviación estándar (SD). Letras minúsculas indican diferencias estadísticamente significativas ( $p < 0,05$ ) entre días de almacenamiento. Letras mayúsculas indican diferencias estadísticamente significativas ( $p < 0,05$ ) entre tratamientos. MAE = Equivalentes de ácido málico; \*Condiciones experimentales TSN = 1,4 W/mL a 51°C por 10 min. Espacio en blanco = Las muestras se descartaron debido a visible deterioro.

nonenzymatic reactions (Valdramidis, Cullen, Tiwari, & O'Donnell, 2010). From the fifth day of storage, NEBI increased significantly ( $p < 0.05$ ) in UPN nectar (Table 4), due to the oxidation of AA and/or polyphenols as mentioned by Peters, Badrie and Comissiong (2000). These authors observed an increase in NEBI in soursop nectar (unpasteurized and pasteurized) with respect to time and the appearing of brown pigmentation. In the TSN up to 45 days of cold storage, it presented a value of 0.022 absorbance units. There is the theory that TS removes oxygen from the environment where it is applied (Cheng, Soh, Liew, & Teh, 2007); therefore, the antioxidants can be kept more stable in a thermosonicated nectar; related results reported Cruz-Cansino et al. (2015) in thermoultrasonicated purple cactus pear juice compared to the treated with pasteurization, mentioning that thermoultrasonication prevented NEBI for 28 days.

The changes in  $L$ ,  $a^*$  and  $b^*$  were lower in TSN compared to UPN during cold storage (Table 4). These results are in concordance with the NEBI and  $\Delta E$  values. The values of  $\Delta E > 3.0$  indicate perceptible changes in color by the consumer (Choi, Kim, & Lee, 2002). In this sense, unlike the UPN that presented a  $\Delta E > 3.0$  after 5 days of storage, the TSN maintained values of  $\Delta E < 3.0$  after 45 days of storage, which indicates a white color with values of  $L$ ,  $a^*$  and  $b^*$  practically unchanged. Comparable results were also observed in ultrasonicated soursop juice (Dias et al., 2015); this means that TSN is stable in terms of enzymatic and nonenzymatic browning (Aadil et al., 2015).

### 3.5. AA content, TSP and AC

On the day of preparation of the nectars, the AA content was 35.68 and 34.48 mg/100 mL in UPN and TSN nectar,

respectively (Table 5). The time of storage also affected ( $p < 0.05$ ) the AA content, which decreased in all treatments during storage. The highest vitamin C loss was observed in the UPN after 3 days of storage (85%, 5.95 mg/100 mL); while in the TSN, only 15% (29.21 mg/100 mL) was lost. Some authors have reported percentage of AA retention greater than 90% and up to 100% when applied TS in fruit or vegetable juices (Abid et al., 2014; Martínez-Flores et al., 2015; Rawson et al., 2011), proposing that the stability of AA can be associated with the elimination of dissolved oxygen from the medium, essential for AA degradation, during the cavitation produced by the TS treatment (Cheng et al., 2007).

Regarding the TSP content, the evaluated treatments also had a significant effect ( $p < 0.05$ ). In the UPN, 348.79 mg GAE/100 mL were quantified. The TSN presented slight variability (1%, 349.99 mg GAE/100 mL) with respect to control nectar. Comparable results have also been reported when TS was applied in juices by Martínez-Flores et al. (2015) for carrot juice (increase of 9%) and Rawson et al. (2011) for watermelon juice (increase of 7%). It has been reported that the increase in TSP by TS is because phenols attached to carbohydrates (pectin, lignin, cellulose and hemicellulose) of the cell wall of vegetables are released, when it is broken by cavitation (Cui et al., 2014).

During storage, all treatments showed a decrease in the TSP content, with a loss of 14% (3 days) and 7% (30 days) for UPN and TSN, respectively. Similar effects in thermosonicated juices of purple cactus pear and carrot by Cruz-Cansino et al. (2015) and Martínez-Flores et al. (2015) have been reported. These authors showed that

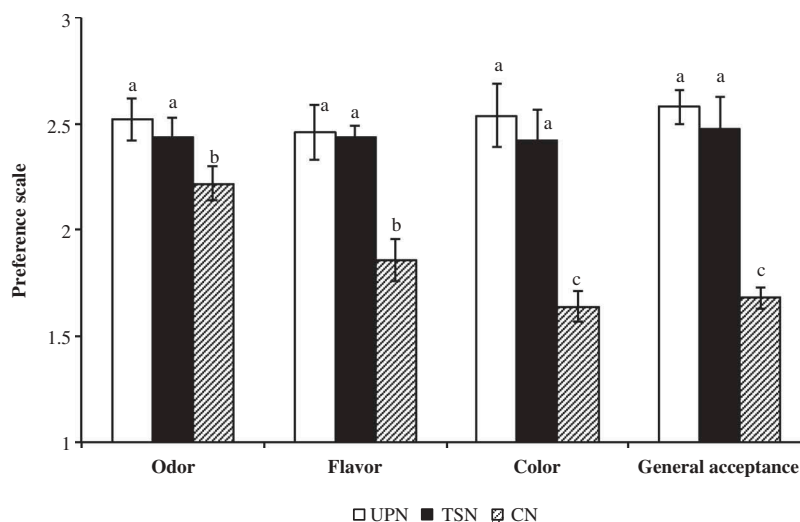
**Table 5.** Ascorbic acid (AA), total soluble polyphenols (TSP) and antioxidant capacity (ABTS, DPPH and FRAP) of unpasteurized (UPN) and thermosonicated (TSN) soursop nectars, on the day of preparation and at the end of the refrigerated storage.

