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# Effect of thermosonication on pectin methylesterase activity and quality characteristics of orange juice[1]

## Efeito da termossonicação na atividade da pectinametilesterase e características de qualidade de suco de laranja

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**ABSTRACT** - Pectin methylesterase (PME) is the enzyme responsible for quality losses in orange juice and pasteurization is used to inactivate PME. This process decreases the sensory and nutritional aspects of the beverage. This study aimed to evaluate the effect of combined ultrasound and moderate temperature on several quality parameters of orange juice, and whether the used treatment resulted in a reduction of PME activity when compared to thermally treated samples and natural juice. Thermosonication is an alternative preservation method that allows a significant decrease in PME activity and a smaller loss of vitamin C. The treatment with 40 kHz ultrasound and a temperature of 60 °C resulted in the highest reduction of PME activity. For ascorbic acid, the lower the heat used in combination with sonication, the smaller the loss of this compound. Soluble solids content, pH, and color of the juice did not alter during processing.

**Key words**: Ultrasound. Food preservation. Pasteurization. Cloudy.

**RESUMO** - A pectinametilesterase (PME) é a principal causadora de alterações em suco laranja. O método geralmente usado para inativação da pectinametilesterase no suco é a pasteurização. Devido a perdas sensoriais e nutricionais métodos não térmicos têm sido testados para inativação da PME. Dentre essas tecnologias, pode-se destacar a sonicação. Este trabalho buscou avaliar o efeito do uso conjugado de ultrassom e temperaturas medianas na redução da atividade da pectinametilesterase em amostras de suco de laranja. Além disso, foram determinados o teor de vitamina C, a cor, o pH, o teor de sólidos solúveis e a estabilidade em relação a turbidez, nas amostras submetidas aos tratamentos e realizada a comparação, tanto com amostras submetidas ao tratamento térmico, como com o suco natural. Observou-se que a aplicação da termossonicação é uma alternativa de método de conservação, permitindo uma redução significativa na atividade da pectinametilesterase e uma menor perda de vitamina C. O tratamento que apresentou melhor redução na atividade de PME foi utilizando ultrassom 40 kHz com temperatura de 60 ºC. Em relação ao ácido ascórbico, quanto menor a temperatura utilizada em conjunto com a sonicação, menor foi a perda deste composto. O teor de sólidos solúveis e o pH e a cor do suco não foram alterados ao longo do processamento.

**Palavras-chave**: Ultrassom. Conservação de alimentos. Pasteurização. Turbidez.

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# INTRODUCTION

Orange juice is a popular beverage consumed around the world mainly because of its good taste and relatively low cost. Additionally, orange juice consumption is increased because it is considered a healthy food and is rich in vitamin C (PANIAGUA-MARTÍNEZ *et al.*, 2018).

During processing, juice quality may be affected by many microbiological, chemical, physical or enzymtic factors (MASTELLO *et al.*, 2018). Among them, pectin methylesterase (PME; EC 3.1.1.11) is the main enzyme responsible for quality losses in orange juice.

PMEs are enzymes originating from plants or microorganisms. In plants, they act in the process of cell growth, elongation of the primary cells, and in the ripening of the fruit by softening the cell walls (RAMADAN, 2019). They catalyze demethylesterification reactions of the galacturonic acid present in pectin, thus reducing its molar mass, releasing methanol and converting pectin to pectate (CHAKRABORTY; RAO; MISHRA, 2019). The free carboxylic groups tend to bind with bivalent calcium ions leading to the formation of a gel network. The presence of carboxylic groups along the polymer leads to calcium crosslinking, causing precipitation and turbidity loss in juice.

The natural cloudiness of juice is due to a complex mixture of components such as proteins, pectin, cellulose lipids, and hemicellulose. In orange juice, this is a desirable characteristic. The Cloudy stability is essential because it affects the color, the taste, the flavor, and the mouthfeel of orange juice. Cloudy losses are the most important physical alteration in this beverage due to PME, which causes pectin demethylation, resulting in turbidity loss and pectin precipitation (MARSZAŁEK *et al.*, 2017).

