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Effect of ultrasound treatment in fresh apple juices from different cultivars

Efeito do tratamento de ultrassom em sucos de maçã de diferentes cultivares

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Abstract

The sonication process is based on the cavitation phenomenon, which allows the extraction of target compounds, homogenization of solutions, and breakdown of plant cells. Thus, this paper aimed to assess the effect of ultrasound treatment in fresh apple juice to control the enzymatic browning effect. Three different apple varieties, Gala, Fuji and Argentina, were used to extract the juices, and were then subjected to ultrasound treatment for 0, 5, 15, and 30 minutes. Total soluble solids, citric acid content, pH, phenolic compound concentration, and color aspects were evaluated. Argentina apple variety had higher total soluble solids and pH values, with no visual differences between treatments. In contrast, ultrasound treatment of the fuji apple had a statistical difference to the control sample, regarding citric acid content. The analysis of the L* parameter, representing luminosity, showed slight variations ranging from 24.78 to 28.27 in all samples, with no significant differences observed among different treatment durations. Furthermore, the a* parameter showed a significant difference only for the Gala apple in the 0 and 5 min treatment. The b* and Chroma value was higher in Argentina variety (5.78 to 7.05 and 6.08 to 7.42, respectively) than in Fuji and Gala. On the other hand, a statistical difference was observed in the °HUE in Gala variety, faced to Fuji and Argentina. The content of phenolic compounds in the apples' varieties remained unaltered by ultrasound treatment, with values ranging from 52.47 to 116.23 mg of gallic acid equivalent L-1. These unexpected results indicated no significant variations between the sonification durations in the evaluated parameters. Thus, it is suggested that a new ultrasound procedure utilizing a probe and/or temperature control may be more effective in diminishing the enzymatic browning effect.

Keywords: Cavitation. Sonication. Color analysis. Polyphenoloxidase.

Resumo

O processo de sonificação é baseado no fenômeno da cavitação, que permite a extração de compostos quimicos, homogeneização de soluções e quebra de células vegetais. Assim, este trabalho teve como objetivo avaliar o efeito do tratamento ultrassônico em sucos de maçã para controlar o efeito de escurecimento enzimático. Três diferentes variedades de maçã, Gala, Fuji e Argentina, foram utilizadas para extrair os sucos e, em seguida, submetidas ao tratamento ultrassônico por 0, 5, 15 e 30 minutos. Foram avaliados sólidos solúveis totais, teor de ácido cítrico, pH, concentração de compostos fenólicos e aspectos de cor. A variedade de maçã Argetina apresentou maiores valores de sólidos solúveis totais e pH, sem diferenças visuais entre os tratamentos. Em contrapartida, o tratamento de ultrassom na maçã fuji apresentou diferença estatística em relação à amostra controle, no que diz respeito ao teor de ácido cítrico. A análise do parâmetro L*, representando a luminosidade, apresentou pequenas variações variando de 24,78 a 28,27 em todas as amostras, não sendo observadas diferenças significativas entre os diferentes tempos de tratamento. Além disso, o parâmetro a* apresentou diferença significativa apenas para a maçã Gala nos tratamentos 0 e 5 min. O valor de b* e Chroma foi maior na variedade Argentina (5,78 a 7,05 e 6,08 a 7,42, respectivamente) do que em Fuji e Gala. Por outro lado, foi observada diferença estatística no HUE na variedade Gala, frente a Fuji e Argentina. O teor de compostos fenólicos nas variedades de maçãs permaneceu inalterado pelo tratamento ultrassônico, com valores variando de 52,47 a 116,23 mg de ácido gálico equivalente L-1. Os resultados obtidos não indicaram variações significativas entre as durações do tratamento de ultrassom nos parâmetros avaliados. Assim, sugere-se que um novo procedimento de ultrassom utilizando sonda e/ou controle de temperatura pode ser mais eficaz na diminuição do efeito de escurecimento enzimático.

Palavras-chave: Cavitação. Sonicação. Análise de cores. Polifenoloxidase.

