Optimization of Thermoultrasound Process of a Soursop (Annona muricata) Nectar and Its Comparison with Pasteurization on Physicochemical Properties and In Vitro Bioaccessibility of Antioxidants

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SUMMARY

Research background. Soursop nectar contains antioxidants and for its conservation the pasteurization is applied. However, this technology decreases its physicochemical properties and bioactive compounds, hence, an alternative is the thermoultrasound which could counter these effects. Therefore, the thermoultrasonicated nectar was compared to a pasteurized one and the in vitro bioaccessibility of antioxidants was estimated.

Experimental approach. The soursop nectar (25 %) was elaborated and the response surface methodology was used. The thermoultrasound (TUS) (75-90 % amplitude; 3.15-15 min) was applied, and 2 % stevia and 6 % of agave inulin were added as sweeteners. The microbiological,
physicochemical, enzymatic and antioxidant properties were analyzed. The nectar was prepared again and control, optimized TUS and pasteurized were compared. In addition to the aforementioned determinations, microstructure, total dietary fiber (TDF) and in vitro bioaccessibility of antioxidants were performed.

Results and conclusions. The response variables that fit the mathematical model were $L^*$, $b^*$, Chroma ($C^*$), total phenolic content (TPC), antioxidant activity by ABTS•$, DPPH•$ and ferric reducing antioxidant power (FRAP). The $L^*$ and DPPH•$ were influenced by quadratic time and TPC by time ($p<0.0001$). The optimal TUS condition was 82 % amplitude at 9.15 min and the responses variables were $L^*$, $b^*$ and $C^*$ (45.48, 3.55 and 3.62, respectively), TPC (38.40 mg GAE/100 mL), ABTS•$ (31.28 μmol TE/100 mL), DPPH•$ (124.22 μmol TE/100 mL) and FRAP (3.06 μmol Fe (II)/100 mL). In comparison with the pasteurized sample, optimized TUS had high value of TDF (3.53%), $L^*$ (45.56) $h°$ (-56.49), TPC (26.63 mg GAE/100 mL), ABTS•$ and DPPH•$ (22.03 and 129.22 μmol TE/100 mL, respectively), FRAP (3.10 μmol Fe (II)/100 mL) and low pectin methylesterase activity (0.28 PMEU/mL). For in vitro bioaccessibility, the optimized TUS nectar showed high absorption of TPC (15.26 GAE/100 mL) and high values in the antioxidant activity by ABTS (34.92 μmol TE/100 mL) and FRAP (7.88 μmol Fe (II)/100 mL).

Novelty and scientific contribution. The thermoultrasound improves the physicochemical properties and in vitro bioaccessibility of antioxidants soursop nectar. On the other hand, as an alternative, this beverage provides low-calorie with prebiotic properties for the benefit of consumer health.

Keywords: functional beverage; safety; optimization; response surface methodology; total dietary fiber; phenolic content

INTRODUCTION

Soursop (Annona muricata) belongs to the Annonaceae family and it is cultivated in Southeast Asia, South America and tropical areas of Africa (1). The fruit is consumed mainly fresh, juice and nectar, its pulp is juicy with a pleasant smell, subacid taste and contains antioxidants such as vitamin E, vitamin C, carotenoids and phenolic compounds, which are related to prevention non-communicable diseases (diabetes and hypertension), as well as antiproliferative effect of cancer cells and induction of apoptosis (2).

In general, to obtain longer shelf life of the processed products such as nectar, pasteurization is used, however, this conventional technology degrades the bioactive compounds, generates losses
of nutrients as well as unfavorable physical properties changes (stability, viscosity, color, among others) due to the application of high temperatures (3). Therefore, several alternatives are explored such as thermoultrasound (TUS), which combines ultrasound application and temperatures below 100 °C (usually 50 and 60 °C) (4). The TUS technology reduces the enzymatic activity and microbiological load, in addition to the release of bioactive compounds of the food matrix with slight changes in its nutritional properties (5). This technology has been applied in studies such as nectar and juice (jackfruit and beetroot) using the response surface methodology, as a tool in research for the optimization of multiple responses simultaneously, with the purpose to reduce the tests and experimental time (3,6).

In addition to the abovementioned, by their importance in human health, it is necessary to estimate the absorption of bioactive compounds and this has been studied in our working in beetroot juice through in vitro bioaccessibility process (6). This methodology provides information on the amount of present compounds in the intestine released by the digestion process (chewing, enzymes, pH), which could be absorbed by intestinal barrier (7). Finally, the high consumption of simple sugars is a worldwide problem, so one of the alternatives could be the addition of natural sweeteners with low calories content with benefits to health, such as stevia (glycoside) and inulin (hydrocolloid) (8,9). These hypocaloric products have been added in different nectars (orange, pomegranate, guava and mango) (10,11). The aim of this study was to determine the optimum condition of thermoultrasound to process soursop nectar considering the microbiology and physicochemical, enzymatic and antioxidant properties, besides its comparison with a pasteurized sample on the in vitro intestinal bioaccessibility of antioxidants.

MATERIALS AND METHODS

Plant material and nectar preparation

According to Codex Stan “the nectar is the unfermented but fermentable product obtained by adding water with or without the addition of sugars, honey and/or syrups and/or food additive sweeteners to a mixture of those products” (12). To soursop nectar elaboration, 10 kg of fruit was acquired in the local market in Pachuca, Hidalgo, Mexico. The fruits with no external damage were selected, and these were purchased in stages of commercial maturity. The fruits were washed and disinfected with a previously prepared solution using the commercial ionized silver to 0.082 % (Microdyn®, Mexico), later peel and seeds of soursop were removed. The nectar was elaborated with a concentration of 25 % adding purified water according to the Codex Stan (12); and was homogenized into a commercial blender for 30 seconds. Immediately after elaboration of the nectar,
for the first stage of study the nectar was subjected to the thermoultrasound treatments to obtain the optimal condition of the process. In the second stage, the nectar preparation was carried out again to have a control (untreated nectar), a pasteurized nectar and the optimized TUS sample for their comparison.