PME inactivation in juice can prevent its negative effects and is generally undertaken by pasteurization (WILIŃSKA *et al.*, 2008). However, in comparison with the methods used e.g. for the degradation of microorganisms, more rigorous processing conditions are necessary for the inactivation of PME (PÉREZ-GRIJALVA *et al.*, 2018). These conditions may cause losses in sensory characteristics in pasteurized juice.

To decrease losses of sensory characteristics and alterations in physicochemical parameters, non-thermal treatment techniques have been tested for PME inactivation (ELEZ-MARTÍNEZ; SUÁREZ-RECIO; MARTÍN-BELLOSO, 2007). Sonication, which consists of the application of high-intensity sound waves to food, is one of those techniques.

High-powered ultrasound is used to induce cavitation in liquids, which is a process of formation and propagation of bubbles, which progressively increase in size until they collapse. This phenomenon generates high-pressure and high-temperature zones in the liquid, producing a localized pasteurization effect, which may inactivate microorganisms and enzymes (TIWARI *et al.*, 2009). Some of the effects of ultrasound technology in juice preservation and on PME inactivation have already been reported in recent studies (LAFARGA *et al*., 2019).

The aim of this study was to evaluate the effect of the combined use of ultrasound and moderate temperatures on the reduction of PME activity in orange juice samples. Furthermore, the vitamin C content, colour, pH, soluble solids content and cloud stability were determined in the samples treated with ultrasound and then compared to samples receiving conventional thermal treatment (pasteurization) as well as untreated juice.

# MATERIAL AND METHODS

## Sample preparation

Oranges (*Citrus sinensis*) were obtained from a local market in Alegre/ES (Brazil) and stored at 5 °C until they were processed into juice. The oranges were washed with tap water, dried with paper towels, cut into two pieces and pressed mechanically in a household juice extractor (Fruit Juice Extractor E-10, Mondial) in the Food Chemistry Laboratory at the Center of Agro-Sciences of the Universidade Federal do Espirito Santo (UFES), Alegre, Brazil. The juice was divided into seven parts: five treatment samples subjected to sonication at different temperatures, one control sample (fresh juice) and one pasteurization treatment sample treated only thermally.

Ultrasonic treatments were carried out in an ultrasonic bath (Soniclean 2PS ultrasonic cleaner, Sanders, 40 kHz). The 10 mL aliquot samples were distributed in Falcon tubes (15 mL), incubated in ultrasonic bath at the treatment temperature (25, 30, 40, 50, and 60 °C), for 10 minutes. The pasteurization treatment sample was treated with 90 °C for 30 sec. The results were compared to the untreated control sample (fresh juice).

## Physicochemical analyses

The soluble solids content was determined by refractometry using a benchtop refractometer (DIGIT). The soluble solids content was expressed in °Brix (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1995).

The pH was determined using the electrometric method with a pH meter (model mPA-210, Tecnopon). For this, 10 mL juice samples were used Association of Official Analytical Chemists (1995).

The ascorbic acid content in the samples was determined with the titrimetric method (967.21) by

Association of Official Analytical Chemistis (1995). The adopted methodology consists of reducing a coloured substance, 2,6-dichlorophenol indophenol (DCPIP), by titration with a solution containing ascorbic acid. The results obtained were expressed as milligrams of ascorbic acid per 100 mL sample, and the calculation was done as stated in equation 1.

$$\text{mg vit.C/100ml of juice} = (V-v)*F*100\,/\,\text{Volume of sample} \quad (1)$$

Where V is the volume of the sample used in titration, v is the volume of the control used in titration, and F is the dye factor.