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INTRODUCTION

The enzymatic browning of vegetables results from physical damages during harvesting, transportation, storage, processing and physiological injuries, presenting as a final and unwanted product, mainly the oxidation of phenolic compounds into quinones. During the processing, cell breakdown occurs, triggering contact between the phenolic compounds and the enzymes responsible for browning, with consequences in the sensory (visual and taste parameters) and nutritional aspects of food products (Moon *et al.*, 2020). The protein polyphenol oxidases (PPO) can be found in plant tissues, vegetables, fruits, animals and some microorganisms, such as fungi and bacteria (Hamdan *et al.*, 2022; Moon *et al.*, 2020).

The browning issue is responsible for quality loss in fruits and vegetables, being this oxidation process is critical in food deterioration because approximately 50% of fresh fruit loss is caused by the enzymatic browning effect (Arnold; Gramza-Michałowska, 2022).

Fruits and vegetables are rich in phenolic compounds, which the PPO oxidizes to quinones, dark-colored compounds called melanin. The reaction occurs after the fruit comes into contact with oxygen, for instance, an apple that, when cut or physically damaged, has a partially white interior and reaches shades of brown after a particular time. However, this reaction does not occur in fresh fruits, once the substrate (phenolic compounds) is allocated in cell compartments apart from the enzyme (Hamdan *et al.*, 2022).

The phenolic compounds are secondary metabolites with recognized antioxidant activity (Ferreira *et al.*, 2021). Chemically, their structure has an aromatic ring with one or more hydroxyl groups, and its composition in fruits and vegetables varies according to the species, cultivation, degree of ripeness, and environmental conditions of development and storage (Saranraj; Behera; Ray, 2019).

Apple fruit and its products, such as puree, fresh-cut, and juices, are rich in phenolic compounds, which can lead to enzymatic browning. However, different cultivars like Gala, Argentina, and Fuji have different chemical compositions. Thus, different enzymatic browning effects (or levels) can be observed in different apple varieties before and after inhibition treatments (Arnold; Gramza-Michałowska, 2022).

Several methods have been used to control or minimize the enzymatic browning process, such as thermal treatment, modified atmosphere storage, low temperature, irradiation, and application of antioxidants (Hamdan *et al.*, 2022). However, most of them require using expensive equipment or adding synthetic substances to the foods. Therefore, alternative methods or proceedings to avoid enzymatic browning are expected in this scenario.

Thus, the cavitation phenomenon produced by the ultrasound equipment is based on the physical principle of low-frequency mechanical waves emitted by the cavitation process. The process begins with the formation and development of a microbubble. The microbubble generated by the device will collapse, and then, around the area, punctual zones of high pressure and temperature will be developed in the solution. The generated microbubbles can cause several characteristics in the samples submitted to the procedure, such as the breakdown of plant cells, the reduction of particulate matter in suspension, and the homogeneity in the sample (Ferreira *et al.*, 2020, 2023).

The ultrasound equipment, mainly bath models, is cheap and easily accessible in laboratories, used primarily for cleaning and disinfecting materials in clinics, microbiology laboratories, and in clinical analysis laboratories. Its use has a few limitations, such as the potency of some ultrasound models and the usability of probe ultrasound (because it is possible to perform one sample at a time). Regarding the effectiveness of the sonication process, this paper aimed to evaluate the effect of ultrasound in fresh apple juice.

2 MATERIALS AND METHODS

For this experiment, three varieties of apples (*Malus domestica Borkh*) were purchased from local commerce in Ponta Grossa (PR): Gala, Fuji, and Argentina. The apples were previously sanitized in a chlorine solution with 100 ppm of active chlorine for 15 minutes to obtain the juices. Afterward, the apples and utensils were rinsed with a 5 ppm active chlorine for 5 minutes. The juices were extracted using a domestic fruit centrifuge (Fama, FC600). The obtained samples were divided into control treatment (0 minutes) and ultrasound bath treatment (model USC-1400A - UNIQUE) for 5, 15, and 30 minutes at a constant room temperature. After the treatments, the juices were stored in hermetically closed plastic bottles in a freezer at -18°C for nine days. Thawing was conducted in refrigeration at ± 4°C for 24 hours.