Thermoultrasound (TUS) and pasteurized treatments

A high intensity ultrasonic equipment (VCX-1500, Sonics Materials, Inc. Newtown, CT, USA) at 1500 W and a probe with a tip diameter of 25-mm connected to an amplitude transformer at a constant frequency of 20 kHz were used. According to previous studies (13, 14), the samples were thermoultrasonicated with different amplitude percentages (72 %, 75 %, 82 %, 90 % and 93 % (corresponding to acoustic energy density of 3.60 W/mL, 3.75 W/mL, 4.10 W/mL, 4.50 W/mL and 4.65 W/mL, respectively) and times of 3.15 min, 5 min, 9.15 min, 13.30 min and 15 min with pulse durations of 2 s on and 4 s off. A sample of 300 mL was heated in a jacketed vessel through which water circulated using a bath water (Cole-Parmer, 12108-10, USA) to obtain a sample outlet temperature of 50±2 °C. On the other hand, the pasteurization process was carried out at 65 °C for 30 min (15) using the same jacketed vessel and volume of sample (300 mL). The treatments (including control) were collected in sterile flasks; immediately after and according to Alizadeh et al. (11), 2 % of stevia as commercial sweetener and 6 % of agave inulin were added. The microbiology, color, pH, total soluble solids, titratable acidity, viscosity and stability were performed fresh, while for the rest of the determinations, the samples were stored frozen at -35 °C for one week for further analysis.

Optimization of thermoultrasonicated soursop nectar

Response surface methodology

A central composite rotatable design was used, where two independent variables (amplitude percentage and sonication time) each one in five levels (−α, −1, 0, +1, +α) were analyzed. The amplitude variables of 75-90 % (X1, %) and time of 3.15-15 min (X2, min) were applied. The design yielded 13 treatments, where 5 were the central points, 4 factorial points and 4 axial points (Table S1), with a distance of α=1.414 between each central design. The multiple linear regression analysis on the dependent variables data obtained by triplicate were performed and the mean value of each attribute was taken as the response (Yi), using JMP® 7.0.2 statistical software (SAS Institute, Cary, NC, USA) (16) and fitted to a second order polynomial model, given by the Eq. 1:

\[
Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{2} \beta_{ij} X_i X_j
\]
where $Y$ and $\beta_0$ correspond to the predicted response and the constant coefficient, respectively; $\beta_i$ and $\beta_{ii}$ are linear and quadratic coefficients, respectively and $\beta_{ij}$ is the interaction coefficient, $X_1$ and $X_2$ are the independent variables that correspond to the amplitude and sonication time, respectively.

To establish the optimal process condition, the response variables that fit the mathematical model with a coefficient of determination ($R^2$) and adjusted $R$ squared (adj-$R^2$), both $\geq 0.80$ were used. Also, the regression coefficient, the significance level ($p$) and the predicted values were obtained.

Three-dimensional and contour figures from SigmaPlot 12.3 software (Systat Software, Inc. San Jose, CA, USA) (17) were elaborated. The optimal TUS processing condition was illustrated by overlapping the contours figures. The reproducibility of the optimal process condition was performed by triplicate ($n=9$) and compared with the predicted values, using a $t$-student with a significance level of $p<0.05$ (SPSS version 15.0, SPSS INC, IL, USA) (18).

Microbiology analysis

Microbiological determination was performed by the microdrop technique pour plate. An aliquot of 100 μL of sample diluted in previously sterilized 0.1% peptone water was prepared. Three decimal dilutions at 1:10, 1:100, 1:1000 and a direct inoculation (20 μL) were carried out. Aerobic mesophilic (AM) microorganisms were determined using plate count agar (PCA) media and were incubated (Labtech, LSI-3016A, Gyeonggi-do, Korea) for 48 h at 30 °C. For Enterobacteriaceae count (EB), the samples were poured in violet red bile glucose agar (VRBG) with an incubation of 24 h at 37 °C. The colony count was expressed as log colony forming units per milliliter (log CFU/mL).

Quality measurements

The pH was measured using a potentiometer (HANNA, PH210, B, Romania), while for total soluble solids (TSS) (expressed in °Brix) a refractometer (Brix/ATC FG-113, Hangzhou Chincan Trading Co., Ltd., China) was employed. The titratable acidity (TA) was performed and the results were expressed in percentage.

Physical and chemical parameters

The browning index (BI) was determined according to the methodology described by Martins et al. (19) with minor modifications. Ten milliliters of sample were centrifuged (6500, Hamilton Bell, NJ, USA) for 10 min at 1321×g with the purpose of removing coarse particles. After, 5 mL of the supernatant were taken and added in another centrifuge tube, plus 5 mL of ethyl alcohol to 95% (Sigma-Aldrich, Dublin, Ireland) and was centrifuged under the aforementioned conditions. After, 200
μL of the supernatant in a microplate were aggregated and the absorbance at 420 nm by means of a microplate reader (Power Wave XS UV-Biotek, software KC Junior, VT, USA) was measured.

The viscosity was carried out with a rotary viscometer (Brookfield Engineering Laboratories, Middleboro, DV3T, MA, USA) using the LV-4 needle at 60 rpm. For analysis, 35 mL of sample at 20 °C in a centrifuge tube of 50 mL was placed. The results were expressed in centipoises (cP).

The physical stability determination was made, where 10 mL of sample in a centrifuge tube (previously weighed) of 15 mL was placed, it was centrifuged (Hamilton Bell, V6500, NJ, USA) at 1321×g for 20 min and the supernatant was eliminated. The stability was calculated by weight difference and was expressed as percentage.

The cloud index (CI) methodology was according to Cervantes-Elizarrarás et al. (14). An aliquot of 5 mL was centrifuged (Hamilton Bell, V6500, NJ, USA) at 1321 × g during 10 min. After, 200 μL of the supernatant in a microplate were added and the absorbance at 660 nm through a microplate reader (Power Wave XS UV-Biotek, Winooski, VT, USA) was measured, taking the absorbance value as the cloud index. Distilled water as blank was used.

The determination of pectin methylesterase (PME) enzyme was performed by titration of the carboxyl group (20). It was carried out by adding 40 mL of 1 % citrus pectin in a 2 mol/L NaCl solution with 10 mL of soursop nectar. The mixture was adjusted with NaOH 1 mol/L to obtain a pH of 7.0, after 1 mL of NaOH at 0.05 mol/L was added and the time was measured until reaching pH 7.0. For the expression of the results, the pectin methylesterase unit (PMEU) was considered, which is equivalent to 1 μmol of the carboxyl group per minute at pH 7.0 at a temperature of 30 °C and was calculated according to the Eq. 2:

\[
\text{PMEU/mL} = \frac{\text{mL of NaOH} \times \text{Normality of NaOH}}{\text{(Minutes) (mL of sample)}} \div 2
\]

**Color parameters**

A portable colorimeter (Minolta CM-80, 500SM-508D, Tokio, Japan) was used to measure \(L^*\) which indicates lightness (values of 0 = black, 100 = white), while that \(a^*\) and \(b^*\) coordinates red-green and blue-yellow, respectively, according to the International Commission on Illumination (CIE). Saturation or chromaticity (\(C^*\)) and hue (\(h^*\)) were calculated using \(a^*\) and \(b^*\) values (Eqs. 3 and 4).

