Research article

Effect of ultrasound and steam treatments on bioaccessibility of β-carotene and physicochemical parameters in orange-fleshed sweet potato juice

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ABSTRACT

Several health benefits have been associated to orange-fleshed sweet potato owing to the existence of various bioactive compounds, including β-carotene. The purpose of this study was to establish the effect of ultrasound and steam treatment on the bioaccessibility of β-carotene, total polyphenols, antioxidant activity, polyphenol oxidase, peroxidase activity, and color in the orange-fleshed sweet potato juice. Sweet potato juice was processed using ultrasound (0.66 W cm−2 and 8 min), steam (2 min), and a combination of steam and ultrasound. The bioaccessibility of β-carotene was increased in processed sweet potato juice, with samples treated by ultrasound showing the highest bioaccessibility (76.6%). Processing had no effect on antioxidant or enzyme activity, but resulted in significant changes in the color of the juice. As a processing technology, ultrasound enables preservation or improvement of the quality of sweet potato juice, and when combined with other treatments, facilitates the development of new products.

1. Introduction

Sweet potato (Ipomoea batatas L.) is known as one of the most important crops around the world, together with rice, wheat, potato, maize, and cassava. It has been cultivated due to its nutritious and health-benefits for humans (Wang et al., 2016). Sweet potato is commonly consumed in México, where it is cooked with molasses or consumed as crystallized candies. To take advantage of its nutritional value, there is a need to identify alternative uses of this product. Ipomoea batatas L. has high concentration of starch, and also contains dietary fiber, vitamins, minerals, and antioxidants, such as carotenoids, anthocyanins, phenolic acids, tocopherol (Teow et al., 2007). In orange-fleshed sweet potato, β-carotene is the main pigment, as well as a major precursor of vitamin A (Kidmose et al., 2007). The favorable effects of bioactive compounds are dependent on their bioavailability; therefore, it is necessary to evaluate the percentage of their liberation from the food source and their capacity to be absorbed through the intestinal wall (Hervert-Hernández et al., 2010). It is possible to examine the bioaccessibility of carotenoids by computing the quantity shifted to the micelle fraction following a simulated in vitro digestion methodology (Pugliese et al., 2013). Previous studies have reported the retention and bioaccessibility of β-carotene in sweet potato and products based on this, subjected to different treatments (Bechoff et al., 2011; Failla et al., 2009; Trancoso-Reyes et al., 2016; Zhang et al., 2018). However, there have been no reports on sweet potato juice.

Juices from fruit and vegetables are commonly used as agents to carry high concentrations of bioactive compounds, since juices are an appropriate form for their intake (Anaya-Esparza et al., 2017). The selection of the processing method is crucial since it can be used to reduce the negative physical and chemical effects induced by excessive thermal treatments. Blanching is an essential phase in the processing of vegetables and vegetable commodities, including juices, to inactivate enzymes and microorganisms, to remove entrapped air, and preserve color (Bhat and Sharma, 2016). Enzyme inactivation, mainly of polyphenol oxidase, represents a quality parameter in the processing of fruits and vegetables. Previous reports have indicated that the inactivation of enzymes is difficult to achieve only through the use of ultrasound (Bi et al., 2015; Cheng et al., 2007; Ríos-Romero et al., 2018). Nevertheless, blanching can also negatively affect the heat sensitive nutrients, texture, water soluble components, and the value and biological activity of the final

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products. This depends on the thermal supply to the product. Alternative technologies are gaining popularity as food processing techniques, such as ultrasonic waves on juices that can not only maintain but also enhance the nutritional value (Jabbar et al., 2014). Ultrasound is a form of vibrational energy in the frequency range of 20–100 kHz with a sound intensity of 10–1000 W cm⁻² (O’Donnell et al., 2010). The benefits of ultrasound are attributed to acoustic cavitation, which consists of the formation, growth, and collapse of microbubbles due to pressure changes (Chemat et al., 2017). Ultrasound treatment can enhance improvements in food products, either by increasing or maintaining the nutritional quality, and increasing the bioaccessibility of bioactive compounds. In this context, the objective of this study was to determine the effect of ultrasound and steam treatment on the bioaccessibility of β-carotene, antioxidant activity, polyphenol oxidase and peroxidase activity, and color in sweet potato juice.