The PME activity was evaluated according to Elez-Martínez, Suárez-Recio and Martín-Belloso (2007). Orange juice aliquot (10 mL) at 30 °C and 20 mL pectin (1%) were homogenized with sodium chloride (1.5%) solution, then incubated at 30 °C. The final solution was adjusted to a pH of 7 using 0.2 N NaOH and then to a pH of 7.7 using 0.05 N NaOH.

After reaching the previously described condition (pH of 7.7 and 30 ºC), 0.1 mL of 0.05 N NaOH was added, and the time required for the pH to return to 7.7 was measured. The PME activity (in units of PME) is described by Eq. (2).

$$A=[N\alpha 0H]*V_{N\alpha 0H}/V_{juice}*t \quad (2)$$

Where [NaOH] is the NaOH concentration, $V_{NaOH}$ is the volume of NaOH used, $V_{juice}$ is the volume of juice used, and t is the time (in minutes) needed for the pH value to return to 7.7. The residual PME activity (RA, %) is described by Eq. (3).

$$RA(\%) = 100 * \frac{A_t}{A_0} \quad (3)$$

Where $A_t$ is the enzymatic activity after applying the treatment to the juice and $A_0$ is the initial enzymatic activity, i.e., the enzyme activity of fresh juice.

Orange juice samples of 10 mL were centrifuged at 1200 rpm for 30 minutes at 20 °C (HERMLEZ 382K, Wehingen, Germany). The cloud value was determined as supernatant absorbance at 660 nm using a spectrophotometer (Bel Photonics 2000 UV) with distilled water as a blank (VERSTEEG *et al.*, 1980).

A colorimeter (Konica-Minolta CM-5 spectrophotometer) with CIEL*a*b system was used to determine the color change in the samples. The color attributes were expressed as L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Additionally, the hue angle (hº), C* (Chroma) and total colour difference (ΔE*) were calculated using equations (4 – 6).

$$h° = \tan^{-1}(b*/\alpha*) \quad (4)$$

$$C* = \sqrt{\left(\alpha^{*2} + b^{*2}\right)} \quad (5)$$

$$\Delta E* = \sqrt{(\Delta\alpha*^2 + \Delta b*^2 + \Delta L*^2)} \quad (6)$$

**Statistical analysis**

All measurements were performed in triplicate. The obtained data were represented as mean value ± standard deviation (SD). Completely randomized design (CRD) was conducted with 3 replicates for each treatment and in triplicate for each experiment. The ultrasound treatment data at different temperatures were subjected to univariate analysis of variance (ANOVA) and regression analysis. The comparison between the ultrasound treatment, the conventional thermal treatment and the control treatment was performed by ANOVA. Tukey's test was used when the ANOVA exhibited a significant difference between the treatments P < 0.05. Statistical analyses were carried out in the software Stasoft Statistica 10.

## RESULTS AND DISCUSSION

No significant differences (p > 0.05) between treatment and control samples for soluble solids content and pH (Table 1), indicating any quality loss due to our treatment in comparison to fresh juice. Additionally, any mathematical model was able to represent the variation of these variables according to the temperature used together with the ultrasound treatment. The values found in the experiment for the studied variables remained statistically constant after the application of the treatments performed in this study. Comparison of the two variables (pH and soluble solids content) between fresh juice and pasteurized juice showed that none of them was significantly affected.

Tiwari *et al.* (2009) also did not observe alterations in the soluble solids content or pH of orange juice treated with sonication. Zafra-Rojas *et al.* (2013) did not notice changes in these two characteristics when evaluating sonication of apple juice and purple cactus pear juice, respectively.

