Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters

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ABSTRACT

The effects of ultrasound process on quality parameters and on peroxidase (POD), polyphenol oxidase (PPO) and ascorbate peroxidase (APx) activities of cantaloupe melon juice were investigated. A factorial central composite design was carried out changing processing time and ultrasound intensity. POD, PPO and APx residual activities, color and phenolic compounds were the responses analyzed. Applying ultrasound power intensity of 376 W/cm² for 10 min resulted in significant reduction of POD and PPO activities and the total inactivation of APx. No color degradation was observed. However, ultrasound (US) processing also caused the reduction on phenolic compounds (30%). The technology showed to be suitable for cantaloupe melon stabilization as alternative to thermal and other treatments that results in quality loss. US processing was also able to improve and keep the juice homogeneity (cloud stability) during 6 weeks of cold storage (4 °C). This is a very interesting advantage for juice processing because cantaloupe pulp settles very fast resulting in a two phase product.

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1. Introduction

Cantaloupe melon (*Cucumis melo* L.) is a very popular and widely consumed fruit in the world. In Brazil, cantaloupe melon represents one of the most valued agriculture commodities with significant volumes exported every year (Brazilian Fruit Yearbook, 2011). The cantaloupe melon has high amounts of functional substances such as carotenoids, which act as an antioxidant, minerals and some amino acids (USDA, 2011). Due to the pleasant aroma and the high sugar content, cantaloupe melon is a suitable raw material for the juice industry (Vaillant et al., 2005).

However, cantaloupe melon is a highly perishable fruit with short periods of storage that are insufficient to allow, without the use of some technology, its commercialization in distant markets (Sá, Silva, Terao, & Oster, 2008). Cantaloupe melon and its products are sensitive to high temperature treatments, such as thermal sterilization. The thermal processing of melon juice results in off-flavor formation, color, vitamins and aromatic compound degradation (Hayashi, 1996). The introduction of new technologies in food industry might reduce the processing time and improve the industrial operating conditions, resulting in high quality products that preserve the natural characteristics

of the food (Butz & Tauscher, 2002; Cárcel, García-Pérez, Benedito, & Mulet, 2011).

The ultrasound processing (US), also called sonication, is a nonthermal technology that has been effective in the inactivation of microorganisms and enzymes related to degradation of fruit juices allowing the treatment of thermo-sensitive food (Rawson et al., 2011). Despite the fact that some authors reported some negative impacts on juice quality due to ultrasound processing, such as: a slight deterioration of sensory quality in calcium added orange juice (Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010) and some off-flavor formation in orange juice (Wong, Vaillant, & Pérez, 2010), according to a recent review published by O'Donnell, Tiwari, Bourke, and Cullen (2010) fruit juices treated with ultrasound suffered minimal effects on the quality of final product and, because of that, this technology has been studied for several applications in food processing.

Ultrasound produces repeated cycles of compression and decompression called acoustic cavitation, which is the process of nucleation, growth and collapse of bubbles in liquids exposed to ultrasonic waves at low frequency (20 kHz–100 kHz) and high power (10–1000 W/cm²). The collapse of the bubbles generates high local temperatures (5000 K) and high pressures (1000 atm) resulting in high shear rates and generating strong micro-streaming, which can contribute for enzyme and microbial inactivation (Apfel, 1981; Mason, 1991). The enzyme and microbial inactivations due to ultrasound have been reported as dependent on the nature of the enzyme; the process

variables (ultrasound power intensity, ultrasound frequency, temperature or pressure); the characteristics of the medium (viscosity, food matrix composition) as well as on the type of connection and chemical reactions that they establish with other molecules (Cárcel et al., 2011; Chemat, Huma, & Khan, 2011; O'Donnell et al., 2010).

Among the food enzymes, peroxidase (POD) and polyphenol oxidase (PPO) are frequently involved in multiple deteriorative changes, such as enzymatic browning, with consequent loss of sensorial and nutritional properties of fruit and vegetables (Oms-Oliu, Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008). These enzymes are usually inactivated by thermal treatments, which demand large amount of energy besides imparting several quality loss (Pereira & Vicente, 2010).

Despite the several works that have been published on ultrasound processing of fruit juices, few are addressed to enzyme inactivation and the effects of sonication on cantaloupe melon juice quality parameters have not been reported elsewhere. Therefore, the purpose of the present work was to find optimum ultrasound operating conditions for the processing of cantaloupe melon juice and to evaluate the effects of US processing on juice quality parameters.

2. Materials and methods

2.1. Juice preparation

Cantaloupe melon juice (C. melo L.) was prepared from frozen fruit pulps obtained from local market without addition of preservatives. The pulp was diluted in potable water adjusting the soluble solid content to 3°Brix (ratio 1:2). The juice was stored at 4 °C prior to processing.

2.2. Sonication treatment

A 500 W ultrasound processor (Unique® DES500, São Paulo, Brazil) with a 1.3 cm diameter probe tip was used for juice sonication. Samples were processed at a constant ultrasound frequency of 19 kHz. Cantaloupe juice samples (150 mL) were placed in a 200 mL glass Becker. The ultrasound probe was submerged to a depth of 25 mm in the sample. All treatments were carried out in triplicate. The intensity of ultrasound power which dissipated from the probe tip was calculated by Eq. (1) (Li, Pordesimo, & Weiss, 2004).

$$I = \frac{P}{\pi r^2} \tag{1}$$

where r is the radius of the titanium tip (cm) and P is the input power level (W). The input power was controlled through amplitude setting and the power levels were adjusted to 20%, 60%, and 100% of total input power (500 W), which was equal to 100, 300 and 500 W. The calculated intensities were 75, 226 and 376 W/cm^2, respectively.