2. Materials and methods

2.1. Materials

HPLC type solvents (methanol, acetoniitrite, and 2-propanol) were acquired from J.T. Baker (Baker-Mallinckrodt, Mexico). The β-carotene standard, hydrogen peroxide (H₂O₂), chlorogenic acid (5-cafeoilquinic acid), ferrous chloride (FeCl₂), linoleic acid, 2,6-Di-tert methyl phenol (BHT), ammonium thiocyanate (NH₄SCN), pyrogallol, 1,2-dihydroxybenzene-butyl-4-(catechol) acid, 2,2’azobis (2-amino-propane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein, porcine pepsin, α-amylase, and pancreatin were obtained from Sigma-Aldrich (St. Louis, MO, USA). The rest of substances used were of analytical brand. The orange-fleshed sweet potatoes variety was from the northeast region of Mexico and was bought in a local supermarket in Durango, Mexico. The same lot was used for all experiments. The roots were stored in a cold room (6 ± 2 °C) until processing.

2.2. Obtention of sweet potato juice

Sweet potatoes were washed, peeled, and cut in 4-cm-wide sections. In order to get the juice, the sweet potatoes were placed in an extractor (TURMIX® D.F. México). The resulting juice immediately was processed and subjected to three types of treatment: (i) ultrasound waves, applied with an ultrasonic device (UP2000H; Hielscher Ultrasound Technology, Germany) attached to 40 mm diameter probe (treatment time of 8 min, ultrasound intensity of 0.66 W cm⁻², and a constant frequency of 26 kHz) (Ríos-Romero et al., 2018); (ii) steam treatment, performed using a domestic steamer for 2 min; (iii) a combination of steam and ultrasound treatment. A sample of juice without treatment was physicochemically characterized and used as a control. Treated juices treatment were kept in the dark at –20 °C up to analysis. For the analysis of in vitro bioaccessibility, the processed sweet potato juices were lyophilized.

2.3. Physicochemical characterization (pH, soluble solids, titratable acidity)

The pH was measured using a potentiometer (Microprocessor pH Meter 212; HANNA Instruments, Romania). Measurements of soluble solids were determined as ‘Brix using a manual refractometer (ATAGO PAL-1; Tokyo Tech. Award, Japan) at 25 ± 1 °C. Titratable acidity (TA) was measured with sodium hydroxide (0.1 N) up to a pH of 8.2 ± 0.1. It was expressed as grams of citric acid per 100 mL of sample.

2.4. Extraction and analysis of β-carotene

The β-carotene extraction from the treated samples was performed as reported by Ríos-Romero et al. (2018). Briefly, sweet potato juice (3 mL), 0.2 g of sodium carbonate and 20 mL of methanol were mixed and filtered. Then, the solids were washed twice using 20 mL of methanol. After, the residue was washed three times with 15 mL of acetone/hexane (1:1, v/v) and BHT (0.1%). The supernatant from the series of washings was placed in a separation funnel containing 20 mL of sodium sulfate solution (10% w/v). Subsequently, 100 mL of distilled water was added and carefully mixed until 400 mL was completed. Then, 10 mL of petroleum ether was poured. Finally, the organic phase was separated and evaporated at 40 °C in a rotary evaporator. Subsequently, the samples were mixed with acetoniitrite and methanol (85:15, v/v). The β-carotene content was measured by ultra-high-performance liquid chromatography (UPLC). The liquid chromatography system involved a sample administrator (5 °C) and a binary solvent administrator connected with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The column used was an Acquity UPLC HSS T3 (2.1 mm × 100 mm × 1.8 μm) (Waters Corp., Milford, MA, USA) at 35 °C. The gradient system with the mobile phase consisted of solvent A (acetoniitrite:methanol) (85/15, v/v) and solvent B (2-propanol, 100%). The ramp program consisted of 95:5 (A:B) at initial conditions, 95:5 (A:B) from 0 to 2.0 min, 50:50 (A:B) from 2 to 3.5 min, and 95:5 (A:B) from 3.5 to 4.5 min, the flow rate was 0.5 mL min⁻¹. The atmospheric pressure chemical ionization conditions were as follows: positive polarity; Corona, 3.5 kV; cone, 30 V; source temperature, 150 °C; probe temperature, 550 °C; desolvation and cone gas, 550 L h⁻¹ and 150 L h⁻¹, correspondingly, and collision gas 0.15 mL min⁻¹. For the determination of β-carotene, the calibration curve was constructed. The method was linear in the range from 4 to 20 μg mL⁻¹ with a correlation coefficient of 0.9979. The UPLC and tandem Xevo TQ-S triple quadrupole mass spectrometer control and data processing were carried out using MassLinx (Water Corp., Milford, MA, USA) software.

