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Effect of thermosonication on polyphenol oxidase inactivation and quality parameters of soursop nectar

Luis M. Anaya-Esparza [a], Rita M. Velázquez-Estrada [a], Sonia G. Sayago-Ayerdi [a], Jorge A. Sánchez-Burgos [a], Marco Vinicio Ramírez-Mares [b], María de Lourdes García-Magaña [a], Efigenia Montalvo-González [a, *]

[a] Instituto Tecnológico de Tepic, Laboratorio de Integral de Investigación de Alimentos, Av. Tecnológico 2595, P.C 63175, Tepic, Nayarit, Mexico
[b] Instituto de Recursos, Universidad del Mar, campus Puerto Ángel, Carretera a Zipolite Km 1.5, C.P. 70902, Puerto Ángel, San Pedro Pochutla, Oaxaca, Mexico

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ABSTRACT

In the present study, the effect of thermosonication on polyphenol oxidase (PPO) inactivation and quality parameters of soursop nectar was evaluated [at 24 kHz, average temperature of 34, 44 and 54 °C, acoustic energy density (AED) (1.1, 1.2 and 1.4 W/mL) and processing time (2, 6 and 10 min)]. Classical thermal pasteurization (65 °C, 30 min) reduced 81% PPO activity and ascorbic acid content by 36% in comparison to fresh nectar. All treatments with thermosonication at 34 or 44 °C decreased the initial PPO activity between 34 and 67%; while treatments at 54 °C decrease PPO activity among 91 and 99%. Also these last treatments had 90% retention of ascorbic acid, without significant changes in quality parameters. In conclusion, thermosonication can be an excellent alternative to total PPO inactivation, without changes in the quality of soursop nectar.

1. Introduction

Juices and nectars are subject to rapid microbial growth, enzymatic browning and chemical and physical deterioration if they are not properly processed (Nwachukwu & Ezeigdo, 2013). Traditionally, the shelf-life stability (microbial and enzymatic) of juices or nectars has been achieved by thermal processing (pasteurization at 60–90 °C and sterilization 90–120 °C). Soursop pulp is susceptible to spoilage and browning catalysed by polyphenol oxidase enzyme (PPO), affecting their sensory and nutritional qualities (Dias et al., 2015). Several authors have studied the microbial and enzymatic stability of different products prepared with soursop fruit: pasteurized juices with or without preservatives (Abbo, Olurin, & Odeyemi, 2006; Nwachukwu & Ezeigdo, 2013) and pasteurized nectars (Peters, Badrie, & Comissiong, 2000). According to these reports, the pasteurization was effective on microbial and enzymatic inactivation of products made with soursop. However, thermal pasteurization caused undesirable alterations reducing the quality and freshness of the products.

Novel technologies have been investigated lately as complete or partial alternatives to conventional heat treatment, highlighting the use of ultrasound in the beverage industry (Aadil et al., 2015). Ultrasonic generators transform electrical energy into ultrasound energy via a piezoelectric transducer. The most significant effect associated with the transmission of ultrasound power inside a liquid is cavitation. The bubbles formed through cavitation increase rapidly in size and collapse under the influence of the acoustic field (Louisnard & González, 2011). However, it has been reported that ultrasound is often more effective when combined with other preservation methods, such as heat (temperature 30–50 °C) to increase the enzymatic and microbial inactivation. Combination of ultrasound and heat is known as thermosonication and products treated by this technology have better results with minimal impact on quality parameters, in comparison to ultrasound alone on PPO inactivation in fruit juice (Aadil et al., 2015). Several studies have been done with different fruit juices treated by thermosonication, finding optimum conditions of processing that ensure both quality and safety, particularly in grape juice (Aadil et al., 2015), apple juice (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014) and mango juice (Kiang, Bhat, Rosma, & Cheng, 2013) among others.

Although several works have been published about ultrasound

* Corresponding author.
E-mail address: efimontalvo@gmail.com (E. Montalvo-González).

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processing of fruit juices, there are few reports on enzymatic inactivation of PPO by thermosonication. In addition, the effect of thermosonication treatment on the quality of soursop nectar has not been reported yet. Therefore, in the present work we studied the processing of soursop nectar using thermosonication and evaluated the inactivation of PPO and its effect on quality parameters.