2.3. Experimental design and data analysis

The sonication conditions were studied through a 2^2 face centered central composite experimental rotated design (CCRD) with 3 central points. The power intensity and processing time were changed from 75 to 376 W/cm^2 and 2 to 10 min, respectively. For all treatments, the initial temperature of the juice was 4 °C. After sonication, the juice temperature was recorded by digital thermometer. The dependent variables were: residual enzymatic activity, reduction of phenolic compounds, color and temperature increase.

2.4. Enzymatic assay

For peroxidase (POD, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.14.18.1) determination, enzyme extraction was done according

to the methodology described by Wissemann and Lee (1980). Ten milliliters of cantaloupe melon juice was mixed with the same volume (10 mL) of potassium phosphate buffer (0.05 M pH 7.0) containing 1% (w/v) of polyvinylpyrrolidone (PVP). The mixture was centrifuged in a Sigma® 6K15 centrifuge (10,733 g for 30 min at 4 °C). The supernatant was used as the enzyme source.

POD activity was monitored at 470 nm in a spectrophotometer (Spectrum® SP200UV) according to the method described by Matsuno and Uritani (1972). The enzyme activity was measured as follows: 2.75 mL of a phosphate (sodium)-citrate (citric acid) buffer (0.1 M, pH 5.0) containing 1% (v/v) of guaiacol and 0.25 mL of hydrogen peroxide 3% (v/v) were added to 1.5 mL of enzyme extract. The assay mixture was incubated at 30 °C for 5 min. The reaction was interrupted with the addition of 1 mL of sodium bisulfate 30% (w/v). One unit of enzyme activity (1 UEA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

PPO activity was measured based on the method reported by Wissemann and Lee (1980). The reaction mixture contained 0.3 mL of enzyme extract and 1.85 mL of a potassium phosphate buffer solution (0.1 M pH 6.0) containing catechol (0.1 M) and KCl (0.1 M). The reaction mixture was incubated at 30 °C for 30 min. The reaction was interrupted with the addition of 0.8 mL of perchloric acid 2 N. One unit of enzyme activity (1 UEA) was defined as the amount of enzyme that causes a change of 0.001 in the absorbance (395 nm) per minute.

The enzyme extract for ascorbate peroxidase (APX, EC 1.11.1.1) activity measurement was prepared as follows: 4 mL of the juice was mixed to 10 mL of potassium phosphate buffer (0.1 M; pH 7.0) containing EDTA (0.01 mM). The mixture was centrifuged (14,000 g for 30 min at 6 °C). The supernatant constituted the enzyme source.

APx activity was assayed according to Nakano and Asada (1981). The reaction mixture composed of 1.3 mL of a potassium phosphate buffer solution (50 mM , pH 6.0) containing EDTA (0.05 mM), 50 μL of ascorbic acid (15 mM), 50 μL H$_2$O$_2$ (0,03 M) and 100 μL of enzyme extract. The reaction was started by adding the H$_2$O$_2$ and ascorbate oxidation was measured at 290 nm for 1 min. Enzyme activity was measured using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}) and the results expressed in mMol H$_2$O$_2$ min^{-1} mL^{-1} of juice taking into consideration that 2 mol ascorbate is required for a reduction of 1 mol H$_2$O$_2$. One unit of enzyme activity (1 UEA) was defined as the amount of enzyme in 1 mMol of H$_2$O$_2$ per minute.

All standards and reagents were of analytical grade and were commercially available from Sigma-Aldrich (Germany).

2.5. Residual enzymatic activity

The residual enzyme activities (RA) of POD, PPO and APx after sonication were calculated according to Eq. (2).

$$RA(\%) = \frac{A_S}{A_0} \times 100. \tag{2}$$

The sub indices 0 and s in Eq. (2) mean the control sample (non treated) and the sonicated one, respectively.

2.6. Temperature effect on enzyme activity

To evaluate the temperature effect on enzyme activity without the ultrasound interference, an assay was carried out at 53 °C, which was the highest temperature, recorded after the cantaloupe melon juice sonication (376 W/cm^2 for 10 min). Samples of 150 mL of the non-sonicated juice were kept in a water bath at the desired temperature for 10 min. Samples were taken every 2 min and the POD, PPO and APx activities were determined as described earlier.

2.7. Total phenolic compounds

Total phenolic compounds were determined using the Folin–Ciocalteau methodology (Obanda & Owuor, 1997). Phenolic extraction was carried out in two steps using a juice aliquot of 15 mL. The first extraction was done adding 5 mL of a methanol:water solution (50:50 v/v) to the sample. The mixture was then centrifuged (10,733 g during 30 min at 20 °C). The supernatant was poured and 5 mL of an acetone:water solution (70:30) was added to the pellet. The mixture was homogenized and centrifuged again as described.

The reaction mixture contained: 250 μL of the phenolic extract, 500 μL of Folin–Ciocalteu reagent (Sigma-Aldrich, Germany), 500 μL of sodium carbonate and 500 μL of distilled water. The mixture was then left in the darkness for 30 min at 25 °C. The absorbance of the sample was measured at 700 nm. Gallic acid (HPLC grade, Sigma-Aldrich) was used as standard. Results were expressed as percentage of reduction of phenolic compounds calculated according to Eq. (3). All measurements were carried in triplicate.

$$Reduction(\%) = \frac{P_0 - P_S}{P_0} \times 100. \tag{3}$$

Where the sub indices 0 and s in Eq. (3) mean the control sample (non-treated) and sonicated one, respectively.