2.5. In vitro digestion of β-carotene

The percentage of β-carotene bioaccessibility in the sweet potato juice was evaluated based on the standardized static in vitro digestion method suggested in a consensus report by Minekus et al. (2014) with some modifications. Samples of sweet potato lyophilized juice (1 g) were exposed to imitated oral, gastric, and small intestinal stages of digestion. Briefly, Simulated Salivary Fluid stock solution (3.5 mL) at pH 7, α-amylase solution of 1500 U mL⁻¹ (0.5 mL), CaCl₂ (25 μL, 0.3 M), and water (975 μL) were added to the sample. Afterwards the mixture was gently shaken at 37 °C for 2 min. Next, oral bolus was combined with 7.5 mL of simulated gastric fluid stock solution at pH 3, 1.6 mL of pepsin solution (25000 U mL⁻¹), and CaCl₂ (5 μL, 0.3 M) adjusted to pH 3 with hydrochloric acid 1 M, followed by the addition of water to obtain a final amount of 20 mL. The mixture was then shaken at 37 °C for 2 h. For the small intestinal stage, 10 mL of simulated intestinal fluid stock solution at 37 °C and pH 7, pancreatin solution 5 mL (800 U mL⁻¹ trypsin activity), bile extract (2.5 mL), and CaCl₂ (40 μL, 0.3 M) were mixed. The pH was settled to pH 7 with NaOH, 1 M and some water was supplemented to obtain a final amount of 40 mL. Finally, the sample was agitated at 37 °C for 2 h. The extraction of carotenoids was performed on the entire micellar fraction left, with the addition of diethyl ether (20mL) and NaCl (10mL, 10%, w/v). The mixture was agitated in a vortex for 1 min and then centrifuged at 4410 × g at 4 °C for 30 min. Then, the micellar fraction was moved to a beaker containing anhydrous sodium sulfate and concentrated by rotary evaporation. Quantification of β-carotene was performed as described in the previous section. The bioaccessibility percentage was calculated using Eq. (1):

\[
%Bioaccessibility = \frac{(β – carotene digested)}{(β – carotene in juice)} \times 100
\]  

(1)

2.6. Analysis of total polyphenols

Analysis of total polyphenols were performed by using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Twenty five microliters
Control 6.00 ± 0.01 a
Ultrasound 5.96 ± 0.03 a
Steam 5.99 ± 0.03 a
Steam + Ultrasound 5.95 ± 0.03 a

Mean ± standard deviation followed by different superscript letter in the same column indicate that values are significantly different (p < 0.05.) by Tukey’s test.

Table 2 shows the β-carotene content in sweet potato juice subjected to various processing methods. Ultrasound process improved the extraction of β-carotene by 21.3% with respect to the control sample. The juice treated with the combination of steam and ultrasound presented an
increase of 16.4% in the β-carotene content in respect to the control sample. Several authors (Abid et al., 2014a, b; Jabbar et al., 2014; Santhirasegaram et al., 2013; Rios-Romero et al., 2018) have pointed out similar increases in the content of carotenoids in sonicated juices attributed to the blow up of the cell tissues. Treatment with steam resulted in a non-significant change of 2.8%, which may be due to the light processing conditions (only 2 min).