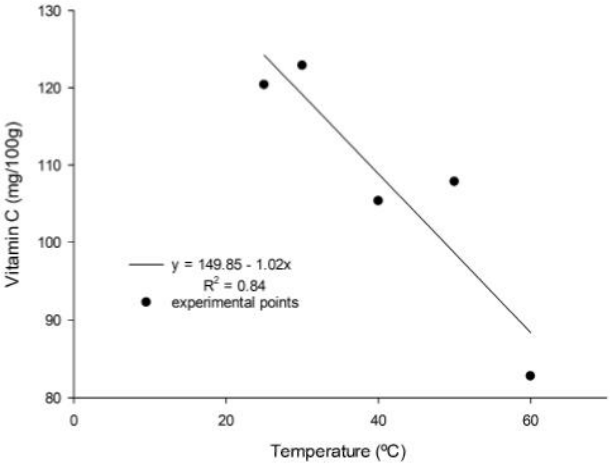
Ascorbic acid is a natural antioxidant present in oranges and its content is another essential characteristic when evaluating the quality of orange juice. Ascorbic acid can be degraded during processing and storage by many factors. High temperatures are one of the most critical factors that accelerate the vitamin C loss (BODINI *et al.*, 2019). According to this study vitamin C declines linearly with increasing temperature, in other words, the higher temperature used in combination with the ultrasound treatment, higher the loss in ascorbic acid in the juice samples (Figure 1). The determination coefficient ($R^2$) for the ascorbic acid model was 0.84. This statistical parameter confirms the consistency of the model, indicating a linear model satisfactorily.

**Table 1** - Effect of treatments applied to orange juice on soluble solids content pH and ascorbic acid

| Treatments* | Soluble Solids (ºBrix)ns | pHns | Ascorbic acid (mg/100 g) |
|---|---|---|---|
| FJ | 9.83 ± 0.94 | 3.33 ± 0.26 | 135.43 ± 7.53 a |
| US25 | 9.92 ± 0.80 | 3.32 ± 0.24 | 120.38 ± 2.85 ab |
| US30 | 9.92 ± 0.82 | 3.31 ± 0.24 | 122.89 ± 8.69 ab |
| US40 | 9.92 ± 0.91 | 3.31 ± 0.25 | 105.33 ±7.52 b |
| US50 | 9.92 ± 0,79 | 3.33 ± 0.22 | 107.84 ± 8.68 b |
| US60 | 9.92 ± 0.84 | 3.30 ± 0.26 | 82.76 ± 7,31 c |
| TT | 9.92 ± 0.80 | 3.28 ± 0.25 | 80.25 ± 4.34 c |

ns Means in the same column does not differ statistically to each other by ANOVA (F test) at the 5% probability level. Means followed by the same letter in the same column are statistically similar to each other by Tukey's test at the 5% probability level.*FJ: Fresh juice; US25 (Ultrasound to 25 ºC); US30 (US to 30 ºC); US40 (US 40 ºC); US50 (US 50 ºC); US60 (US 60 ºC); TT (thermal treated)

**Figure 1** - Vitamin C content according to the temperature used together with the ultrasound treatment




Vitamin C content of the samples treated with ultrasound at 25 and 30 °C did not differ significantly from fresh juice ($p > 0.05$), while the higher temperature-treatments resulted in decreased vitamin C content ($p > 0.05$). The ultrasound treatment at 60 °C exhibited the highest loss, with approximately 39% less ascorbic acid than fresh orange juice (Table 1).

Pasteurized sample presented the lowest value in vitamin C content compared to the other ones, however, not differing from the 60 °C ultrasound sample. All other treatments yielded lower reductions in vitamin C content compared with the pasteurized treatment ($p < 0.05$). The treatments with ultrasound at 25 °C and 30 °C retained approximately 89% of vitamin C content, applying 40 or 50 °C kept about 80% of the acid ascorbic while the pasteurization process kept only 60%. Thus, the use of thermosonication with up to 50 °C can be used as an alternative method for better preservation of ascorbic acid. In addition, ultrasound-treated samples could exhibit better vitamin C retention (TIWARI *et al*., 2009).