2.8. Color

The color of the cantaloupe melon juice was determined using a Minolta CR300 colorimeter (Tokyo, Japan). The colorimeter was calibrated using the illuminant D65, and measurements were made through an 8-mm port/viewing area (Minolta, 1998). The reflectance instruments determined three color parameters: lightness (L*), redness (a*), and yellowness (b*). Numerical values of L*, a* and b* were converted into ΔE (total color difference), ΔC (chroma) and hue angle (h°) according to Eqs. (4)–(6), respectively. The reference value for ΔE was the non-sonicated juice. Color measurements were taken in quintuplate.

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{4}$$

$$\Delta C = \sqrt{(a_0^{*2} + b_0^{*2}) - (a_s^{*2} + b_s^{*2})} \tag{5}$$

$$h° = \tan^{-1}(b^*/a^*). \tag{6}$$

The sub indices 0 and s in Eq. (5) mean the control sample (non treated) and sonicated one, respectively.

2.9. Cloud stability

After the sonication, samples were stored at 4 °C in 100 mL graduated cylinders. Sodium azide (0.1% w/v) was added to each sample to avoid microbial contamination during the storage. The graduated cylinders were covered with polyethylene film. Cloud stability was determined after 6 weeks of refrigerated storage. Aliquots (5–7 mL) of the stored juice were drawn from the upper portion of the graduated cylinders. The turbidity of the juices was determined reading the absorbance at 660 nm (Randall, Robert, & Karel, 1997; Yemenicioglu, Günaydin, & Cemeroglu, 2000). Distilled water was used as a reference.

2.10. Quantification of sugars after sonication

Sugars (glucose, fructose and sucrose) were quantified in fresh juice (non treated) and sonicated juice at the best conditions for enzyme inactivation (376 W/cm for 10 min) by high performance liquid chromatography (HPLC). A Varian ProStar system equipped with two high-pressure pumps model ProStar 210, refraction index detector model ProStar 355 RI and column oven (Timberline) was used. Separation was achieved using an Aminex HPX 87 C (300 mm × 7.8 mm) column at 80 °C. Ultra pure water at 0.6 mL/min was used as eluent and the detector temperature was 35 °C. All samples were analyzed in triplicate. The software ProStar WS 6.1 was used to acquire and handle the data.

2.11. Statistical analysis

Except color determination, which was taken in quintuplicate, all other assays were carried out in triplicate. Results were expressed as mean ± SD. Statistical analysis of the experimental data was carried out using the software. Statistica 7.5 (Statsoft). F-test and ANOVA analysis were used as significant criteria for the fitted models.

3. Results and discussion

3.1. Effect of ultrasound process on enzymatic activity

The enzyme activity in the non sonicated juice (control sample) are: 207.95 ± 2.09 EAU/mL (POD), 240.95 ± 4.09 EAU/mL (PPO) and 0.54 EAU/mL (APx). The residual activities of cantaloupe melon juice enzymes (POD, PPO and APx) after the sonication treatments are presented in Table 1. Fig. 1 depicts the Pareto chart of the effects of independent variables on the studied responses.

Fig. 1a shows the effects of independent variables on peroxidase (POD) activity reduction. At a confidence level of 90%, the effect of processing time (linear and quadratic) and the effect of the interaction of power intensity and processing time were significant on the enzyme activity reduction. Thus, a simultaneous increase of US power intensity and time favored the reduction of POD activity. On the other hand, the effect of power intensity (linear and quadratic) was not significant on PPO activity reduction at the given confidence interval.

Fig. 1b shows the effect of the independent variables on polyphenol oxidase (PPO) activity reduction. At a confidence level of 95%, time (linear and quadratic) and power intensity (linear) had a significant effect on enzyme activity reduction. The quadratic effect of ultrasound intensity revealed that, for the experimental domain evaluated herein, higher power intensity at low processing times did not affect PPO activity reduction, since this effect was small and not significant. Pareto chart (Fig. 1c), shows that all independent variables and their interaction had a significant effect on APx at 95% of confidence level. The effect of processing time (linear and quadratic) was higher than power intensity.

Table 1
Experimental design and responses of the influence of ultrasonic treatment on the enzymatic activity (POD, PPO, APx) of cantaloupe melon juice.

| Power intensity (W/cm²)	Process time (min)	Assay	Residual activity (%)		
			POD	PPO	APx
75	2	1	114.34 ± 2.67	88.18 ± 1.80	43.26 ± 1.83
75	10	2	121.03 ± 2.22	85.66 ± 1.92	35.23 ± 1.46
373	2	3	119.38 ± 1.63	84.14 ± 1.05	41.12 ± 2.43
373	10	4	23.29 ± 3.40	70.48 ± 1.46	1.56 ± 0.00
75	6	5	113.40 ± 1.95	89.12 ± 0.95	51.67 ± 1.49
373	6	6	127.36 ± 0.48	88.46 ± 1.15	36.26 ± 1.82
226	2	7	114.62 ± 1.67	90.81 ± 1.26	26.00 ± 2.30
226	10	8	96.41 ± 0.87	77.60 ± 1.53	1.05 ± 0.00
226	6	9	126.22 ± 0.81	87.85 ± 1.83	43.38 ± 1.14
226	6	10	126.71 ± 1.50	89.54 ± 1.33	39.51 ± 1.77
226	6	11	125.44 ± 1.69	87.78 ± 1.73	44.46 ± 1.09