**Tabla 5.** Ácido ascórbico (AA), polifenoles solubles totales (TSP) y capacidad antioxidante del néctar de guanábana sin pasteurizar (UPN), y termosonicado (TSN), en el día de su elaboración y al final de su almacenado en refrigeración.

Treatment	Days of evaluation	AA (mg AA/100 mL)	TSP (mg GAE/100 mL)	ABTS (mmol TE/mL)	DPPH (mmol TE/mL)	FRAP (mmol TE/mL)
UPN	0	35.68 ± 1.48 <sup>a</sup>	348.79 ± 3.70 <sup>a</sup>	22.66 ± 0.64 <sup>a</sup>	6.14 ± 0.77 <sup>a</sup>	4.93 ± 0.50 <sup>ab</sup>
	3	5.95 ± 0.70 <sup>c</sup>	297.09 ± 3.70 <sup>c</sup>	14.19 ± 0.79 <sup>b</sup>	0.37 ± 0.03 <sup>c</sup>	3.95 ± 0.10 <sup>c</sup>
*TSN	0	34.48 ± 1.37 <sup>a</sup>	349.99 ± 3.23 <sup>a</sup>	22.29 ± 0.54 <sup>a</sup>	7.00 ± 0.11 <sup>a</sup>	5.56 ± 0.55 <sup>a</sup>
	30	29.21 ± 1.18 <sup>b</sup>	323.88 ± 2.79 <sup>b</sup>	17.43 ± 0.52 <sup>b</sup>	4.08 ± 0.39 <sup>b</sup>	4.55 ± 0.31 <sup>b</sup>

Values are the average ( $n = 6$ ) ± standard deviation (SD). Different letters indicate significant statistical differences ( $p < 0.05$ ). GAE: Gallic acid equivalents; AA: ascorbic acid; TE: trolox equivalent. \*Experimental conditions TSN = 1.4 W/mL at 51°C for 10 min.

Los valores son el promedio ( $n = 6$ ) ± desviación estándar (SD). Letras diferentes indican diferencias estadísticamente significativas ( $p < 0,05$ ). GAE = Equivalentes de ácido gálico; AA = Ácido ascórbico; TE = Equivalentes de Trolox; \*Condiciones experimentales TSN = 1,4 W/mL a 51°C por 10 min.



**Figure 3.** Sensory evaluation of unpasteurized soursop nectar on the day of preparation (UPN) and thermosonicated (TSN) after 30 days of storage at 4°C and a commercial nectar (CN). Values are the averages of triplicate determinations from two different experiments ( $n = 6$ ) ± standard deviation (SD). Different lowercase letters indicate significant differences between treatments ( $p < 0.05$ ).

**Figura 3.** Evaluación sensorial del néctar de guanábana en el día de su elaboración, sin pasteurizar (UPN) y termosonicado (NTS) después de 30 días de almacenamiento a 4°C, y un néctar comercial (CN). Los valores son promedio de tres determinaciones de dos diferentes experimentos ( $n = 6$ ) ± desviación estándar (SD). Letras minúsculas distintas indica diferencias significativas entre tratamientos ( $p < 0,05$ ).

during storage, there is a gradual release of phenolic compounds and amino acids, caused by the senescence and decomposition of the cell structure, although extended periods of exposure of these compounds to oxygen can oxidize them. It was clear that TSN kept these compounds bioactive and available, for a longer time than pasteurized nectar.

AC was affected by storage time ( $p < 0.05$ ). AC values for all the treatments decreased with the ABTS assay, from 22.02–22.66 to 14.19–17.43 mmol/mL at the end of storage. The same was true for the DPPH and FRAP tests, the initial AC values (6.14–7.00 and 4.93–5.56 mmol/mL, respectively) decreased to 0.37 and 3.95 mmol/mL in the UPN and 4.08 and 4.55 mmol/mL in the TSN. The results are in agreement with the decrease in TSP content. TSN had higher antioxidant values than the untreated control (UPN). It has been shown that polyphenols can act by multiple mechanisms to neutralize radicals such as chelating metals, transferring electrons or donating hydrogen ions. Differences in AC between trials can be attributed to different concentrations and type of polyphenols in the extract evaluated (Pérez-González, Rebollar-Zepeda, León-Carmona, & Galano, 2012).

### 3.6. Sensory evaluation

The UPN and TSN received high scores in all attributes evaluated (Figure 3). Regarding to the CN, the preservation treatment to which the nectar is subjected generates a darker color and diminishes the flavor of soursop although received higher score in terms of odor than the samples. Dias et al. (2015) carried out a sensorial evaluation on untreated and ultrasonically treated soursop juice and there were not significant differences in the attributes of color, aroma and flavor.

## 4. Conclusions

The TS applied in this work turned out to be a viable technology for the processing of soursop nectar with microbiological stability, inactivation of the PPO and a greater retention of bioactive compounds during the storage at 4°C for 30 days. In addition, the TSN presented better sensory attributes than the CN; therefore, it is a good alternative to extend the shelf life of minimally processed soursop nectar. Nevertheless, further studies are required to establish the most effective conditions that can prolong the shelf life of soursop-based beverages.



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## Disclosure statement

The authors have declared that no conflict of interest exists.

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