The pH was determined in a digital pH instrument (KASVI). The total acidity, expressed in citric acid, was determined by titrimetry (Lutz, 2005), with the results expressed in mg g^{-1}. The total soluble solids were measured using a portable refractometer.

The color parameters measured using a Hunter Lab Ultra Scan Pro colorimeter were analyzed, including L* (representing brightness on a scale from 0 to 100, where 0 is black and 100 is white); a* (indicating the hue variation from green to red) b* (indicating the hue variation from blue to yellow), the angle ºHUE (indicating the hue; $h^* = \tan^{-1}(b^*/a^*)$), CHROMA (color saturation; $C^*=(a^{*2}+b^{*2})^{1/2}$) and ΔE (total color variation of the sample in a given time interval; $\triangle E^*_{ab} = ((\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2)^{1/2}$) (Botrel *et al.*, 2012; Mooz; Castelucci; Spoto, 2012).

The total phenolic content was determined using the Folin-Ciocateau method (Singleton; Rossi, 1965), with absorbance readings at 760 nm. The results were quantified using an analytical curve using gallic acid as reference (mg GAE L^{-1} of sample).

Statistical analysis was performed on the data, and the results are presented as mean ± standard deviation. In addition, the Tukey test was performed using SASM – Agri software to determine any significant differences among the means.

3 RESULTS AND DISCUSSION

The juice samples of the Fuji variety varied pH by around 4% above the control sample value, which obtained a pH of 4.03 (Table 1). For the Gala variety, the range of variation was inferior, with a pH of 4.0 for the 5- and 30-minutes treatments and 3.99 and 3.98 for the control and 15-minute treatments, respectively.

The Argentina apple variety also did not show variation to the control sample concerning the pH (Table 1), since the treatments of 15 and 30 minutes had a pH of 4.22. For the control treatment (0 min) and the time of 5 min, pHs of 4.32 and 4.23 were observed, respectively. Therefore, it was observed that the sonication treatment at different durations did not affect these parameters. This suggests that the initial pH characteristics of the raw material remained unchanged.

Table 2 shows the citric acid contents for the different treatments. Concerning the control sample, only the Fuji variety decreased 35% in citric acid content to the control sample. On the other hand, Gala and Argentina apples did not show variation in citric acid content, considering the standard deviation and the Tukey test. Besides, it is observed that the ultrasound treatment did not interfere with the citric acid content.

Table 1. Total soluble solids content (Brix %) and pH values of ultrasound-treated apples

Apple Varieties	Total soluble solids (%)				pH values			
	Treatments							
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
Fuji	11.8	11.8	12.1	11.0	4.03	4.21	4.21	4.21
Gala	11.1	11.1	11.2	11.2	3.99	4.00	3.98	4.00
Argentina	15.2	15.1	15.1	15.0	4.32	4.23	4.22	4.22

Also, the results suggest low variation in total soluble solids content (Table 1) since the ultrasound process cannot remove solids distributed in the solution. Besides, the treatment time did not promote the samples' concentration.

Table 2. Citric acid content of ultrasound-treated apples

Apple Varieties	Citric acid content (mg g^{-1})			
	Treatments			
	0 min	5 min	15 min	30 min
Fuji	2.54 ± 0.21^a	1.61 ± 0.12^b	1.75 ± 0.13^b	1.65 ± 0.02^b
Gala	2.66 ± 0.11^a	2.70 ± 0.13^a	2.54 ± 0.01^a	2.52 ± 0.19^a
Argentina	2.29 ± 0.11^a	2.23 ± 0.11^a	2.22 ± 0.11^a	2.18 ± 0.26^a

Equal letters in the same line for the same apple variety indicate statistical similarity at the 5% significance level.

The Argentina apple variety had the highest total soluble solids content. Due to its total soluble content, the juice extraction procedure was challenging, confirming its higher solids content.

Regarding the color, the 0 and 15-minute treatments showed superior in luminosity compared to the other treatment for Fuji apples, as indicated by the L* parameter (Table 3). The Gala variety juices did not have differences in the L* results regarding enzymatic browning. However, significant and negative differences were observed for the 5 and 30-minute treatments in Fuji apples and the 5-minute treatment in Argentina apples. These treatments exhibited inferior results regarding the L* parameter, indicating a higher degree of enzymatic browning.