A

B

C
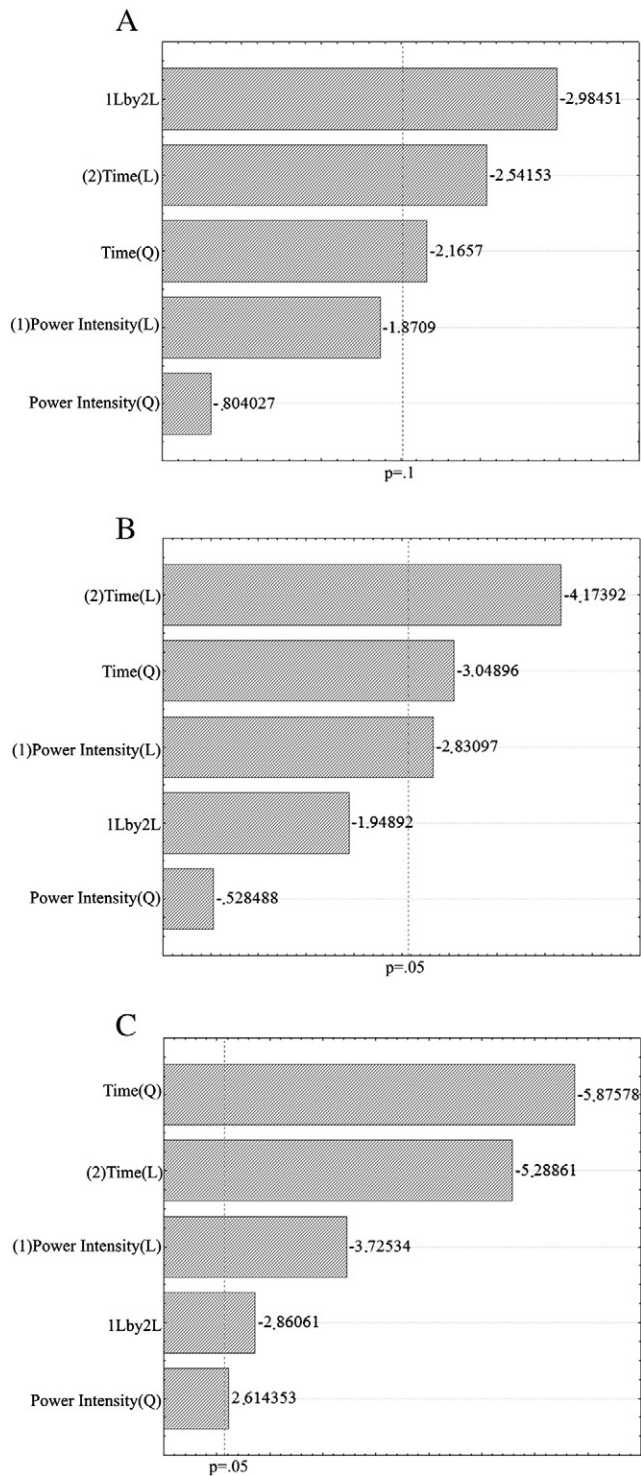
Fig. 1. Pareto chart for residual activity of POD (A), PPO (B) and APx (C) after US processing of cantaloupe melon juice. Linear (L) and quadratic (Q) responses. (1) Processing time, (2) power intensity; 1L by 2L interaction between processing time and power intensity.

The fitted models for residual enzymatic activity (POD, PPO and APx) as a function of US power intensity and processing time were expressed on Eqs. (7)–(9), respectively.

$$POD_{RA(\%)} = 44.27 + 0.33X - 3.8 \times 10^{-4}X^2 + 22.67Y - 1.4Y^2 - 4.27 \times 10^{-2}XY \tag{7}$$

$$PPO_{RA(\%)} = 80.53 + 2.41 \times 10^{-2}X - 4.00 \times 10^{-5}X^2 + 3.94Y - 0.35Y^2 - 4.63 \times 10^{-3}XY \tag{8}$$

$$APx_{RA(\%)} = 23.81 - 0.15X - 3.90 \times 10^{-4}X^2 + 16.56Y - 1.38Y^2 - 1.37 \times 10^{-2}XY. \tag{9}$$

Where:

X   Power intensity (W/cm^2);
Y   Processing time (min).

All models were statistically significant at 95% of confidence interval since the calculated F values (5.13 for Eq. (7); 8.10 for Eq. (8) and 17.20 for Eq. (9)) were higher than the listed F value ($F_{5.5} = 5.05$). Good correlation coefficients were also obtained ($R^2 = 0.84$ for Eq. (7); $R^2 = 0.90$ for Eq. (8) and $R^2 = 0.94$ for Eq. (9)). Fig. 2 shows the surface response graphs built from Eqs. (7)–(9).

An increase in peroxidase (POD) activity was observed for low processing times (Fig. 2a). Increasing the processing time the POD activity decreases. This behavior is because, at a first, US treatment causes cell disruption of the fruit pulp (Fernandes, Gallão, & Rodrigues, 2009). Thus, the intracellular enzyme is released in the liquid medium increasing the enzyme activity. In fact, for low exposition times, the rate of enzyme release is higher than the rate of enzyme denaturation. For longer exposition times, the rate of enzyme denaturation overcomes the enzyme release. Most studies reported that prolonged exposure periods were necessary to inactivate enzymes using high power ultrasound (O'Donnell et al., 2010). As shown in Fig. 2a, higher POD inactivation was observed for processing times higher than 6 min and power intensity higher than 226 W/cm^2. The minimal residual activity (23.29%) was obtained at the graph edge with the most drastic treatment (376 W/cm^2 for 10 min).

Polyphenoloxidase (PPO) enzyme activity reduction was observed in the whole experimental domain (Fig. 2b), even at low processing times and power intensity. The surface graph is similar to that obtained for POD since PPO residual activity decreased by increasing processing time and power intensity. The best condition that favored the reduction of the activity PPO was the same to that found for POD (376 W/cm^2 for 10 min). However, higher residual activity was found for PPO (70.48%) compared to POD (23.29%).