2. Materials and methods

2.1. Chemical reagents

All chemicals and solvents were of HPLC or analytical grade, Catechol, Bradford reagent, ascorbic acid standard, 2,4-dinitrophenyl hydrazine (DNPH), albumin bovine serum, phenolphthalein and thiourea were purchased from Sigma-Aldrich Chemical Company (St. Lois, MO, USA).

2.2. Preparation of soursop nectars

Mature soursop fruits were obtained from an orchard located in the village El Tonino, near Tepic, Nayarit, Mexico. The pulp was diluted with purified water (350 g/L); subsequently the mixture was homogenized, and then adjusted with sucrose (50 g/L), according to the requirements of the *Codex Alimentarius* for Fruit Juices and Nectars (CODEX STAN 247-2005). Two control soursop nectars were considered; one fresh unpasteurized nectar (without treatment) and the other thermally pasteurized at 65 ± 1 °C for 30 min, this particular temperature and time were chosen to simulate the conventional batch pasteurization process as mentioned by Bermúdez-Aguirre, Mawson, and Barbosa-Cánovas (2011).

2.3. Thermosonication treatment

The utrasonic processor (Hielsher UP400S, Teltow, Germany) used in this study consisted of a sonotrode attached to an electroacoustic transducer. The sonotrode (Hielscher H7 Tip 7, Teltow, Germany) has a tip diameter of 7 mm and an acoustic power density of 300 W/cm^2; the maximum immersion depth is 90 mm. The ultrasonic processor had a fixed working frequency of 24 ± 1 kHz, with continuously adjustable output amplitude (20–100%). The unit was used at maximum output, which corresponded to a specified power consumption of 400 W. A shaking water bath (Thermo Scientific 2870, Ohio, USA) was used to maintain a constant temperature. For each treatment, 200 mL of soursop nectar was placed in a 250 mL beaker. The samples were treated by thermosonication for 2, 6 or 10 min; at initial temperatures of 30, 40 or 50 °C, respectively (water bath) and the acoustic energy density (AED) was adjusted to 1.1, 1.2 and 1.4 W/mL respectively, according to equation (1) as reported by O'Donnell, Tiwari, Bourke, and Cullen (2010). These AED values correspond to 210, 240 and 270 W of ultrasound power respectively. Temperatures inside the samples were checked and recorded before (initial) and after (final) treatment as can be seen in Table 1. The average increase of temperature was 0.7 °C (2 min), 2.4 °C (6 min) and 6.4 °C (10 min). The average temperature was considered as the treatment temperature.

$$AED = P/V \quad (1)$$

AED = Acoustic energy density (W/mL).
P = Absolute ultrasound power (W).
V = Volume of the medium (mL).

2.4. Polyphenol oxidase and residual enzymatic activity

Enzymatic extraction was done according to the method described by Bora, Holschuh, and da Silva (2004). The enzymatic extract was obtained by mixing 5 mL of soursop nectar with 5 mL of phosphate buffer (pH 7.5), and then centrifuged (Hermle Z306, Wehingen, Germany) at 9380 *g* (40 min at 4 °C). PPO activity was determined in the supernatant using catechol as a substrate. The absorbance was measured immediately after the addition of the crude enzymatic extract at 420 nm (Jenway 6705 spectrophotometer, Felsted, UK) at intervals of 15 s for at least 3 min. The linear portion of the curve was used to compute the enzyme activity units (U) by plotting absorbance as a function of time. One unit of activity was considered as the quantity of enzyme, which caused an increase of 0.001 of absorbance per minute per millilitre (Guerrero-Beltrán, Barbosa-Cánovas, Moraga-Ballesteros, Moraga-Ballesteros, & Swanson, 2006). Results were expressed as specific activity of PPO (U/mg protein). Total protein concentration (mg) was determined by the Bradford method (Bradford, 1976). Residual enzymatic activity of PPO (PPO_{RA}) (%) was calculated according to equation (2) (Fonteles et al., 2012).

$$PPO_{RA}\ (\%) = (As/Ao) \times 100 \quad (2)$$

As = PPO specific activity of thermosonicated sample.
Ao = PPO specific activity of control sample (untreated).