To compare color differences, the control and pasteurized nectars values as reference were used. The total color difference (TCD) or \(\Delta E\) was obtained employing the \(L^*, a^*, b^*\) values (21) by means of the Eq. 5:

\[
C^* = (a^{*2} + b^{*2})^{1/2} \quad \text{/3/}
\]

\[
h^* = \tan^{-1} (b'/a) \quad \text{/4/}
\]
\[ \Delta E = \left( (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right)^{\frac{1}{2}} \]

Total phenolic content, antioxidant activity analysis by ABTS\(^+\), DPPH\(^-\) and ferric reducing antioxidant power (FRAP)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu procedure of Stintzing et al. \((22)\). The solution of Folin-Ciocalteu 1:10 (Folin-Ciocalteu:distilled water) was prepared and a calibration curve at concentrations of gallic acid of 0, 100, 200 and 300 mg/L was performed. Briefly, in an Eppendorf tube, 100 µL of the sample, 500 µL of Folin–Ciocalteu (1:10) solution plus 400 µL of sodium carbonate (7.5 %) were added, vortexed and incubated for 30 min at room temperature. Later, the absorbance (765 nm) in a microplate reader (Power Wave XS UV-Biotek, Winooski, VT, USA) was measured. The results were expressed as milligrams of gallic acid equivalents per 100 milliliters (mg GAE/100 mL).

The antioxidant activity by ABTS was performed according to Kuskoski et al. \((23)\). The radical cation ABTS\(^+\) was produced by reaction of two compounds (7 mmol/L ABTS and 2.45 mmol/L potassium persulfate in distilled water) in dark conditions at room temperature for 16 h before use. After, the ABTS\(^+\) solution was diluted with deionized water until reaching an absorbance of 0.70±0.10 at 754 nm. A calibration curve at concentrations of 0, 5, 10, 20, 30, 40 and 50 µmol/L Trolox equivalent was carried out. Then, in an Eppendorf tube, 100 µL of sample and 900 µL of diluted ABTS\(^+\) were added and incubated at room temperature for 7 min, the mixture at 754 nm in a microplate reader (PowerWave XS UV-Biotek, Winooski, VT, USA) was measured. The results were expressed as micromoles of Trolox equivalents per 100 milliliters (µmol TE/100 mL).

To antioxidant activity by DPPH\(^-\) was determined according to the methodology described by Morales and Jiménez-Pérez \((24)\). The stable DPPH radical was prepared in ethanol (7.4 mg/100 mL). The calibration curve was prepared with Trolox at different concentrations (0, 50, 100, 200 and 300 µmol/L). Then, in an Eppendorf tube, 100 µL of sample and 500 µL of DPPH\(^-\) solution were mixed and was left to stand 1 h at room temperature. The absorbance at 520 nm using a microplate reader (PowerWave XS UV-Biotek, Winooski, VT, USA) was measured. The results were expressed as micromoles of Trolox equivalents per 100 milliliters (µmol TE/100 mL).

With respect to FRAP (ferric reducing antioxidant power), it was performed with the established by Thaipong et al. \((25)\). A FRAP solution was prepared from a mixture 3 solutions (10:1:1) as follow: an acetate buffer (300 mmol/L) with 3.1 g C\(_2\)H\(_3\)NaO\(_2\) • 3H\(_2\)O and 16 mL C\(_2\)H\(_4\)O\(_2\) (pH 3.6); a TPTZ (2,4,6-tripyridyl-s-triazine) solution 10 mmol/L in 40 mmol/L HCl and another solution 20 mmol/L of FeCl\(_3\)•6H\(_2\)O. After, the FRAP solution was placed in a water bath at 37 °C. A calibration curve at
concentrations of 0, 20, 30, 40 and 50 µmol/L with FeSO₄ was performed. Subsequently, in an Eppendorf tube 30 µL of sample plus 90 µL of distilled water and 900 µL of the FRAP solution were added, mixed and stored for 10 min in dark conditions. Finally, absorbance at 593 nm with a microplate reader (Power Wave XS UV-Biotek, Winooski, VT, USA) was measured and the results were expressed as micromoles of iron (II) per 100 milliliters (µmol Fe (II)/100 mL).

Comparison of parameters between the optimized TUS and pasteurized soursop nectar

After the optimal condition of TUS was obtained, it was compared with a pasteurized soursop nectar and the untreated (control). In addition to the determinations described above, dietary fiber content (total, soluble and insoluble), microstructure and in vitro intestinal bioaccessibility of antioxidants were performed.

Dietary fiber content

A lyophilized sample of 5 g (7753020, Labconco, MO, USA), milled (IKA® A11 basic, Wilmington, NC, USA) and sieved (500 µm) was used for total dietary fiber (TDF) and soluble dietary fiber (SDF). Enzymatic-gravimetric method with a commercial kit for total dietary fiber assay (TDF-100A, Sigma Chemical Company, St Louis, USA) was used under the specifications established by the manufacturer following the method 985.29 of AOAC (26). The results were expressed in percentage (%). The insoluble dietary fiber (IDF) was calculated by difference between the total and soluble fiber with the following equation:

\[
% \text{IDF} = \frac{(\text{TDF} - \text{SDF})}{6}
\]

Microstructure analysis by scanning electron microscopy (SEM)

To determine the microstructure of the soursop nectar, a scanning electron microscope (SEM) (JSM-IT300, Jeol, USA) with a scope of 250 and 500 magnifications was used. A lyophilized sample on a double-sided graphite tape was placed and was coated with a 1 mm thin layer of gold into a sputter coater (Denton Vacuum LLC, Moorestown, NJ, USA), a pressure of 20 millitorr and with a current of 20 milliams for 4 min was applied.

In vitro intestinal bioaccessibility determination of total phenolic and antioxidant activity

It was estimated using an in vitro digestion model followed by dialysis based on the method described by Ramirez-Moreno et al. (27). A sample of 20 mL at pH 2.0 (with 6 mol/L of HCl) was adjusted. Then, the sample was incubated held under continuous shaking (Allegra 25TM, Beckman Coulter, CA, USA) with 120 µL of pepsin solution (40 mg pepsin-Sigma Aldrich P-7000-per mL 0.1
mol/L HCl) at 37 °C for 2 h. After, 1.5 mL of pancreatin, sodium cholate and sodium deoxycholate solution (5 mg of pancreatin, 12.5 mg of sodium cholate hydrate and 12.5 mg of sodium deoxycholate per mL 0.1 mol/L NaHCO₃) were added. The solution was placed in a dialysis membrane (12–14 KDa molecular weight cut-off, width 35 mm; Sigma Aldrich) and was dialyzed in 250 mL of sodium bicarbonate solution at pH 7.5 for 16 h and gentle stirring (60 rpm). Aliquots of dialyzed fraction (bioaccessible fraction) were taken and phenolic compounds and the antioxidant activity (ABTS•⁺, DPPH•⁻ and FRAP) were determined. The estimation of bioaccessibility (in the small intestine) of TPC and antioxidant activity was measured as a result of the difference of antioxidants in the original sample (before in vitro digestion) and the antioxidants in the dialyzed fraction.