The ultrasound treatment was able to promote the complete inactivation of the enzyme APx (assays 4 and 8). In the other experimental conditions, the residual activity of APx did not exceed 52%. The most sensitive enzyme towards sonication was APx because significant inactivation was achieved even at low power and processing times. The effect of enzyme release due to cell disruption and further inactivation due to longer exposition periods can be also seen in the surface graph of APx residual activity (Fig. 2c.). However, the surface showed a saddle point near to the central point of the experimental domain. This saddle point is a local minimum for power intensity and a local maximum for processing time, showing that when keeping the power intensity near 226 W/cm^2, residual activity increased for processing times higher and lower than 6 min. On the other hand, sonication with power intensity higher or lower than 226 W/cm^2 for 6 min decreased the residual activity. APx activity was minimized by applying power intensities higher than 226 W/cm^2 for 10 min. The results obtained in the present study are in agreement with Zapata, Sabater, and Martin (1998), who reported that ascorbate peroxidase is more unstable than other peroxidases.

The cavitational intensity has been one of the main mechanisms proposed to explain enzymatic inactivation of foods subjected to ultrasound treatment. The formation of cavitation bubbles results in shear force that can change the conformation of the proteins. Enzymes can also be denatured by free radicals generated during
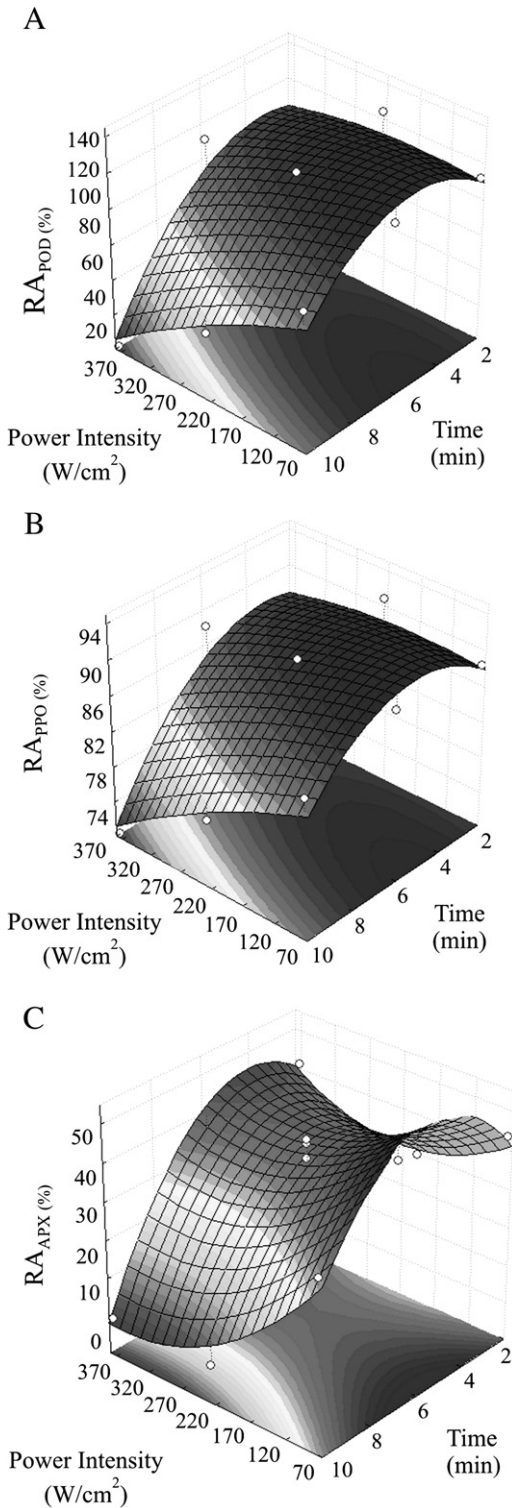
Fig. 2. Response surface plots showing effects of ultrasound intensity power, process time and their interaction on the residual activity of POD (A), PPO (B) and APx (C).

sonolysis of water molecules (Mason, 1998; Suslick, 1989). Previous studies reported that the activity of free enzymes may also increase under moderated ultrasound irradiation (Choi & Kim, 1994; Sakakibara, Wang, Takahashi, Takahashi, & Mori, 1996).

There was a reduction in PPO activity in all assays of experimental design. However, in assays 4 and 8, the reduction of PPO activity was higher, reaching 70.48% and 77.60% of residual activity, respectively (Table 1). However, the activity of PPO of the control sample was

240.95 ± 4.09 EAU/mL, a value considered low compared to other fruits such as: peach (476 EAU/mL) and apple (1499 EAU/g fresh fruit) (Rocha & Morais, 2001).

Jang and Moon (2011) studied the inactivation of the PPO and POD in apples through the combined use of US treatment and ascorbic acid. The results revealed that the isolated use of ultrasound was not effective in the inactivation of the enzymes leading to a slight increase of PPO activity. On the other hand, treatment with the simultaneous use of ultrasound and ascorbic acid had synergistic inhibitory effect on the enzymes involved in browning of the fruit. Sala, Burgos, Condón, Lopez, and Raso (1995) reported the use of ultrasound associated with heat to reduce the severity of heat treatment. Herein, good enzyme inactivation was observed without the use of chemical additives or external heating.