2.5. Colour, total colour difference and non-enzymatic browning index

Soursop nectar colour (L, a b) was measured using a Minolta Colorimeter (Konica Minolta CR-400, Osaka, Japan). Total colour difference (TCD) was determined using equation (3), which indicates the magnitude of colour change after treatment (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). Non-enzymatic browning (NEBI) was measured using the method of Meydav, Saguy, and Kopelman (1977), that consists in evaluating the non-enzymatic browning of nectar, measuring the absorbance change in an ethanol extract with inactivated PPO.

$$TCD = \sqrt{(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2} \quad (3)$$

Where: L_o, a_o and b_o are colour values of untreated nectar and L, a and b are values of treated nectar.

2.6. Ascorbic acid content

The ascorbic acid (AA) content was measured according to the method of Rahman, Mizanur, and Khan (2007). In this method bromine water oxidizes ascorbic acid to dehydroascorbic acid in the presence of acetic acid. After coupling with 2,4-dinitrophenyl hydrazine at 37 °C for 3 h, the solution is treated with H_2SO_4 solution (1.0 mol/L) to produce a red colour complex and the absorbance was spectrophotometrically measured at 521 nm. AA content was calculated using a calibration curve of standard ascorbic acid. Results were expressed as gram of AA per liter of nectar (g AA/L).

2.7. Total soluble solids, titratable acidity and pH

Total soluble solids (TSS) were determined by refractometry, using the Abbe refractometer (Abbe 315RS, Royal Tunbridge Wells, England). Titratable acidity (TA) was measured in 5 mL of nectar mixed with 25 mL of distilled water and titrated using an automatic

Table 1
Experimental matrix and thermosonication treatments (TS1 - TS9) obtained from 3^{3-1} fractioned factorial design and temperature before and after of treatments.

Treatments	Time (min)	Acoustic energy density (W/mL)	Initial temperature (°C)	Final temperature (°C)	Average temperature (°C)
Fresh control	–	–	–	–	–
Pasteurized	30	–	65	66.3 ± 0.3	–
TS1	2	1.1	30	31.0 ± 0.3	34 ± 3.0
TS2	6	1.4	30	33.3 ± 0.6	
TS3	10	1.2	30	37.0 ± 0.5	
TS4	2	1.4	40	41.0 ± 0.3	44 ± 2.5
TS5	6	1.2	40	44.1 ± 0.5	
TS6	10	1.4	40	46.0 ± 0.6	
TS7	2	1.2	50	51.0 ± 0.3	54 ± 2.5
TS8	6	1.4	50	53.1 ± 0.7	
TS9	10	1.4	50	56.4 ± 0.5	

Values in final temperature are the average and SD of triplicate determination from three different experiments (n = 9).

titrator (SCHOTT Instruments, Berlin, Germany) with NaOH 0.1 mol/L and using phenolphthalein as indicator. Results were reported as equivalent gram of malic acid per liter (g MAE/L) of nectar. pH was measured using a pH meter (HANNA Instruments, HI 221, Bedford, UK). All analyses were done in triplicate. All the measurements were performed according to the official AOAC methods (AOAC, 2005).

2.8. Experimental design and statistical analysis

All experiments were performed using a fractional factorial design (3^{3-1}), considering as factors at different levels: amplitude, time and temperature as shown in Table 1. Data analysis (pH, titratable acidity, total soluble solids, colour attributes (L, a, b), non-enzymatic browning index, ascorbic acid and polyphenol oxidase residual activity) was examined by analysis of variance (One-way ANOVA) and Tukey test ($p < 0.05$). Additionally, response surface methodology (RSM) was used to investigate the effect of three independent processing parameters (acoustic energy density, average temperature and time) on PPO residual activity. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out to obtain correlations between all variables and estimate the relationship between soursop nectar samples (control, pasteurized and thermosonication treatments). All data were analysed by the Statistica software, (v.10 Statsoft®, Tulsa, USA). The experiments were performed in triplicate three times and the nine samples analysed. Results obtained were presented as means ± standard deviation (SD).