Other factors leading to the degradation of ascorbic acid can occur when ultrasound is applied to liquid systems, such as thermal effects due to bubble implosions, mechanical stresses induced by sound waves, and free radical production. With respect to vitamin C degradation, the most likely degradation mechanism is free radical production, which leads to the oxidation of ascorbic acid (VERCET; BURGOS; LÓPEZ-BUESA, 2001).

By processing orange juice in 20 kHz ultrasound equipment at different amplitude, temperature and treatment time conditions, Valdramidis *et al.* (2010) observed significant changes in the vitamin C content in samples treated with ultrasound compared with natural juice. The greatest loss was observed using the highest temperature tested in the experiment (30 °C), leading to a 15% loss of ascorbic acid content compared with unprocessed juice. Furthermore, as the temperature increased, the loss of vitamin C also increased.

Gómez-López *et al.* (2010) evaluated ascorbic acid degradation using 20 kHz ultrasound. The authors found that ascorbic acid degradation due to ultrasound depended on the treatment time. Tiwari *et al.* (2009) and Adekunte *et al.* (2010) also found results similar to Gómez-López *et al.* (2010) and Valdramidis *et al.* (2010), but for orange and tomato juice, respectively.

On the other hand, Aadil *et al.* (2015) and Abid *et al.* (2013) observed a significant increase in the ascorbic acid content in grapefruit, apple, lemon and guava juice samples, respectively, following treatment with ultrasound. All authors attributed these increases to the elimination of dissolved oxygen, which is essential for the degradation of ascorbic acid during cavitation.

PME residual activity in the treatment with ultrasound in combination with temperature decreased with increasing temperature. It was evident that the treatment at 25 °C, i.e., treated only with ultrasound, did not reduce the

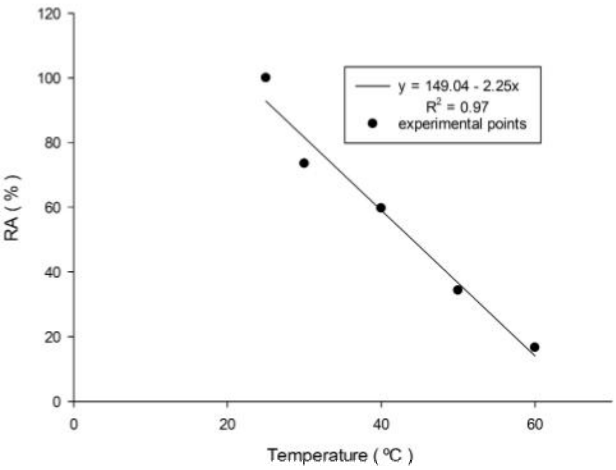
PME activity. The best result was found using ultrasound and a temperature of 60 °C, which yielded a residual activity of 16.5% (Figure 2). The mathematical equation that best described this phenomenon was a linear model, with an $R^2$ of 0.97, indicating a strong correlation between the experimental points and the predicted by the equation.

The PME residual activity results (Table 2) showed non-significant differences ($p > 0.05$) in control (fresh juice) and 25 ºC sonicated juice. All the remaining treatments exhibited reduced residual PME activity, as observed for the ultrasound treatments with temperatures of 30 ºC and 40 ºC, for which the reductions were statistically similar, even as treatments using ultrasound at 50 ºC and 60 ºC.

Furthermore, it was observed that pasteurization and treatment with ultrasound at 60 ºC was efficient in the PME inactivation, no significant difference in the reduction in PME activity was observed.

Ultrasound acts on the enzyme through mechanical and chemical processes induced by cavitation (CHEN *et al.*, 2019; SWAMY; MUTHUKUMARAPPAN; ASOKAPANDIAN, 2018). According to Snir *et al.* (1995), the effect of thermosonication on PME inactivation is more pronounced when lower temperatures are used. O'Donnell *et al.* (2010) commented that one explanation for this effect is that higher temperatures increase the vapour pressure inside the bubbles, causing a damping effect and decreasing the efficiency of bubble collapse in cavitation.