Statistical analysis

All determinations were performed by triplicate. The optimized TUS soursop nectar, control and the pasteurized were analyzed by means of ANOVA for the comparison of the differences between means ± standard deviation (SD) (n=9), Duncan test at level significance at p<0.05 was applied, using the SPSS® 15.0 Software for Windows (SPSS INC, Armonk, NY, USA) (18).

RESULTS AND DISCUSSION

Optimization of thermoultrasound process

As previously described, to obtain the optimal condition of the thermoultrasound (TUS) process of soursop nectar, the response surface methodology was applied. According to the experimental design and mathematical model, the response variables (dependents) that obtained a determination coefficient (R²) and adjusted coefficient of determination (adjusted-R²) ≥ 0.80 (80 %) were used. The above indicates that dependent variable behavior is attributed to the independent variables. Therefore, in the present study the response variables that fit the mathematical model were L*, b*, C*, TPC, antioxidant activity by ABTS•⁺, DPPH•⁻ and FRAP (will be described in more detail starting in the color section). The response variables that did not fit the mathematical model (data not shown) were microbiology, pH, total soluble solids (TSS), titratable acidity (TA), browning index (BI), viscosity, stability, cloud index (CI) and pectin methylesterase (PME). However, the results obtained of these variables are described below.

Effect of thermoultrasound on aerobic mesophilic and enterobacteriaceae of soursop nectar

The untreated soursop nectar (control) had values of 5.24 log CFU/mL in aerobic mesophilic (AM) and 5.00 log CFU/mL in enterobacteriaceae count (EB). All thermoultrasonicated nectars showed a reduction of 1.42-3.54 log CFU/mL for AM with respect to control sample, being the treated
sample by TUS at 75 % of amplitude for 13.30 min with less load microbial with 1.70 log CFU/mL. Notably, there was no EB growth for TUS treatments at 75 % of amplitude for 13.30 min, 82 % of amplitude for 9.15 and 15 min, and 93 % of amplitude for 9.15 min (Table S1). Except for the latter for AM, these treatments were within of the international criteria (<2 log CFU/mL) for AM and EB in pasteurized juices (28). The reduction in bacterial load could be due to the weakening of the cell wall, since, thermoultrasound creates cavitation (regions with temperatures and pressures high (microstreaming)), generating intracellular lesions, perforation and exposure of the cytoplasmic content (5).

**pH, total soluble solids (TSS), titratable acidity (TA) and browning index (BI) of thermoultrasonicated soursop nectar**

The samples (including the control) had pH values between 3.71 and 4.37. In relation to TSS, all samples presented 11 °Brix, while in TA were between 0.13 to 0.27 %. The untreated soursop nectar had 0.05 of browning index (BI), while thermoultrasonicated samples reported values from 0.02 to 0.11 (Table S1). A similar behavior was reported in another study of thermosonicated soursop nectar with 35 % pulp (15).

**Effect of thermoultrasound on viscosity, physical stability, cloud index (CI) and pectin methylesterase (PME) of soursop nectar**

The viscosity and CI values of the treated samples by TUS were high (309.33 to 432.67 cP and 0.16 to 0.25, respectively) in comparison with the control (220.67 cP and 0.15, respectively). While in physical stability, thermoultrasonicated samples presented low values (43.98 to 62.72 %) with respect to untreated nectar (63.24 %). Regarding the activity of PME, the control sample obtained 0.34 PMEU/mL and treated soursop nectars had 0.22 to 0.33 PMEU/mL (Table S1). The increase of the viscosity in the treated samples is because the ultrasound generates the fragmentation of large particles by the effect of cavitation, as well as the temperature during sonication that increases the solubility of the pectin particles (29). With respect to increase of CI in thermoultrasonicated soursop nectars, it may be due to the collapse of bubbles formed during cavitation, which disintegrates molecules and particles (30). Regarding inactivation of PME, it is attributed to free radicals generated during thermoultrasonication, also by mechanical effect that denatures the enzyme (31).

**L*, a*, b*, chroma, hue and total color difference (ΔE) of thermoultrasonicated soursop nectar**
The values of $L^*$ (lightness), $a^*$ (green-red) and $b^*$ (blue-yellow), as well as chroma ($C^*$) and hue ($h^*$) were obtained (Table S2). The control nectar was slightly darker showing low values in $L^*$ parameter (40.30) than the treated samples by TUS (44.46 to 50.70). For the $a^*$ and $b^*$ coordinates, the untreated nectar exhibited values of -0.57 and 3.83, respectively, while the thermoultrasonicated samples were in ranges of -1.03 to -0.07 and 2.38 to 3.99, respectively, placing all samples in the green-yellow quadrant. Respect to $C^*$ and $h^*$, the control sourssop nectar had 3.87 and -81.59, respectively, which were between the ranges found in the samples by TUS (2.52 to 4.01 and -88.93 and -70.75, respectively). The $L^*$, $b^*$ and $C^*$ parameters presented $R^2$ of 0.94, 0.93, 0.95 and adj-$R^2$ of 0.89, 0.88 and 0.91, respectively, showing high correlation degree. A According the regression coefficient results, time in its quadratic term ($\beta_{22}$) significantly ($p<0.0001$) influenced in the $L^*$ parameter (Table 1). This can be seen in Fig. 1a, where to longer exposure thermoultrasound time, the luminosity increases. The $b^*$ coordinate was affected ($p<0.001$) by amplitude*time interaction ($\beta_{12}$), observing in Fig. 1b that increasing amplitude and time, the $b^*$ value was high. For chroma, the linear term of amplitude ($\beta_1$), the interaction term ($\beta_{12}$), as well as the time in its quadratic term $\beta_{22}$, significantly ($p<0.001$) impacted (Table 1). The positive effect of $\beta_1$ and $\beta_{12}$ can be seen in Fig. 1c, where to greater the amplitude and time, the chromaticity increased.