3. Results and discussion

3.1. Polyphenol oxidase activity, residual enzymatic activity and response surface methodology

Table 2 shows PPO_{RA} in treated TS and control nectars, respectively. The pasteurized nectar (65 ± 1 °C) had PPO_{RA} of 19.4% (1.4 U/mg protein) in comparison with fresh control nectar (7.3 U/mg protein). The reduction of PPO_{RA} of the thermosonicated samples was dependent on experimental conditions. The thermosonicated samples with average temperature of 34 °C or 44 °C for any AED and time (TS1, TS2, TS3, TS4, TS5), had values of 34–67% (2.4–4.6 U/mg protein) of PPO_{RA}. For treatments TS6, TS7 and TS8, PPO_{RA} (average temperature 54 °C) was from 9.5 to 19% (0.7–1.4 U/mg protein). Although there was enzymatic activity this means that in these treatments there was a partial inactivation of PPO enzyme (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014). The lower PPO_{RA} was obtained at conditions of 1.4 W/mL AED, average temperature of 54 °C for 10 min (TS9), showing a reduction of PPO from 99% (0.03 U/mg protein). Similar results were reported by Sulaiman, Soo, Farid, and Silva (2015) by thermosonication treatment (24 kHz, 1.3 W/mL of AED, 10 min and 72 °C) during inactivation of PPO on fruit purees. Despite the temperature increases in the thermosonication process, the cavitation effect was not attenuated (Ugarte-Romero, Feng, & Martin, 2007). PPO inactivation by thermosonication has been attributed to an additive effect between cavitation and heat, causing protein denaturation, which is related to the reduction of specific enzyme activity and therefore, to the residual enzymatic activity (Aadil et al., 2015; Taylor, Islam, Zhang, & Adhikari, 2014). Dias et al. (2015) reported that ultrasound application at room temperature (25 °C) on soursop juice only reduced 15% of PPO activity, maintaining a PPO_{RA} of 85%. According to Knorr et al. (2011) if ultrasound is combined with temperatures ≥ 50 °C, the enzymatic inactivation range increases considerably compared to ultrasound alone. In some research when thermosonication was applied to grape (Aadil et al., 2015) and apple (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014) juices, a value of <10% of residual enzymatic activity of PPO was reported.

Table 2
Polyphenol oxidase residual activity (PPO_{RA}) and ascorbic acid (AA) content measured in fresh, pasteurized and thermosonicated (TS) soursop nectars.

Treatments	PPO_{RA} (%)	AA (g/L)
Fresh control	100	0.4 ± 0.01^{a}
Pasteurized	19.4 ± 0.92^{e}	0.2 ± 0.02^{d}
TS1	67.1 ± 1.74^{a}	0.3 ± 0.01^{ab}
TS2	51.0 ± 0.59^{b}	0.3 ± 0.01^{a}
TS3	34.0 ± 1.39^{d}	0.4 ± 0.02^{a}
TS4	52.0 ± 1.92^{b}	0.3 ± 0.01^{c}
TS5	39.4 ± 2.17^{c}	0.3 ± 0.02^{ab}
TS6	19.0 ± 1.81^{e}	0.4 ± 0.02^{a}
TS7	12.4 ± 0.96^{f}	0.3 ± 0.01^{bc}
TS8	9.5 ± 1.05^{g}	0.3 ± 0.01^{ab}
TS9	0.5 ± 0.08^{h}	0.4 ± 0.02^{a}

Values are the average of triplicate determination from three different experiments (n = 9) ± standard deviation (SD). Means in a column with different letters are significantly different ($p < 0.05$). TS1-TS9 = the key to the samples numbers can see in Table 1.

Polyphenol oxidase inactivation was observed in the whole experimental domain, even at low processing times and temperature. According to surface response methodology the better conditions to inactivate PPO were 1.3 W/mL, 8 min at 51 °C with a PPO_{RA} from 0.98% (0.06 U/mg protein). Also the regression model, the PPO_{RA} in soursop nectar can be predicted using the following polynomial equation (4) ($R^2 = 0.97$ with 95% confidence level):

$$PPO_{RA}(\%) = 597.3 - 932.9AED + 315.1AED^2 - 0.10T^2 + 3.80t - 0.61t^2 + 4.3AED^{*}T \quad (4)$$

"AED" Acoustic energy density (W/ml) "T" is the temperature (°C) and "t" is the treatment time (min).