The Chroma value of apple varieties increased with the duration of ultrasound treatment, suggesting a noticeable alteration in color perception. Fuji exhibited the highest degree of darkening after 30 minutes (ΔE 2.87), followed by Gala (ΔE 1.81) and Argentina (ΔE 1.63). The °HUE showed differences in the control to the 5, 15 and 30 min treatments in Fuji and Gala apples varieties. No HUE differences were observed to the Argentina variety. These results corroborated with previous studies that reported the preservation of the luminosity (L*) and chroma in sweet potato after ultrasound treatment (40 kHz for 10 minutes), as well as the significant variation in the ΔE parameter (from 21.54 to 8.48) in fresh-cut quince fruit after 14 days of storage (Pan *et al.*, 2020; Yildiz; Izli; Aadil, 2020). In addition, these studies have suggested a link between ultrasound treatment and browning inhibition, attributed to the reduction of polyphenol oxidase activity and an improvement in antioxidant activity, which helps combat oxidative stress (Pan *et al.*, 2020).

Regarding the content of polyphenolic compounds, apples and their products suffer from the degradation of phenolic compounds, which are substrates for polyphenol oxidase action. This effect leads to the production of *o*-quinones (already dark in color), which by secondary chemical reactions, produce melanin, a brown pigment. This phenomenon is known as enzymatic browning (Arnold; Gramza-Michałowska, 2022). Therefore, changes in the content of phenolic compounds can indirectly indicate the inactivation of polyphenol oxidase and evaluate the proposed US treatment.

Table 3. Color analysis of ultrasound-treated apples

Apple Varieties	Treatments	L	a*	b*	ΔE	Chroma	HUE
Fuji	0 min	27.77 ± 0.17 ^{a}	0.81 ± 0.13 ^{a}	3.12 ± 0.23 ^{b}	2.87	3.22 ± 0.25 ^{b}	1.32 ± 0.03 ^{b} 76°
	5 min	25.90 ± 0.13 ^{b}	0.52 ± 0.11 ^{a}	3.12 ± 0.23 ^{b}		3.17 ± 0.21 ^{b}	1.40 ± 0.05 ^{a} 80°
	15 min	28.26 ± 0.23 ^{a}	0.43 ± 0.04 ^{a}	4.34 ± 0.06 ^{a}		4.36 ± 0.06 ^{a}	1.47 ± 0.01 ^{a} 84°
	30 min	25.41 ± 0.25 ^{b}	0.80 ± 0.27 ^{a}	4.75 ± 0.76 ^{a}		4.82 ± 0.80 ^{a}	1.41 ± 0.03 ^{a} 81°
Gala	0 min	25.18 ± 0.97 ^{a}	0.02 ± 0.01 ^{b}	3.10 ± 0.55 ^{c}	1.81	3.10 ± 0.55 ^{c}	1.56 ± 0.00 ^{a} 90°
	5 min	25.21 ± 0.17 ^{a}	0.47 ± 0.09 ^{ab}	3.76 ± 0.05 ^{bc}		3.79 ± 0.06 ^{bc}	1.45 ± 0.02 ^{b} 83°
	15 min	24.78 ± 0.52 ^{a}	0.76 ± 0.35 ^{a}	4.96 ± 0.49 ^{a}		5.02 ± 0.51 ^{a}	1.42 ± 0.06 ^{b} 81°
	30 min	26.06 ± 0.39 ^{a}	0.94 ± 0.10 ^{a}	4.38 ± 0.08 ^{ab}		4.48 ± 0.06 ^{ab}	1.36 ± 0.03 ^{b} 78°
Argentina	0 min	27.36 ± 0.50 ^{a}	1.65 ± 0.20 ^{a}	5.88 ± 0.24 ^{b}	1.63	6.11 ± 0.28 ^{b}	1.30 ± 0.02 ^{a} 74°
	5 min	26.31 ± 0.14 ^{b}	1.85 ± 0.46 ^{a}	5.78 ± 0.74 ^{b}		6.08 ± 0.84 ^{b}	1.26 ± 0.04 ^{a} 72°
	15 min	27.36 ± 0.33 ^{a}	1.93 ± 0.05 ^{a}	6.79 ± 0.06 ^{ab}		7.06 ± 0.06 ^{ab}	1.29 ± 0.01 ^{a} 74°
	30 min	28.27 ± 0.39 ^{a}	2.33 ± 0.16 ^{a}	7.05 ± 0.34 ^{a}		7.42 ± 0.37 ^{a}	1.25 ± 0.01 ^{a} 72°

Equal letters in the same column for the same apple variety indicate statistical similarity at the 5% significance level.