The bioaccessibility of carotenoids from vegetable commodities is the main consequence from the disruption of cells during preparation, such as processing and chewing (Zaccari et al., 2015). The effect of treatment in the percentage of bioaccessibility is presented in Table 2. A notable increase in the bioaccessibility of β-carotene was detected in the processed samples. Samples processed with ultrasound showed the highest increase in the percentage of bioaccessibility, reaching a value of 76.64%. The improvement in the bioaccessibility of β-carotene could be because the acoustic waves, which, under the given conditions, did not damage the structure of the β-carotene. However, when the steam and ultrasound treatments were combined, β-carotene could undergo thermal and chemical oxidation, decreasing its bioaccessibility. Food processing modifies the structure of the food matrix, which may cause changes in the bioaccessibility of their bioactive compounds. Specifically, ultrasound treatment may improve the bioaccessibility of carotenoids, as reported by Campoli et al. (2018) in sonicated guava juice, they reported an important increase in lycopene bioaccessibility. Additionally, Gille et al. (2019) reported that the ultrasound extraction of carotenoids from P. tricornutum biomass caused a significant increase of up to three-fold in the bioaccessibility of β-carotene. Similarly, Mercado-Mercado et al. (2018) observed that the ultrasound treatment of mango by-products improved the bioaccessibility of β-carotene, with an increase of 32.6% in mango peel and 44.0% in mango paste compared with the control sample. The mechanisms proposed for the liberation of carotenoids are as follows: (1) the modification of the cell by the cavitation phenomenon; (2) breakdown of the glycolysic linkages in dietary fiber; 3) the exposure of ester bonds, lipids, and peptides to the outside; (4) an increment in the enzyme activity of pepsin and pancreatin; (5) hydrolysis of α1–4 glycosidic bonds present in the amylase and amylepectin chains; (6) decrease in the viscosity of digestible fiber in the intestinal fragment; (7) liberation of carotenoids present in soluble digestible fiber (Mercado-Mercado et al., 2018).

### 3.3. Processing effect on total polyphenols, antioxidant activity, and enzymatic activity

The effect of ultrasound and steam processing on the total polyphenols in the sweet potato juices is indicated in Table 3. During the cavitation phenomenon, the formation of free radicals results in a reduction in the amount of bioactive compounds. This was detected in the samples of sweet potato juice treated with ultrasound (a reduction of 11.9% in the total polyphenols content). In addition, the degradation of these compounds may occur as a result of oxidation caused by the enzymes released in the process but which were not inactivated. In previous studies, Dias et al. (2015) and Fonteles et al. (2012) also communicated a reduction in the content of these compounds. The results concerning the effect of steam and the combination of steam and ultrasound treatment on sweet potato juice showed that phenolic compounds were not significantly different. Ma et al. (2013) reported that blanching in a water bath at 86 °C for 10 min increased the total polyphenol content, whereas blanching assisted in the dissolution of polyphenols in the juice. Bhat and Sharma (2016) evaluated the effect of combining blanching and ultrasound treatment on total polyphenols in gourd juice and found that the extraction and preservation of total polyphenols was appreciably higher in the processed samples than in the control sample.

The antioxidant activity of sweet potato juices is shown in Table 3. Due to its lipophilic characteristics and its capacity to scavenge peroxyl radicals, the ferric thiocyanate method, which has the ability to capture peroxyl radicals and to react with polyunsaturated fatty acids, was used to measure the antioxidant activities of carotenoids in sweet potato juice. The inhibition of linoleic acid oxidation in sweet potato juices processed with ultrasound and steam presented insignificant changes compared to the control sample. This insinuates that the existence of non-phenolic components aided to the antioxidant activity of the juice. The ORAC method was used to evaluate the antioxidant activity attributed to the polyphenolic compounds. This assay tests the ability of antioxidants present in juice to preserve fluorescein from oxidative injury. According to the results obtained using the ORAC method, the antioxidant activity of the treated samples showed insignificant changes in comparison to the control sample. The antioxidant activity was mainly attributed to non-phenolic compounds, such as β-carotene, but also to the phenolic compounds present in the juice. In a previous study, Rios-Romero et al. (2018) reported the same tendency in sweet potato juice sonicated under different conditions.

With regards to enzyme inactivation (Table 4), the processing conditions did not inactivate polyphenol oxidase or peroxidase, most likely due to the changes in protein conformation, which promoted the combination of substrate and enzyme. Earlier studies (Yu et al., 2013; Wang et al., 2018) have indicated that ultrasound under mild conditions does not inactivate enzymes. Even in the current study, ultrasound treatment combined with steam was not enough to enhance the inactivation of enzymes. Failure to inactivate the enzymes can have detrimental effects
Table 4. Effect of processing on the residual activity of polyphenol oxidase and peroxidase in sweet potato juice.

| Process           | Polyphenol oxidase residual activity (%) | Peroxidase residual activity (%) |
|-------------------|-----------------------------------------|----------------------------------|
| Control           | 100.0 ± 0.00                         | 100.0 ± 0.00                     |
| Ultrasound        | 98.7 ± 3.20                           | 97.8 ± 3.00                      |
| Steam             | 108.4 ± 6.40                          | 108.1 ± 7.80                     |
| Steam + Ultrasound| 92.0 ± 5.10                           | 104.5 ± 7.90                     |

Mean ± standard deviation followed by different superscript letter in the same column indicate that values are significantly different (p < 0.05) by Tukey's test.