However, the increase of processing time and power intensity caused significant increase of the juice temperature (ΔT °C) as shown in Table 2. Fig. 3a shows the Pareto chart of the effect of power intensity and processing time on temperature. At 95% of confidence interval, except for the quadratic effect of processing time, all other effects were significant on juice temperature increase (ΔT °C). Linear effects (processing time and power intensity) and their interaction were positive, while the quadratic effect was negative. Eq. (10) presents the fitted model for the juice temperature increase. The model was validated and showed statistical significance at 95% of confidence level according to ANOVA analysis and F-test (calculated $F_{5,5} = 88.38$, $R^2 = 0.99$).

$$\Delta T(°C) = -13.59 + 0.14X - 3.00 \times 10^{-4}X^2 + 3.10Y - 0.12Y^2 + 9.60 \times 10^{-3}XY \qquad (10)$$

X   Power intensity (W/cm^2);
Y   Processing time (min).

The increase of processing time and power intensity increased the juice temperature. The maximal temperature was observed at the edge of the surface at the most drastic treatment (376 W/cm^2 for 10 min), which is the same condition that presented the lower residual activity for the enzymes studied. Thus, the energy liberated by the sonication may have caused a synergistic effect on enzyme denaturation.

Fig. 4 shows the residual activities of POD, PPO and APx obtained keeping the juice at 53 °C for 10 min. As can be seen, the maximal temperature recorded after the juice sonication was unable to denature the studied enzymes attesting that inactivation shown in Fig. 2 was mainly due to the sonication.

Table 2
Influence of US process on the concentration of cantaloupe melon juice phenolic compounds.

Power intensity (W/cm^2)	Process time (min)	Assay	ΔT (°C)	Total phenolics (µg gallic acid 100 mL^{-1})	Reduction (%)
		Control	[a]	14.69 ± 0.56	[a]
75	2	1	4.0 ± 0.5	9.69 ± 0.60	34.02 ± 2.10
75	10	2	21.0 ± 0.8	11.86 ± 0.23	19.24 ± 1.62
373	2	3	9.0 ± 0.7	10.62 ± 0.15	27.72 ± 1.04
373	10	4	49.0 ± 1.2	11.42 ± 1.09	22.26 ± 0.99
75	6	5	12.0 ± 0.6	10.27 ± 0.28	30.07 ± 1.04
373	6	6	31.0 ± 0.4	11.53 ± 0.77	21.50 ± 0.87
226	2	7	9.0 ± 0.6	10.89 ± 0.39	25.87 ± 0.77
226	10	8	44.0 ± 0.9	11.52 ± 1.61	21.59 ± 1.34
226	6	9	30.0 ± 0.7	12.64 ± 0.16	14.78 ± 1.09
226	6	10	31.0 ± 1.1	12.43 ± 0.17	15.37 ± 1.16
226	6	11	30.0 ± 0.8	12.28 ± 0.47	16.38 ± 1.20

[a] The value does not apply for the control sample.
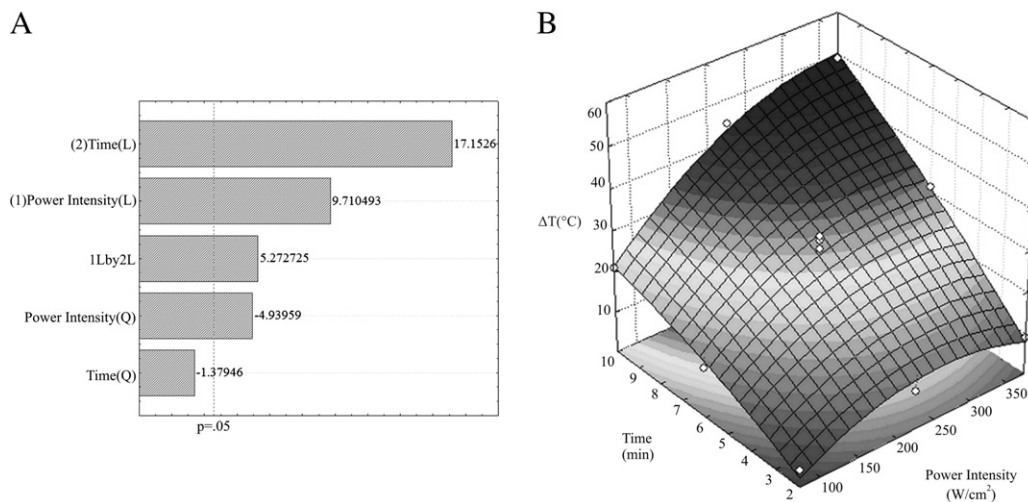
A

B

Fig.3. Pareto chart for the temperature increase after juice sonication (A). Response surface of the temperature increase after juice sonication (B).

3.2. Phenolic compounds

The response surface methodology was not applied to evaluate the reduction of phenolic compounds in sonicated juice because the processing time and the ultrasound power intensity effects on total phenolic content were not statistically significant. (Table 2). Thus, response surface analysis cannot be applied. In all assays there was a reduction in the levels of these compounds, reaching 30% of reduction (Table 2). The formation of free radicals may have affected the phenolic compounds of the cantaloupe melon juice since – OH radicals formed during cavitation can affect the bioactive compounds such as phenolics (Wan et al., 2005). The cavitational bubbles formed by sonication may be filled with water vapor or other gases dissolved in the juice, such as O_2 and N_2 (Dubrović, Herceg, Jambrak, Badanjak, & Dragović-Uzelac, 2011), which could have favored the oxidative degradation of phenolic compounds.