The content of phenolic compounds in the Fuji apple variety after treatment for 30 minutes was lower than the content in the 0 min treatment, suggesting a potential consumption of the PPO substrate. These results are consistent with Yildiz and Aadil (2022), who investigated the application of ultrasound for 15 min in fresh-cut mango storage for 0, 7 and 14 days. They concluded that ultrasound decreases PPO enzyme content of 311.13, 412.11, and 595.12 U mL^{-1} to 76.17, 101.35, and 134.12 U mL^{-1} after 0, 7 and 14 days, respectively.

After 5 minutes of treatment, the Fuji variety had a reduction of 13 % in the polyphenolic compounds. Similarly, Qiao et al. (2021) evaluated the application of *Sonchus oleraceus L.* extract and ultrasound treatment for 5 min and observed a control in polyphenol oxidase activity, preventing enzymatic browning. Therefore, the sonication method is an alternative to the traditional chemical methods.

According to

Table 4, the content of phenolic compounds showed no significant differences between treatment times. The Gala variety showed a difference of 26% regarding the 30 to 0 min treatment, increasing the content of phenolic compounds similar to Yildiz et al. (2020), which used the ultrasound bath (28 kHz frequency, 50 W power) for 15 min to evaluate the shelf life of fresh-cut quince fruit (Cydonia oblonga Mill.). After 14 days of storage at 4 °C, the content of the total phenolic compounds increased from 13.72 (control treatment) to 23.87 gallic acid equivalents $100g^{-1}$ of sample fresh weight. These results indicate low effectiveness in the ultrasound process in both studies.

Concerning the Argentina apple variety, the content of phenolic compounds was preserved after the 30-minute treatment, suggesting that phenolic oxidation did not occur differently from the control.

However, no statistical differences were observed concerning the 95% of Tukey's test confidence level. So, it is possible to verify no mathematical alterations regarding the enzymatic browning effect by ultrasound.

Table 4. Phenolic compounds from different apple varieties after US treatment

Phenolic compounds (mg GAE L^{-1})				
Apple Varieties	Treatments			
	0 min	5 min	15 min	30 min
Fuji	60.70±21.66[a]	52.78±7.26[a]	54.75±9.95[a]	56.66±10.75[a]
Gala	52.47±9.89[a]	56.12±18.61[a]	66.91±3.72[a]	70.90±13.18[a]
Argentina	110.36±5.91[a]	116.23±11.34[a]	103.66±6.03[a]	111.56±5.20[a]

Equal letters in the same line for the same apple variety indicate statistical similarity at the 5% significance level.

The results of total phenolic compounds follow the global color variation data for the Argentina apple variety, showing less variation, indicating the maintenance of phenolic compounds without being consumed by the PPO enzyme.

Regarding the temperature of inactivation of PPO above 50 °C (Zawawi *et al.*, 2022), using the sonication process through ultrasound equipment is a simple, cheap, and efficient method, mainly considering the quality of some processed foods, such as fruits and vegetables, avoiding using high temperatures in their industrial process.

4 CONCLUSION

There were no satisfactory results regarding enzymatic inactivation using the sonication technique in samples of apple juice from different varieties, demonstrated mainly through colorimeter analyses, where a differential was expected, mainly concerning the parameter L* (luminosity). Therefore, although the application of different times did not influence the enzymatic browning process, an alternative using this same method would be the application of probe ultrasound or heating during the sonication treatment, allowing more easily the inactivation of thermolabile enzymes and thus inhibiting the enzymatic browning process caused by polyphenol oxidase enzyme.

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