Fitted function showed that reduction of PPO_{RA} is mainly dependent of AED, time and temperature (AED*T). Evelyn and Silva (2016) observed that an increase of AED during TS treatment produced a 6-fold reduction compared to the thermal process at the same temperature (75 °C). Similar trends were reported by Sulaiman et al. (2015) during TS (24 kHz, 1.3 W/g of AED and 10 min) for inactivation of PPO in pear, apple and strawberry purees, additionally, they mentioned that TS enhanced the PPO inactivation compared to thermal processing alone (60–75 °C 30 min) and that the inactivation of PPO by TS showed a linear behaviour. Table 3 shows the predicted and actual PPO_{RA} values in thermosonicated soursop nectar and similar values were observed in both cases. The Pareto chart (Fig. 1) shows the effect of independent variables on PPO_{RA}, at a confidence level of 95%, AED, time, temperature and additional interaction between AED-Temperature had a statistically significant effect ($p < 0.05$) on PPO_{RA}. Similar trends were observed by Fonteles et al. (2012) during the inactivation of PPO in cantaloupe melon juice treated by ultrasound. Recently, Baltacioglu, Bayindirly and Severcan (2017) investigated the effect of TS (24 kHz, 210 μm, 30 min and 60 °C) on the structure of mushroom PPO by using FTIR spectroscopy and reported that PPO inactivation was mainly due to the global conformation change of the enzyme and not by a minor change in the active site.

3.2. Ascorbic acid content

Ascorbic acid (AA) is considered an indicator of quality in fruit juices. For this reason, AA stability is important after any preservation treatment (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014). Fresh nectar control had an AA content of 0.4 g/L (Table 2) and similar results were reported in fresh soursop juice by Othman, Fabian, and Lugwisha (2014). The highest loss of ascorbic acid was 50% for pasteurized nectar, while TS1, TS2, TS4, TS5, TS7 and TS8 treatments reduced the value by 25% in comparison to fresh nectar. The AA content in the rest of the thermosonication treatments was similar at fresh nectar. Under thermosonication conditions, AA degradation is dependent on amplitude, acoustic energy density, temperature, and treatment time (Cruz, Vieira, & Silva, 2008; Tiwari, Patras, Brunton, Cullen, & O'Donnell, 2010). In this experiment operation conditions for TS3, TS6 and TS9 conserved AA content. It is possible this was because cavitation (10 min) facilitated the elimination of the dissolved oxygen in the medium, avoiding AA degradation and thus contributing to the stability of the compound in some thermosonicated samples (Cheng, Soh, Liew, & Teh, 2007). Several authors have applied thermosonication with different processing conditions to fruit juices, and they reported reductions of ascorbic acid less than 10% in grape (Aadil et al., 2015), apple (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014) and watermelon (Rawson et al., 2011) juices. Moreover, Martínez-Flores, Garnica-Romo, and Bermúdez-Aguirre (2015) reported retention of 100% of AA in carrot juice treated by thermosonication.

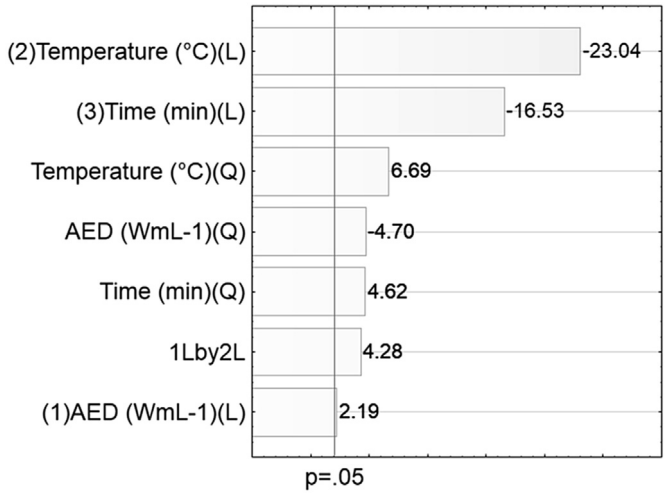

Fig. 1. Pareto chart of effects on residual enzymatic activity (PPO_{RA}) in thermosonicated soursop nectar (AED = acoustic energy density).

3.3. pH, titratable acidity and total soluble solids

The pH, TA and TSS values of fresh, pasteurized and thermosonicated soursop nectars are shown in Table 4. The values in control (fresh and pasteurized) nectars were similar to the data reported for fresh soursop juice (Falguera et al., 2012) and pasteurized soursop nectar (Peters et al., 2000). Thermosonication did not promote significant changes ($p > 0.05$) in pH, TA and TSS in samples, unlike pasteurization, which presented a slight increase in TSS ($p < 0.05$) with 15.6 °Brix in comparison to fresh. Similarly, other

Table 3
Predicted and actual values of polyphenol oxidase residual activity (PPO_{RA}), after thermosonication on soursop nectars.