Rawson et al. (2010) also observed a decrease in the phenolic content of sonicated watermelon juice when the temperature was increased from 25 to 45 °C, with the temperature effect more pronounced at higher processing times (10 min). On the other hand, Lieu and Le (2010) evaluated the application of ultrasound treatment in grape juice and found that the sonicated samples had an increase of 114.3% in the concentration of total phenolics, making evident that
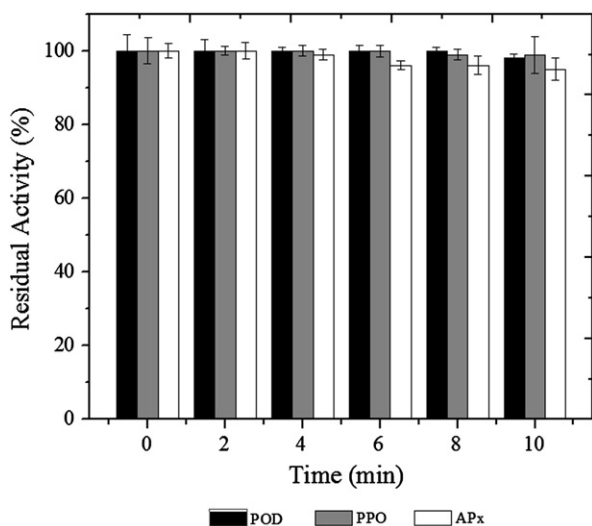
the effect of sonic waves in foods depends on the food matrix besides processing conditions.

3.3. Effects on color parameters

Melons of the variety Cantaloupe are orange colored mainly due to the presence of carotenoids such as β-carotene (Ibdah et al., 2006). The response surface methodology was not used for color analysis because the effects were not statistically significant. Results are presented in Table 3. There was an increase in redness (a* parameter) in assays 2, 6, 7, 8, 9, 10 and 11 (Table 3) when compared to the control (non sonicated juice). For the other assays, no significant differences from control were observed. With respect to parameter b*, treatments 1,3, 4 and 5 showed values closer to the fresh juice (control). Assays 2, and 6 to 11 presented an increase in the juice yellowness compared to the control.

According to Ahmed, Shivhare, and Kaur (2002) any change in a* and b* parameter values is associated with a simultaneous change in the value of luminosity (L*). Lightness (L*) in assays 6 to 11 was significantly lower than the fresh juice. During the US process, there is a disruption of cell membranes forming carotenoid–protein complexes that confer a greater homogenization and subsequent intensification of the orange color of the juice due to the sonication. On the other hand, Chen, Peng, and Chen (1995) reported that the extreme conditions of temperature and pressure that occur during sonication can accelerate the isomerization of carotenoids. Thus, the intensification of the red color of the juice can also be associated to the isomerization of carotenoid compounds present in the variety of melon used in this study. Sun, Ma, Ye, Kakuda, and Meng (2010) demonstrated that the rate of degradation of β-carotene is reduced when there is an increase in US power intensity and temperature. The authors affirm that at higher power ultrasound, the bubbles formed during cavitation may be too large since these bubbles collapse or collapse less violently, which can cause the reduction of the cavitation effects. Under these conditions, the bubbles can also disrupt the propagation of ultrasound waves. Adekunte, Tiwari, Cullen, Scannell, and O'Donnell (2010) also reported changes in the color of tomato juice after ultrasound process. However, sonication resulted in a decrease in the parameter L*, a* and b* values and an increase in the ΔE value, indicating a degradation of lycopene.

The saturation index (ΔC) and the hue angle (h°) may improve the understanding of color variations found in sonicated cantaloupe melon juice. The ΔC indicates the degree of variation in the intensity of the chroma (a* and b*) of the US treated sample with relation to fresh sample. The lower the value of ΔC, the less the variation. Thus,

Fig. 4. Effect of thermal processing (53 °C/10 min) on POD, PPO and APx activities.

Table 3
Effect of US treatment on color of cantaloupe melon juice.

Assay	L*	a*	b*	h°	ΔC	ΔE
Control	71.00 ± 0.79	7.27 ± 0.44	22.70 ± 0.89	71.97 ± 0.82	–	–
1	72.13 ± 0.90	6.81 ± 0.65	21.22 ± 1.02	72.25 ± 0.83	−0.87 ± 0.28	1.74 ± 0.43
2	68.55 ± 1.00	9.95 ± 0.74	25.94 ± 0.87	69.04 ± 0.79	4.63 ± 0.71	5.67 ± 0.99
3	73.72 ± 0.38	6.41 ± 0.29	20.37 ± 0.55	72.53 ± 0.32	−1.79 ± 0.41	2.85 ± 0.98
4	70.10 ± 0.56	8.26 ± 0.47	22.15 ± 0.68	70.57 ± 0.50	0.49 ± 0.15	1.70 ± 0.41
5	72.95 ± 0.61	6.98 ± 0.49	21.40 ± 0.68	71.92 ± 0.50	−0.66 ± 0.23	1.78 ± 0.70
6	67.00 ± 0.81	11.37 ± 0.58	26.71 ± 0.53	66.94 ± 0.64	5.88 ± 0.60	7.75 ± 0.99
7	69.65 ± 0.54	9.16 ± 0.40	24.70 ± 0.52	69.65 ± 0.42	3.18 ± 0.63	3.90 ± 0.50
8	67.40 ± 0.74	10.54 ± 0.69	24.69 ± 0.72	67.35 ± 0.75	4.21 ± 0.33	6.32 ± 0.30
9	68.10 ± 0.68	11.47 ± 0.48	27.20 ± 0.43	67.13 ± 0.54	6.37 ± 0.59	7.64 ± 0.68
10	65.90 ± 0.31	12.65 ± 0.33	27.94 ± 0.31	66.39 ± 0.33	7.51 ± 0.42	9.91 ± 0.55
11	66.58 ± 0.44	11.90 ± 0.27	27.56 ± 0.18	66.64 ± 0.34	6.86 ± 0.27	8.88 ± 0.51

the samples processed in treatments 1, 3, 4 and 5 (the lowest ΔC values) showed little difference of these samples compared to control. The values of hue angle (h°) confirm the data obtained for a* and b* since treatments 1, 3, 4 and 5 showed slight differences compared to control (Table 3). On the other hand, the increase of ΔC shows the color enhancement. Thus, samples submitted to the treatment conditions of assays 1, and 6 to 11 had their orange color enhanced due to the increase of redness and yellowness. The juice color was also more intense in these assays because these samples presented lower L* values when compared to the control. The hue angle (h°) showed variation of less than 5°, indicating that the characteristic color of the juice (orange) was maintained for all treatments.