Table 1 Effect of thermoultrasound on total phenolic content (TPC), antioxidant activity by ABTS\textsuperscript{•+}, DPPH\textsuperscript{•} and FRAP of sourssop nectar

The values of TPC and antioxidant activity (ABTS\textsuperscript{•+}, DPPH\textsuperscript{•} and FRAP) are presented in Table S2. In TPC and FRAP, the thermoultrasonicated sourssop nectars had high values (30.12 to 43.45 mg GAE/100 mL and 2.13 to 3.59 µmol Fe (II)/100 mL, respectively) in comparison with control sample (25.90 mg GAE/100 mL and 1.83 µmol Fe (II)/100 mL, respectively). While in antioxidant activity by ABTS\textsuperscript{•+} and DPPH\textsuperscript{•}, the untreated nectar showed 33.36 µmol TE/100 mL and 90 µmol TE/100 mL, respectively, and were within the ranges obtained of the treated samples by TUS (24.56 to 41.69 µmol TE/100 mL and 42.89 to 141.50 µmol TE/100 mL, respectively).

On the other hand, all response variables showed a correlation of $R^2$ between 0.91 and 0.95, while adj-$R^2$ of 0.83 to 0.91 (Table 1). According to the regression coefficient, the linear term of time ($\beta_2$) had a significant ($p<0.0001$) influence on TPC (Table 1), presenting an increase in phenolic content to longer the thermoultrasound treatment time (Fig. 2a). Regarding antioxidant activity by ABTS\textsuperscript{•+}, the linear terms of amplitude ($\beta_1$) and time ($\beta_2$) were affected ($p<0.0001$ and $p<0.001$, respectively) (Table 1), appreciating it in Fig. 2b, that with a greater amplitude as well as
thermoultrasound time, antioxidant activity increases. Respect to activity by DPPH•, the quadratic term of time ($\beta_{22}$) had a negative effect ($p<0.0001$), while amplitude*time interaction ($\beta_{12}$), an opposite effect ($p<0.05$) was presented (Table 1). This means that increasing the time in the thermoultrasound causes a lower antioxidant activity by DPPH•, while the combination of amplitude with time generates a higher inhibition of the DPPH• radical (Fig. 2c). In relation to FRAP, significantly influenced ($p<0.001$) the linear term of $\beta_2$ and interaction term ($\beta_{12}$) at $p<0.01$ (Table 1), causing a high antioxidant activity (Fig. 2d).

**Optimal condition of TUS process and reproducibility of the study**

As mentioned earlier, the response variables that fit the mathematical model were color ($L^*$, $b^*$ and $C^*$), TPC, antioxidant activity by ABTS••, DPPH• and FRAP. Hence, optimal condition of the TUS process obtained was of 82 % of amplitude for 9.15 min and the predicted values of response variables were 45.48±0.74, 3.55±0.17, 3.62±0.14 for $L^*$, $b^*$ and $C^*$, respectively. In TPC was 38.40±1.39 mg GAE/100 mL, while to antioxidant activity by ABTS•• had 31.28±1.52 µmol TE/100 mL, DPPH• of 124.22±14.54 µmol TE/100 mL and FRAP of 3.06±0.18 µmol Fe (II)/100 mL.

Contour figures of each response variable to make an overlap between them and observe the optimal process condition (Fig. 3) were elaborated. To verify the reproducibility of the optimal process condition of thermoultrasound (82 % of amplitude for 9.15 min), the experimental values with the predicted values were compared. Some color parameters ($L^*$ and $b^*$ coordinate) and antioxidant activity (measured by DPPH• and FRAP) showed no significant differences ($p>0.05$), indicating that it is possible to reproduce this optimal TUS condition, while $C^*$, TPC and ABTS•• had significant differences ($p<0.05$) (data not shown).

**Comparison between optimal condition of TUS with pasteurized soursop nectar**

**Effect of treatments on aerobic mesophilic and Enterobacteriaceae count in soursop nectars**

The pasteurized soursop nectar and optimized TUS sample presented total inactivation for aerobic mesophilic (AM) and Enterobacteriaceae count (EB), while the untreated soursop nectar had 3.65±0.06 log CFU/mL and 3.72±0.06 log CFU/mL for AM and EB, respectively (data not shown). Similar results were observed in a thermosonicated soursop nectar at 87.5 % of amplitude for 10 min (32). The Center for Food Safety establishes as "satisfactory" category to foods with values less than 5.7 log CFU/mL and <3.7 log CFU/mL to AM and EB, respectively (33), therefore, the treated nectars
complied with the limits established. The bacterial inactivation in pasteurized soursop nectar is due to this heat treatment alters the cell membrane stability, reducing the thickness of the phospholipid bilayer, and in consequence the increases of its permeability (34). While in the optimized TUS sample, the damage is caused by the shearing and cavitation where pressure changes occur (compression and decompression of gas bubbles) and high temperatures, generating cell lysis (5).

**Effect of treatments on color parameters in soursop nectar**

The lightness ($L^*$), $a^*$ and $b^*$ coordinates; $C^*$ and $h^\circ$, as well as total color difference ($\Delta E$) are presented in Table 2. The optimized TUS soursop nectar had the high value ($p<0.05$) of $L^*$ (45.56) in comparison with the other samples. Similar behavior was reported in a thermosonicated soursop nectar (32). The increase of $L^*$ in the optimized TUS nectar is attributed to partial precipitation of suspended particles that are not stable (34). In $a^*$ and $b^*$ coordinates, as well as $C^*$ parameter, the control sample showed significantly ($p<0.05$) high values with respect to treated samples. Different results were obtained in $a^*$ and $b^*$ in the thermosonicated soursop nectar (35). Regarding the $h^\circ$ parameter, the optimized TUS treatment expressed higher value ($p<0.05$) than the pasteurized and untreated nectar. The different trends with the other studies are probably due to the equipment used to the processing conditions, to the origin of the fruit and the nectar formulation. For the total color difference ($\Delta E$), taking as reference the control sample, the pasteurized soursop nectar obtained low value in comparison to the optimal thermoultrasound treatment. For $\Delta E$ using the pasteurized sample as reference, the optimized TUS nectar had slightly higher value unlike the untreated nectar (Table 2). If the value of $\Delta E$ is > 3.5, the color difference is perceivable by the human eye (36), so that the difference between the optimized TUS sample and the pasteurized it is not appreciated.