Treatments	Predicted PPO_{RA} (%)	Actual PPO_{RA} (%)
TS1	66	66
TS2	52	51
TS3	25	34
TS4	53	52
TS5	39	39
TS6	20	19
TS7	12	12
TS8	8	9
TS9	0.5	0.5

Correlation coefficient $R^2 = 0.99$. TS1-TS9 = the key to the samples numbers can see in Table 1.

Table 4
Physicochemical parameters measured in fresh, pasteurized and thermosonicated (TS) soursop nectars.

Treatments	pH	Titratable acidity (g MAE/L)	Total soluble solids (°Brix)
Fresh control	3.7 ± 0.1^a	3.8 ± 0.5^a	14.4 ± 0.2^a
Pasteurized	3.6 ± 0.1^a	3.9 ± 0.3^a	15.6 ± 0.4^b
TS1	3.7 ± 0.1^a	3.6 ± 0.4^a	14.4 ± 0.1^a
TS2	3.7 ± 0.1^a	3.4 ± 0.4^a	14.4 ± 0.2^a
TS3	3.6 ± 0.1^a	3.7 ± 0.2^a	14.5 ± 0.3^a
TS4	3.7 ± 0.1^a	3.3 ± 0.4^a	14.5 ± 0.3^a
TS5	3.7 ± 0.1^a	3.2 ± 0.6^a	14.6 ± 0.2^a
TS6	3.8 ± 0.1^a	3.2 ± 0.6^a	14.7 ± 0.2^a
TS7	3.8 ± 0.2^a	3.2 ± 0.1^a	14.9 ± 0.1^a
TS8	3.9 ± 0.2^a	3.0 ± 0.2^a	14.9 ± 0.1^a
TS9	3.9 ± 0.2^a	3.0 ± 0.4^a	14.9 ± 0.1^a

Values are the average of triplicate determination from three different experiments (n = 9) ± standard deviation (SD). Means in a column with different letters are significantly different ($p < 0.05$). MAE = malic acid equivalent. TS1-TS9 = the key to the samples numbers can see in Table 1.

Table 5
Colour (*L a b*), total colour difference (TCD) and non-enzymatic browning index (NEBI) obtained in fresh, pasteurized and thermosonicated (TS) soursop nectars.

Colour attributes					
Treatment	*L*	*a*	*b*	TCD	NEBI
Fresh control	42.4 ± 1.7^{abc}	-0.8 ± 0.1^{a}	1.1 ± 0.1^{bc}	–	0.02 ± 0.001^{a}
Pasteurized	44.0 ± 1.5^{abc}	-1.2 ± 0.3^{a}	0.9 ± 0.1^{ab}	1.7	0.01 ± 0.002^{b}
TS1	42.0 ± 1.7^{a}	-0.8 ± 0.2^{a}	0.9 ± 0.02^{abc}	1.4	0.02 ± 0.001^{a}
TS2	42.0 ± 0.5^{ab}	-0.7 ± 0.2^{a}	0.7 ± 0.1^{a}	1.1	0.02 ± 0.001^{ab}
TS3	44.1 ± 1.0^{abc}	-1.1 ± 0.5^{a}	0.6 ± 0.03^{a}	1.8	0.02 ± 0.003^{ab}
TS4	45.0 ± 0.7^{abc}	-1.1 ± 0.3^{a}	1.2 ± 0.1^{c}	2.2	0.02 ± 0.004^{a}
TS5	44.4 ± 1.4^{abc}	-1.2 ± 0.6^{a}	0.9 ± 0.04^{ab}	2.2	0.02 ± 0.001^{ab}
TS6	46.4 ± 0.9^{c}	-1.2 ± 0.3^{a}	0.8 ± 0.01^{ab}	3.3	0.01 ± 0.001^{b}
TS7	45.2 ± 0.1^{bc}	-1.2 ± 0.1^{a}	0.8 ± 0.02^{a}	2.9	0.02 ± 0.001^{a}
TS8	45.1 ± 0.7^{abc}	-1.2 ± 0.3^{a}	0.9 ± 0.01^{abc}	2.8	0.02 ± 0.002^{ab}
TS9	45.9 ± 0.8^{abc}	-1.25 ± 0.19^{a}	0.83 ± 0.1^{ab}	2.6	0.01 ± 0.002^{c}