**Figure 2** - Residual PME activity according to the temperature used in combination with the ultrasound treatment




Enzymatic inactivation mainly occurs by protein denaturation caused by free radical formation, water sonolysis, or shear forces resulting from bubble formation and collapse (LAFARGA *et al.*, 2019; O'DONNELL *et al.*, 2010). The application alone is less effective than thermosonication, which involves the combination of sonication and heat. Several authors have reported their use in preserving and enhancing attributes (DOLAS; SARAVANAN; KAUR, 2019; TIWARI *et al.*, 2009).

The results (Table 2) indicate that the application of ultrasound at 25 ºC was not able to sufficiently inactivate PME. However, it was possible to obtain an 83.5% reduction by applying ultrasound at a temperature of 60 °C, evidencing that thermosonication is a feasible process.

Terefe *et al.* (2009), Tiwari *et al.* (2009) and Koshani *et al.* (2015) studied the effect of thermosonication on PME inactivation of vegetable/fruit juices using probe ultrasound employing frequencies of 20 and 24 kHz with temperatures up to 75 °C. Tiwari *et al.* (2009) showed that the application of 20 kHz ultrasound with temperatures below 50 °C resulted in an PME inactivation of 62%. Terefe *et al*. (2009), using 20 kHz and temperatures above 60 °C, observed a 6-fold decrease in PME activity. Wu *et al*. (2008) used 24 kHz ultrasound and temperatures of 60-65 and 70 °C for various durations (41.8, 11.7, and 4.3 min) and found a reduction of enzymatic activity by 90%. Finally, Koshani *et al*. (2015) observed about 91% inactivation with controlled temperatures ranging from 63-75 °C. All studies followed the same synergistic effect. High frequency and temperature

**Table 2** - Effects of treatments applied to orange juice on residual PME activity and cloudy stability

| Treatments | Residual PME Activity (%) | Cloud Stability[ns] |
|---|---|---|
| FJ | 100.00 ± 0.0 a | 0.63 ± 0.06 |
| US25 | 100.00 ± 4.9 a | 0.76 ± 0.06 |
| US30 | 73.50 ± 17.9 b | 0.97 ± 0.03 |
| US40 | 59.60 ± 15.1b | 0.92 ± 0.05 |
| US50 | 34.20 ± 8.9 c | 0.90 ± 0.04 |
| US60 | 16.50 ± 2.5 cd | 1.23 ± 0.08 |
| TT | 0.00 ± 2.0 d | 1.09 ± 0.07 |

[ns] Means in the same column does not differ statistically to each other by ANOVA (F test) at the 5% probability level. Means followed by the same letter in the same column are statistically similar to each other by Tukey's test at the 5% probability level

**Table 3** - Effect of treatments on orange juice color

| Treatments | Color | | | | | |
|---|---|---|---|---|---|---|
| | L*ns | a*ns | b*ns | h°ns | c*ns | ΔE* |
| FJ | 20.05 ± 0.56 | 8.74 ± 0.74 | 31.66 ± 0.66 | 75.25 ± 0.94 | 32.84 ± 0.83 | ------ |
| US25 | 22.00 ± 1.51 | 8.65 ± 0.65 | 34.03 ± 0.05 | 75.76 ± 1.00 | 35.11 ± 0.20 | 3.33 ± 0.85 |
| US30 | 21.70 ± 1.00 | 8.38 ± 0.38 | 33.10 ± 0.10 | 75.79 ± 0.58 | 34.14 ± 0.19 | 2.21 ± 2.01 |
| US40 | 21.46 ± 0.46 | 8.17 ± 0.17 | 32.99 ± 0.99 | 76.08 ± 0.12 | 33.98 ± 1.00 | 2.02 ± 0.38 |
| US50 | 20.09 ± 0.09 | 8.29 ± 0.29 | 31.52 ± 0.52 | 75.26 ± 0.26 | 32.59 ± 0.57 | 0.47 ±1.03 |
| US60 | 21.35 ± 0.35 | 8.13 ± 0.13 | 33.13 ± 0.13 | 76.21 ± 0.16 | 34.11 ± 0.15 | 2.05 ± 0.49 |
| TT | 21.81 ± 0.81 | 7.77 ± 0.77 | 33.74 ± 0.74 | 76.71 ± 0.96 | 34.62 ± 0.89 | 2.89 ± 0.74 |