**Table 2**

<table>
<thead>
<tr>
<th>Viscosity, physical stability and pectin methylesterase activity in treated soursop nectars</th>
</tr>
</thead>
<tbody>
<tr>
<td>The pasteurized soursop nectar showed significantly ($p&lt;0.05$) high viscosity, followed by the optimized TUS sample (Table 3). An opposite behavior was reported in a thermosonicated carrot juice with orange pulp (37). The viscosity increase in pasteurized nectar, possibly due to the effect of temperature on pectin and other solids in the medium, increasing the degree of solubility (38). The high values of viscosity in the optimized TUS soursop nectar compared to the control, may be due to the particle size reduction by the disruptive effect of ultrasound, where interactions between small particles result in an increase of viscosity (29).</td>
</tr>
</tbody>
</table>

**Table 3**
Regarding physical stability, the untreated nectar and the optimized TUS soursop nectar presented similar values (p>0.05), while the pasteurized sample obtained high stability (p<0.05) (Table 3). Similar behavior was reported in a thermoultrasonicated prickly pear juice (13). According to the pectin methylesterase activity values obtained in the control soursop nectar (0.40 PMEU/mL), the pasteurized sample and the optimized TUS nectar reduced (p<0.05) the PME enzyme at 0.20±0.02 and 0.28±0.02 PMEU/mL, corresponding to 50 % and 69 % residual activity, respectively (data not shown). In thermoultrasonicated jackfruit nectar a similar behavior was described (3). The enzyme inactivation in pasteurized soursop nectar, is due to heat that causes hydrogen bond breakage, unfolding of protein structures and deamination in the enzyme (39). While thermoultrasound provokes enzyme denaturation by interaction with free radicals and shearing originating from cavitation (31).

Total, soluble and insoluble dietary fiber content in treated soursop nectars

The Table 3 shows the data obtained for total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Control soursop nectar and optimized TUS sample were significantly (p<0.05) a high content of TDF with respect to the pasteurized nectar. The percentage of SDF in the optimized TUS nectar was significantly (p<0.05) high in comparison with the other samples, while in IDF was the untreated nectar. The values obtained in the control, optimized TUS and pasteurized samples for TDF and SDF were higher than described by Anaya-Esparza et al. (15). It has been reported that the addition of inulin increases the soluble fiber content (40). The increase of SDF in the optimized TUS soursop nectar, may be due to acoustic and thermal energy together, breakdown of plant membranes, thus, increasing solubility in fibrous structures (41).

Effect of treatments on microstructure of soursop nectar

Images obtained of soursop nectars through scanning electron microscopy (250x and 500x magnifications) are presented in Fig. 4. Amorphous structures, possibly cellulose, starch and fiber in all the samples can be observed. The control sample (Fig. 4a and Fig. 4b) and pasteurized soursop nectar (Fig. 4e and Fig. 4f) presented flake-like lamella structures with smooth surfaces, while the optimized TUS sample (Fig. 4c and Fig. 4d) showed greater fragmentation of the structures and a rough shape. Which probably allowed the release of bioactive compounds from the food matrix. This last was observed in phenolic content and antioxidant activity increase (see following section).
Effect of treatments on total phenolic content (TPC), antioxidant activity and its in vitro bioaccessibility in soursop nectar

The Fig. 5 shows the antioxidants before (original sample) and after the digestion process (bioaccessible or dialyzed fraction). In the original sample, the optimized TUS nectar exhibited the highest value (p< 0.05) of TPC content (26.63±0.92 mg GAE/100 mL), while the untreated nectar and pasteurized sample had similar content (p>0.05) (18.65±2.51 mg GAE/100 mL and 17.73±0.54 mg GAE/100 mL, respectively) (Fig. 5a). A different result was reported in thermosonicated soursop nectar by Anaya et al. (32). The high content of TPC in the optimized TUS nectar may be due to the cell walls rupture, releasing the phenolic compounds towards of the medium (42).

In the in vitro intestinal bioaccessibility results (bioaccessible fraction), a partial bioaccessibility in all samples was observed. The optimized TUS nectar (15.26±0.82 mg GAE/100 mL) showed higher value (p<0.05) than the control and pasteurized (Fig. 5a), with an absorption of 57.29 % with respect to its original sample. A similar behavior in a thermosonicated tomato juice was found (43). Partial absorption is related to the quantity of polyphenols that are released by the digestion process and solubilized in the intestinal fluid (44).

The antioxidant activity results from the three methodologies are presented in Fig. 5. ABTS•⁺, DPPH• and FRAP in the original sample (before in vitro intestinal bioaccessibility analysis) were significantly (p<0.05) higher in the optimized TUS soursop nectar (22.03±2.24 µmol TE/100 mL, 129.21±8.94 µmol TE/100 mL and 3.10±0.09 µmol Fe(II)/100 mL, respectively) than control sample (17.27±1.28 µmol TE/100 mL, 118.61±6.93 µmol TE/100 mL and 2.41±0.10 µmol Fe(II)/100 mL, respectively) and pasteurized nectar (13.45±1.16 µmol TE/100 mL, 104.51±6.56 µmol TE/100 mL and 2.08±0.11 µmol Fe(II)/100 mL, respectively) (Fig. 5b, Fig. 5c and Fig. 5d). Different results were found in a thermosonicated soursop nectar (35). The higher antiradical activity in ABTS•⁺ and DPPH• in the optimized TUS, is attributed to the effect of sonication which generates hydroxylation in the ortho or para positions of aromatic ring of phenolic compounds, increasing the antioxidant activity (45) and it has been reported that phenolic acids found in soursop correlate with antioxidant activity by FRAP (46).

After of digestion process (in vitro intestinal bioaccessibility), the optimized TUS nectar had high (p<0.05) antioxidant activity by ABTS•⁺ (34.92±1.76 µmol TE/100 mL) in comparison with the other samples (Fig. 5b). In all nectars, the percentage of the antioxidant activity in the dialyzed fraction was more than 100 % (in relation to the original samples). Similar behavior was described in a thermoultrasonicated beetroot juice (6). Respect to DPPH•, antioxidant activity was not found in the
nectars (Fig. 5c). Finally, in the results of FRAP, the optimized TUS sample was significantly high (7.88±0.59 µmol Fe(II)/100 mL) in relation with the other samples (Fig. 5d). All treatments with respect to its original sample, showed more than double of the antioxidant activity (FRAP) in its dialyzed fraction. The high antioxidant activity in ABTS\textsuperscript{•+} and FRAP could be due to deprotonation of hydroxyl groups after passing from an acidic to an alkaline environment, increasing hydrogen donation (46). But, also depends to the antioxidant selectivity of each methodology, e.g., the ABTS\textsuperscript{•+} method is more reliable due to its solubility in both aqueous and organic solvents, in addition to a fast reaction with lipophilic and hydrophilic antioxidants compared to DPPH\textsuperscript{•} (27).

CONCLUSIONS

The results demonstrated that in thermoultrasound technology applied to soursop nectar reduces aerobic mesophilic (complying with European Commission regulations), even some treatments presented a total inactivation in enterobacteriaceae. Almost all the treatments remained unchanged or slight increases in physicochemical parameters. The application of the response surface methodology is an adequate tool for the optimization of the thermoultrasound process in soursop nectar for color ($L^*$, $b^*$, $C^*$), total phenolic content and antioxidant activity (ABTS\textsuperscript{•+}, DPPH\textsuperscript{•} and FRAP). High correlation degree, adequate fit of the mathematical model and an optimal condition of thermoultrasound processing of 82 % of amplitude for 9.15 min was obtained.