Values are the average of triplicate determination from three different experiments (n = 9) ± standard deviation (SD). Means in a column with different letters are significantly different ($p < 0.05$). *L* (whiteness or brightness); *a* (redness/greenness); *b* (yellowness/blueness). TS1-TS9 = the key to the samples numbers can see in Table 1

studies have not shown significant changes in pH, TA and TSS produced by thermosonication in different experimental conditions for fruit juices such as grape, carrot and apple (Aadil et al., 2015; Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014; Martínez-Flores et al., 2015).

3.4. Colour, total colour difference and non-enzymatic browning index

Colour is an important indicator of quality in fruit juices (Tiwari et al., 2010). The effect of thermosonication treatment on the colour of soursop nectar is given in Table 5. A tendency to increase *L* values can be observed for all samples treated with thermosonication, but no significant differences ($p > 0.05$) were observed in comparison to fresh and pasteurized nectars. Also, there were no significant differences ($p > 0.05$) in *a* values among treatments, but the treatments TS2, TS3 and TS7 showed significant differences ($p < 0.05$) in *b* values compared with the fresh control. According to Tiwari et al. (2008) differences in visual colour can be classified measuring total colour difference (TCD) as very distinct (TCD > 3), subtle (1.5 < TCD < 3) and small difference (TCD < 1.5). Therefore, all thermosonication treatments on soursop nectar, except TS6, presented a TCD <3.0, thus classifying as subtle or small visual colour difference. Aadil et al. (2015) reported that thermosonication caused an increase of compounds related to the colour, providing better uniformity and homogenization in colour. In addition, the increase in *L* might be caused by partial precipitation of unstable and suspended particles in the nectar due to the effect of temperature (Quek, Chin, & Yusof, 2012). These results are in agreement with those reported by Dias et al. (2015) in treated soursop juice with only ultrasound.

Fresh control nectar had a NEBI (Table 5) value of 0.02 and the pasteurized nectar of 0.01. Similar results were reported in soursop nectar by Peters et al. (2000). In samples treated with thermosonication there was no difference ($p > 0.05$) with respect to control nectars, except for TS9 with a lower NEBI value (0.01). The results of this work coincided with Walkling-Ribeiro et al. (2009) who reported no effect on NEBI by thermosonication in orange juice. Thus, treatments used in this work did not cause non-enzymatic changes.

3.5. Principal component analysis and hierarchical cluster analysis

Principal component analysis (PCA) was applied to determine pattern recognition between variables and treatments with thermosonication on soursop nectars (Fig. 2). Principal components (PC) 1 and 2 had a high percentage of the total variance (46% for PC1 and 24 for PC2); the graph of quality parameters is presented in Fig. 2a and the graph location of working treatments is shown in