Table 5. Effect of processing on the color characteristics in sweet potato juice.

| Process          | Color characteristics | TCD | Chrome | Hue angle |
|------------------|-----------------------|-----|--------|-----------|
|                  | L*                    | a*  | b*     |           |
| Control          | 47.7 ± 0.50           | 21.6 ± 0.80 | 35.3 ± 0.70 | 41.0 ± 1.50 | 58.1 ± 0.40 |
| Ultrasound       | 46.2 ± 0.60b          | 20.9 ± 0.80b | 33.6 ± 0.20b | 2.5 ± 0.20b  | 38.4 ± 2.00 | 58.0 ± 0.60b |
| Steam            | 45.5 ± 0.80bc         | 19.3 ± 0.70bc | 31.6 ± 0.50bc | 4.9 ± 0.90bc | 37.3 ± 1.70bc | 58.5 ± 0.40bc |
| Steam + Ultrasound| 44.9 ± 0.50bc         | 19.7 ± 0.40bc | 32.3 ± 0.70bc | 4.5 ± 0.80bc | 37.8 ± 0.90bc | 58.5 ± 0.10bc |

Mean ± standard deviation followed by different superscript letter in the same column indicate that values are significantly different (p < 0.05) by Tukey's test.

3.4. Effect of processing on color

Color is a visible signal of the grade of fruit juices and represents an important characteristic in consumer pleasure. Thus, color can point up the acceptance degree of fruit juice (Dias et al., 2015). The effect of the processing treatments on the color of sweet potato juice are summarized in Table 5. The processed samples showed lower color results for brightness (L*), redness (a*), and yellowness (b*) with respect to the control. A similar trend of reducing color values was formerly found by Aadil et al. (2013) and Song et al. (2018) in grapefruit and sweet corn juice, respectively. Differences in visible color can be categorized based on the total color difference (TCD). Choi et al. (2002) pointed out that TCD values > 2 corresponded to perceptible differences in product impression. In this study, the processes induced “visible” color changes in sweet potato juice, with noticeable differences between the applied processes. Processing can cause physical, chemical, and biological reactions that affect the color of sweet potato juice. In this investigation study, the changes observed in the color of the sweet potato juice may be caused by cavitation or thermal degradation, which result in the destruction of pigments. Chrome specify the grade of saturation, purity, or visible strength of color, and is described as the level of alteration from gray to a pure chromatic color (Cruz-Cansino et al., 2015). Therefore, the control presented the highest value, representing a high color saturation. Similarly, Mohideen et al. (2015) observed lower Chrome values in blueberry juice processed using ultrasound. With regard to Hue angle, which represents tonality, none of the juices presented any significant changes, and tonality was maintained for all treatments. Ornelas-Paz et al. (2007) reported elevated a* results and small Hue angle values in mango, which were corresponded with a high β-carotene results in mango flesh. However, in the present study, because of the slight changes in the color parameters, it was not possible to investigate this behavior.

4. Conclusions

In the present investigation, the effects of steam and ultrasonic treatments on the bioaccessibility of β-carotene, antioxidant activity, enzyme activity, and color in orange-fleshed sweet potato juice were investigated. As a result, the bioaccessibility of β-carotene was found to increase after processing, with sonication being the most effective. All of the treatments applied successfully preserved the antioxidant properties of the juices. However, not all of the conditions use for the treatments were effective in inactivating polyphenol oxidase and peroxidase. After processing, the samples of sweet potato juice preserved their characteristic color, and our results indicate that the best processing method was ultrasound. The results presented in this study provide an insight into the advantages of consuming sweet potato juice combined with other juices, enhancing the taste and functional characteristics of this juice due to the high carotenoid content.

Declarations

Author contribution statement

Evelyn Alicia Rios-Romero: Performed the experiments; Wrote the paper.
Luz Araceli Ochoa-Martínez: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Luis Arturo Bello-Pérez: Conceived and designed the experiments.
Juliana Morales-Castro: Contributed reagents, materials, analysis tools or data.
Armando Quintero-Ramos: Performed the experiments.
José Alberto Gallegos-Infante: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.
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