The samples that were treated with optimal condition of thermoultrasound and pasteurization had similar behavior in terms of microbiological quality (total microorganisms inactivation), reduction of pectin methylesterase and color ($\Delta E$). Both technologies complied with international requirements in total microorganisms. The optimized TUS sample exhibited pronounced changes in its microstructure, as well as high content of antioxidant compounds before (original sample) and after (dialyzed fraction) in vitro intestinal bioaccessibility. Therefore, the optimal condition of thermoultrasound of soursop nectar could be considered a drink with greater effects on the consumer health. Further research is necessary such as product acceptability, shelf life, identification of bioactive compounds and impact on the heath.

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CONFLICT OF INTEREST
The authors report no potential conflict of interest relevant to this article.

SUPPLEMENTARY MATERIALS
All supplementary materials are available at: www.ftb.com.hr.

AUTHORS’ CONTRIBUTION
Project administration, conceptualization, methodology and supervision were performed by Nelly del Socorro Cruz-Cansino and Ernesto Alanís-García. Data collection, data and formal analysis were performed by Esther Ramírez-Moreno and José Alberto Ariza-Ortega. Experimental laboratory-data collection and validation tools were performed by José Luis Jiménez Hernández. The interpreting data and first draft of the manuscript was written by Quinatzin Yadira Zafra-Rojas, Enrique Javier Olloqui and Juan Carlos Moreno-Seceña. All authors participated in reviewing, editing and approved the final manuscript.

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Table 1. Model regression coefficient and significance for the response variables of thermoultrasonicated soursop nectar

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$L^*$</th>
<th>$b^*$</th>
<th>$C^*$</th>
<th>TPC*</th>
<th>ABTS</th>
<th>DPPH</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$ (intercept)</td>
<td>45.48ª</td>
<td>3.55ª</td>
<td>3.62ª</td>
<td>38.40ª</td>
<td>31.28ª</td>
<td>124.22ª</td>
<td>3.06ª</td>
</tr>
<tr>
<td>$\beta_1$ (amplitude)</td>
<td>0.02</td>
<td>0.30c</td>
<td>0.30b</td>
<td>-0.26</td>
<td>4.21ª</td>
<td>-6.94</td>
<td>0.02</td>
</tr>
<tr>
<td>$\beta_2$ (time)</td>
<td>0.18</td>
<td>0.15d</td>
<td>0.18c</td>
<td>4.04ª</td>
<td>2.94b</td>
<td>4.39</td>
<td>0.43b</td>
</tr>
<tr>
<td>$\beta_{12}$ (amplitude*time)</td>
<td>1.41c</td>
<td>0.49b</td>
<td>0.43b</td>
<td>0.78</td>
<td>-2.00d</td>
<td>21.35d</td>
<td>0.31c</td>
</tr>
<tr>
<td>$\beta_{11}$ (amplitude*amplitude)</td>
<td>0.90d</td>
<td>-0.21d</td>
<td>-0.21c</td>
<td>-3.53b</td>
<td>1.75d</td>
<td>-7.44</td>
<td>-0.20d</td>
</tr>
<tr>
<td>$\beta_{22}$ (time*time)</td>
<td>2.46ª</td>
<td>-0.30c</td>
<td>-0.28b</td>
<td>-0.51</td>
<td>1.22d</td>
<td>-40.25ª</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

$R^2$ | 0.94 | 0.93 | 0.95 | 0.95 | 0.91 | 0.91 | 0.91 |
| Adj-$R^2$ | 0.89 | 0.88 | 0.91 | 0.91 | 0.90 | 0.84 | 0.83 |

Significance levels: *$p<0.0001$; †$p<0.001$; ‡$p<0.01$; ¶$p<0.05$. *TPC: Total phenol content

Table 2. Color parameters of soursop nectar samples

<table>
<thead>
<tr>
<th>Coordinates</th>
<th>Control</th>
<th>Optimized TUS</th>
<th>Pasteurized</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$</td>
<td>40.45±0.35c</td>
<td>45.56±0.30ª</td>
<td>42.57±0.28b</td>
</tr>
<tr>
<td>$a^*$</td>
<td>-1.24±0.06ª</td>
<td>-1.58±0.04c</td>
<td>-1.39±0.06b</td>
</tr>
<tr>
<td>$b^*$</td>
<td>5.32±0.19ª</td>
<td>3.68±0.36c</td>
<td>4.25±0.02b</td>
</tr>
<tr>
<td>$C^*$</td>
<td>5.29±0.20ª</td>
<td>3.37±0.17c</td>
<td>4.47±0.02b</td>
</tr>
<tr>
<td>$h^\circ$</td>
<td>-75.63±0.74c</td>
<td>-56.49±0.88ª</td>
<td>-71.54±0.37b</td>
</tr>
<tr>
<td>$\Delta E$ Control</td>
<td>-</td>
<td>5.39±0.32</td>
<td>2.38±0.26</td>
</tr>
<tr>
<td>$\Delta E$ Pasteurized</td>
<td>2.38±0.39</td>
<td>3.07±0.31</td>
<td>-</td>
</tr>
</tbody>
</table>
Optimized TUS (82 % of amplitude for 9.15 min), Pasteurized (65°C for 30 min), $L^*$: lightness; $a^*$: red-green; $b^*$: yellow-blue; $C^*$: Chroma; $h^\circ$: Hue; $\Delta E$ Control: total color difference relative to control; $\Delta E$ Pasteurized: color difference relative to pasteurized. Mean ± standard deviation. $a$-$c$ Different letters between rows indicate significant difference ($p<0.05$)

Table 3. Viscosity, stability and dietary fiber content in soursop nectar

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity (cP)</th>
<th>Stability (%)</th>
<th>TDF (%)</th>
<th>SDF (%)</th>
<th>IDF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>151.56±11.61c</td>
<td>55.09±2.87b</td>
<td>3.58±0.01a</td>
<td>3.27±0.01b</td>
<td>0.31±0.03a</td>
</tr>
<tr>
<td>Optimized TUS (82 % of amplitude for 9.15 min)</td>
<td>186.89±10.54b</td>
<td>54.68±1.35b</td>
<td>3.53±0.05a</td>
<td>3.32±0.02a</td>
<td>0.22±0.06b</td>
</tr>
<tr>
<td>Pasteurized (65°C for 30 min)</td>
<td>274.44±32.75a</td>
<td>67.37±1.15a</td>
<td>3.12±0.02b</td>
<td>2.94±0.03c</td>
<td>0.19±0.02b</td>
</tr>
</tbody>
</table>