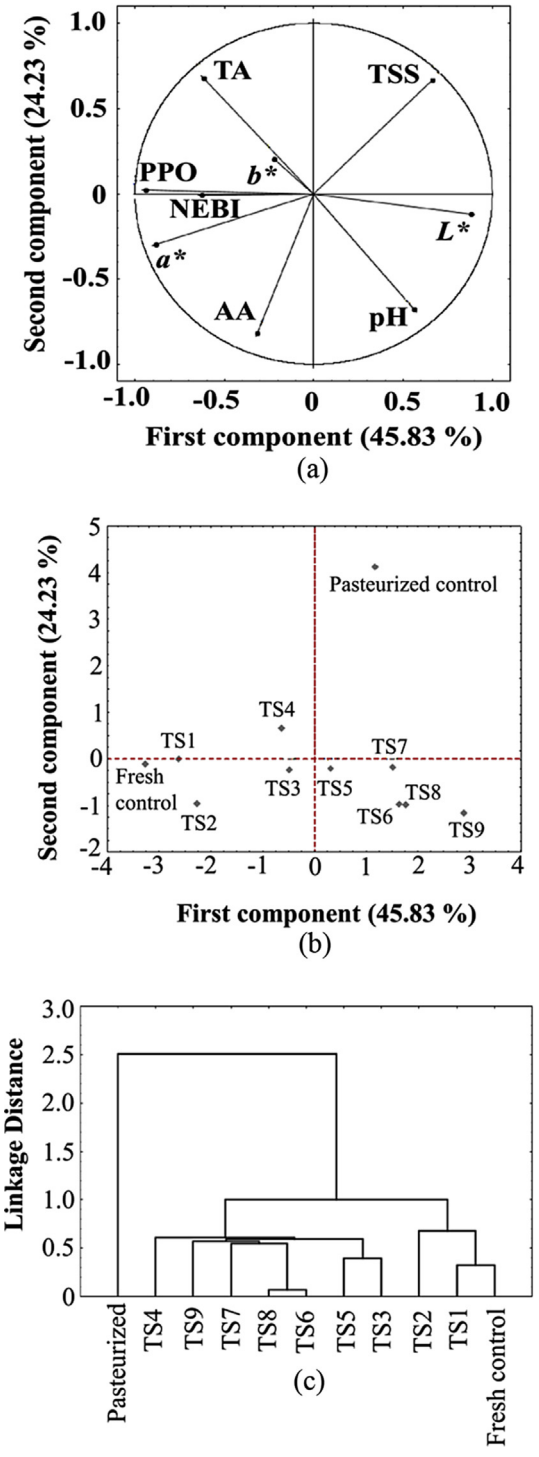

Fig. 2. Principal component analysis (PCA) plots of soursop nectar samples; location of different quality parameters (a), location of different treatments (b) and dendrogram of hierarchical cluster analysis (c) (AA = ascorbic acid; PPO = polyphenol oxidase activity, NEBI = Non-enzymatic browning, TA = titratable acidity, TSS = Total soluble solids, L^* = whiteness or brightness, a^* = redness/greenness, b^* = yellowness/blueness, TS1-TS9 = the key to the samples numbers can see in Table 1).

Fig. 2b. Thus in a scatter plot of the score values of all quality parameters projected in PC1 and PC2 planes, TSS (0.66), lightness (0.88) and pH (0.56) tends to increase, while PPO_{RA} content (−0.94) generally decreases, proceeding from positive to negative values of PC1 (Fig. 2a). From the results in Fig. 2b, it is clear that PCA is able to distinguish between treated nectars from fresh control and pasteurized soursop nectars. PC1 and PC2 showed a separation between pasteurized control and fresh and thermosonicated treatments. According to the factors coordinates: TS1 (−2.62) and TS2 (−2.26) have a closer relationship with the fresh control (−3.26), indicating no change in values for the measured parameters after processing. On the other hand, the pasteurized control (1.16) showed differences from fresh (−3.26) and thermosonicated samples (2.89), proceeding from positive to negative values of PC1. Thermosonication treatments, particularly TS9 demonstrated enhanced effects on NEBI, AA, colour attributes and PPO inactivation.

Additionally, in the present study, hierarchical cluster analysis (HCA) output was computed using PCA scores from quality attributes. The results are displayed as a dendrogram in Fig. 2c, indicating that the samples within the same group are more similar to each other than to samples in different groups. Pasteurized soursop nectar (first cluster) is clearly discernible from the other treatments. A second cluster consists of TS4, TS9, TS8, TS7, TS6, TS5 and TS3; while third cluster includes TS1, TS2 and fresh control. Multivariate tools such as PCA and HCA have been used to estimate the relationship between the effect on quality parameters and different condition treatments using emerging technologies such as thermosonication, high hydrostatic pressure and UV-C irradiation on fruit juices like grape (Aadil et al., 2015), carrot (Jabbar et al., 2015), apple (Abid, Jabbar, Hu, Hashim, Wu, Lei et al., 2014; Abid, Jabbar, Hu, Hashim, Wu, Wu et al., 2014) and lemon-melon juice (Kaya, Yıldız, & Ünlütürk, 2015).

4. Conclusions

According to surface response methodology, thermosonication was effective in reducing PPO activity in soursop nectar. Also it was conserved 90% of ascorbic acid and there were only slight changes in the physicochemical quality of the thermosonicated nectar. This work is important because demonstrates that thermosonication is a useful tool for controlling browning in soursop nectars and can maintain high levels of bioactive compounds, which make these products potentially functional.

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