ns Means in the same column does not differ statistically to each other by ANOVA (F test) at the 5% probability level

control could contribute to increasing cavitation rate/speed resulting in increased inactivation.

Using a bath ultrasound system, Aadil *et al.* (2015) observed the synergistic effect between temperature and ultrasound. They saw a 90% reduction in PME activity at 72 °C for 25.3 min in a 20 kHz ultrasound bath; a 96.8% reduction at 50 kHz and 80°C for 20 min and a 91% at 28 kHz, 60 °C and 60 min, respectively.

Therefore, the results presented in this work as well as the literature agree that the use of thermosonication in juice processing may be a viable alternative to conventional thermal treatment because it yields satisfactory results for PME inactivation.

Cloud loss in juice generally occurs because of the precipitation initiated by PME. No significant differences ($p > 0.05$) in the cloud stability between the tested samples (Table 2). There was no correlation between cloud stability and the temperature used in combination with ultrasound; the loss of cloudiness was practically constant across all treatments analyzed in this experiment.

Higher cloud stability in juices treated with ultrasound is frequently reported in the literature. (TIWARI *et al.*, 2009) noticed an increase in the stability of orange juice samples treated with ultrasound compared to untreated control sample. Abid *et al.* (2013) also observed that sonication significantly increased the stability of orange juice compared with the control sample. According to the authors, the high-pressure gradient formed during cavitation is responsible for the cloud stability effect. This high-pressure gradient leads to colloidal disintegration and causes macromolecules to breakdown, providing greater homogeneity in the juice.

Juice color is predominantly dependent on the presence of various color bearing compounds, including polyphenols (i.e., anthocyanins), carotenoids, and chlorophylls. Ultrasound processing of liquid foods is reported to have minimal color degradation during processing. This positive effect on color retention is mainly due to the effective removal of occluded oxygen from fruit juices (KNORR *et al.*, 2004). Therefore, color can constitute a valuable tool to determine the quality and nutritional losses of liquid foods during processing and subsequent storage. For this reason, it has been implemented in the quality control of different juice industries.

The values found in this study for the color of the various treated orange juice samples did not differ statistically from the control sample or from each other (Table 3).

Valero *et al.* (2007) also found that the treatment did not have any effect on the color under the tested conditions. Tiwari *et al.* (2009) noticed color alterations in orange juice treated with sonication. They found that a* and b* values decreased when applying more intense ultrasound treatment (amplitude levels and time), while L*, total color differences, browning, and turbidity increased. The authors attributed these changes to cavitation, which promotes many physical, chemical, and biological reactions, such as increased chemical reaction rates that may cause color alterations.

Gómez-López *et al.* (2010) also observed alterations in the color of sonicated orange juice. The samples increased in lightness, greenness, and yellowness. However, according to the authors, these changes were not sensorially perceptible.

## CONCLUSIONS

1. Soluble solids content, pH, and color of all treatment samples did not present statistical differences when compared to control. Vitamin C content, on the other hand, was profoundly affected by temperature. The lower the temperature used together with sonication, the better was the retention of ascorbic acid;

2. The use of 40 kHz and 60 °C thermosonication presented the most significant reduction in PEM activity. Thus, thermosonication is a feasible alternative preservation method that allows a substantial reduction in PME activity in orange juice and a better vitamin C retention compared to only thermal treatments.

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