Mean ± standard deviation. TDF: total dietary fiber, SDF: soluble dietary fiber, IDF: insoluble dietary fiber. $a$-$c$ Different letters between rows indicate significant difference ($p<0.05$)
Fig. 1. Effect of thermoultrasound on: a) $L^*$, b) $b^*$ and c) chroma in soursop nectar
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Fig. 2. Effect of thermoultrasound on: a) total phenolic content and antioxidant activity by b) ABTS, c) DPPH and d) FRAP of soursop nectar

Fig. 3. Superposition of contour plots for the response variables ($L^*$, $b^*$, $C^*$, TPC: GAE/(mg/100 mL), ABTS: TE/(μmol/100 mL), DPPH: TE/(μmol/100 mL) and FRAP: Fe(II)/(μmol/100 mL)) and optimum condition (82 % of amplitude for 9.15 min) for the thermoultrasonicated soursop nectar
Fig. 4. Scanning electron microscopy (SEM) of freeze-dried soursop nectar: a) and b) control at 250 and 500×, respectively, c) and d) optimized TUS (82 % amplitude for 9.15 min) at 250 and 500×, respectively, and e) and f) pasteurized (65 °C for 30 min) at 250 and 500×, respectively
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Fig. 5. Total phenolic content and antioxidant activity in the original sample and bioaccessible fraction of optimized TUS (82% amplitude for 9.15 min) and pasteurized (65 °C for 30 min) soursop nectar: a) TPC, b) ABTS, c) DPPH and d) FRAP. Different letters indicate significant differences (p<0.05) between samples. A-C indicate significant differences (p<0.05) between bioaccessible fractions. ND: not detected.
Table S1. Experimental design of thermoultrasoicated soursop nectar on microbiological and physicochemical parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pattern</th>
<th>Amplitude /%</th>
<th>t/min</th>
<th>AM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EB&lt;sup&gt;b&lt;/sup&gt;</th>
<th>pH</th>
<th>TSS&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Titratable acidity</th>
<th>BI&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Viscosity&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Physical stability&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Cloud index</th>
<th>PME&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>5.24±0.34</td>
<td>5.00±0.43</td>
<td>3.91±0.03</td>
<td>11±0.00</td>
<td>0.25±0.04</td>
<td>0.05±0.00</td>
<td>220.67±7.02</td>
<td>63.24±1.14</td>
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</tr>
<tr>
<td>1</td>
<td>+</td>
<td>90</td>
<td>5.00</td>
<td>3.78±0.02</td>
<td>3.74±0.05</td>
<td>3.85±0.03</td>
<td>11±0.00</td>
<td>0.27±0.00</td>
<td>0.06±0.00</td>
<td>341.33±9.02</td>
<td>60.19±1.85</td>
<td>0.22±0.01</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>2</td>
<td>a0</td>
<td>72</td>
<td>9.15</td>
<td>3.64±0.16</td>
<td>3.60±0.05</td>
<td>3.87±0.01</td>
<td>11±0.00</td>
<td>0.22±0.04</td>
<td>0.08±0.00</td>
<td>309.33±4.16</td>
<td>60.03±0.53</td>
<td>0.21±0.00</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>3</td>
<td>0a</td>
<td>82</td>
<td>3.15</td>
<td>3.82±0.02</td>
<td>3.88±0.01</td>
<td>3.79±0.02</td>
<td>11±0.00</td>
<td>0.27±0.00</td>
<td>0.06±0.00</td>
<td>350.67±8.08</td>
<td>62.72±1.10</td>
<td>0.21±0.00</td>
<td>0.33±0.00</td>
</tr>
<tr>
<td>4&lt;sup&gt;i&lt;/sup&gt;</td>
<td>00</td>
<td>82</td>
<td>9.15</td>
<td>1.85±0.21</td>
<td>0.0±0.00</td>
<td>4.06±0.01</td>
<td>11±0.00</td>
<td>0.22±0.04</td>
<td>0.06±0.00</td>
<td>408.00±24.25</td>
<td>59.71±1.13</td>
<td>0.25±0.00</td>
<td>0.28±0.02</td>
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<tr>
<td>5&lt;sup&gt;i&lt;/sup&gt;</td>
<td>00</td>
<td>82</td>
<td>9.15</td>
<td>3.57±0.02</td>
<td>3.38±0.05</td>
<td>3.78±0.01</td>
<td>11±0.00</td>
<td>0.13±0.00</td>
<td>0.05±0.00</td>
<td>329.33±31.13</td>
<td>61.80±0.23</td>
<td>0.23±0.01</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>6</td>
<td>0A</td>
<td>82</td>
<td>15.00</td>
<td>1.85±0.21</td>
<td>0.0±0.00</td>
<td>4.04±0.02</td>
<td>11±0.00</td>
<td>0.20±0.00</td>
<td>0.06±0.00</td>
<td>380.67±1026</td>
<td>59.28±0.42</td>
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<td>0.22±0.01</td>
</tr>
<tr>
<td>7</td>
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<td>90</td>
<td>13.30</td>
<td>2.81±0.19</td>
<td>2.65±0.05</td>
<td>3.71±0.01</td>
<td>11±0.00</td>
<td>0.16±0.04</td>
<td>0.06±0.00</td>
<td>364.00±11.14</td>
<td>56.69±0.93</td>
<td>0.23±0.03</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>8</td>
<td>--</td>
<td>75</td>
<td>5.00</td>
<td>2.20±0.17</td>
<td>1.80±0.58</td>
<td>3.75±0.01</td>
<td>11±0.00</td>
<td>0.20±0.00</td>
<td>0.02±0.00</td>
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<td>0.16±0.01</td>
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<sup>a</sup>Central points. Mean ± standard deviation; <sup>b</sup>AM: Aerobic Mesophilic (log CFU/mL); <sup>c</sup>EB: Enterobacteriaceae count (log CFU/mL); <sup>c</sup>TSS: Total Soluble Solids (°Brix); <sup>d</sup>BI: Browning Index; <sup>e</sup>Viscosity (cP); <sup>f</sup>Physical stability (%); <sup>g</sup>PME: Pectin methylesterase (pectin methylesterase units (PMEU))
Table S2. Experimental design of thermoultrasonicated soursop nectar on color, TPC and antioxidant activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amplitude/ %</th>
<th>t/min</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>C*</th>
<th>Hue (°)</th>
<th>TPC±</th>
<th>ABTS±</th>
<th>DPPH±</th>
<th>FRAP±</th>
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<tbody>
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<td>3.66±0.04</td>
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*Central points. Mean ± standard deviation; C*: Chroma; aTPC Total Phenol Content; GAE: Gallic acid equivalents; bTE: Trolox equivalents; cFe (II) ferrous ion