Choi, Kim, and Lee (2002) suggested that a ΔE > 2 corresponds to visually perceptible differences in various products. Thus, the color of assays 1, 3, 4 and 5 resulted in juices that did not show visual difference compared to the control. For the other assays, a visual difference between the color of sonicated juice and the control was observed. However, as previously commented, these differences are positive and a result of the color enhancement.

The condition of ultrasound that resulted in better retention of the color of the melon juice was the greatest intensity ultrasound treatment and the longest time (376 W/cm² for 10 min). Tiwari, O'Donnell, and Cullen (2009) obtained similar results with the sonication of blackberry juice. The juice has retained a significant content of anthocyanins (>94%) in extreme processing conditions (22.79 W/cm² for 10 min), indicating the stability of anthocyanins during sonication.

3.4. Effects on cloud stability

The turbidity of fruit juices is due to finely divided particles of pectin, cellulose, hemicellulose, proteins and lipids in suspension
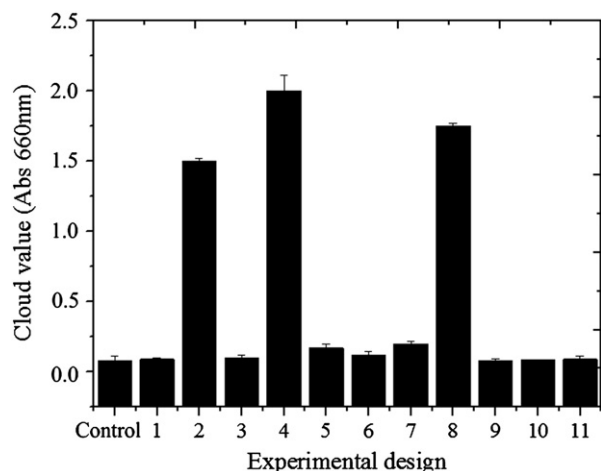

Fig. 5. Effect of ultrasonic power intensity on the cloud stability of melon juice.

(Irwe & Olsson, 1994; Klavons, Bennett, & Vannier, 1994). The cloud stability is a desirable feature in fruit juices because it favorably affects the flavor and color of the juice.

Cloud values are presented in Fig. 5. As can be seen, for assays 2, 4 and 8 the cloud value was much higher than the control (non-sonicated juice) at the end of the tested period (6 weeks in refrigerated storage). For these samples, the juice homogeneity was kept during the whole evaluated period. For the others, a two phase product was obtained. In these experiments, the juices were treated for 10 min in ultrasound intensities of 75, 376 and 226 W/cm², respectively, confirming that the cloud stability of the juice has a greater effect of treatment time in relation to the US power intensity.

The ultrasound processing reduces the size of the suspended particles in a liquid providing better uniformity and stability. This size reduction increases the number of individual particles leading to a reduction of average distance and an increase in the total surface area of the particles (Rao, 1999). The results of this study are consistent with the results published by Tiwari, Muthukumarappan, O'Donnell, and Cullen (2009), who observed greater stability of orange juice subjected to sonication. The authors attribute the greater stability of particulate matter from the juice to the particle reduction after ultrasound treatment. Moreover, they suggest that the activity of the enzyme pectin methylesterase (PMEs) and their interactions with its substrate (pectin) had a great impact on the stability of orange juice. Conversely, Wu, Gamage, Vilkhu, Simons, and Mawson (2008) observed that reducing the size of the particles in sonicated tomato juice proved to be more dependent on the US amplitude than the inactivation of PME.

3.5. Sugar concentration

In comparison with the control sample, the sonicated cantaloupe melon juice resulted in a significant (p < 0.05) increase of sucrose (53.60%) and glucose (4.24%) concentrations (Table 4). These results agreed with previous researches, which reported that ultrasound promotes high extractability for sugars (Fernandes et al., 2009; Lieu & Le, 2010). Due to the cell disruption, US treatment has been applied in extraction protocols (Eh & Teoh, 2012; Paniwnyk, Cai, Albu, Mason, & Cole, 2009; Rodrigues, Pinto, & Fernandes, 2008). The increase on sugar concentration is due to cell disruption promoted by the US treatment which releases the intracellular sugar to the liquid.

Table 4
Concentration of sugars of cantaloupe melon juice after US processing (376 W/cm² for 10 min).

	Control	US
Sucrose	5.00 ± 0.05a	7.68 ± 0.01b
Glucose	5.75 ± 0.01a	6.00 ± 0.08b
Fructose	6.40 ± 0.03a	6.58 ± 0.05b

Different letters in the same line mean significant differences according to Tukey Test (p < 0.05).

4. Conclusions

The experimental condition that favored the decreased POD, PPO and APx activities was 376 W/cm^2 for 10 min. The cloud stability of the melon juice was improved due to the ultrasound treatment. The juice remained completely homogeneous during 6 weeks of refrigerated storage after the same processing conditions. Although sonication caused some phenolic degradation, this technology proved to be suitable for cantaloupe melon juice processing due the pulp, color stability and enhancement.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.foodres.2012.02.013.

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