Effect of thermosonication on enzymatic oxidation and physicochemical properties of soursop (*Annona muricata*) pulp

Efecto de la termosonicación en la oxidación enzimática, y las propiedades físico-químicas de la pulpa de guanábana (*Annona muricata*)

Victor Manuel Gelvez Ordóñez¹, Ivan Daniel López Castilla², Luis Eduardo Ordoñez-Santos³

Abstract

Soursop is an exotic tropical fruit with marked commercial importance. However, its post-harvest presents losses of around 30%. The objective of this research was to study the effect of thermosonication (TS) on physicochemical properties and enzymatic oxidation of soursop pulp. Four treatments were evaluated: control (0 min), ultrasound at 20 °C (US20), 40 °C (US40), and 60 °C (US60) for 25 minutes. Concentration of vitamin C, and peroxidase (POD) and polyphenoloxidase (PPO) inactivation in the soursop pulp, were assessed. The results indicate a loss of vitamin C of 2.63%; 13.16% and 28.95% for temperatures at 20, 40 and 60 °C, respectively. On the other hand, partial inactivation of 48.41%; 69.83% and 74.24% of POD, and 56.38%; 74.47% and 81.91% of PPO enzymes were achieved at temperatures of 20, 40 and 60 °C, respectively. Finally, it was concluded that ultrasound combined with heat (TS) can inactivate POD and PPO enzymes in the soursop pulp, achieving considerable retention of vitamin C.

Keywords

pH • Soluble solids total • Polyphenoloxidase • Peroxidase • Vitamin C

³ Universidad Nacional de Colombia. Facultad de Ingeniería y Administración. Departamento de Ingeniería. Colombia. leordonezs@unal.edu.co
Resumen

La guanábana es una fruta tropical exótica tiene una marcada importancia comercial, sin embargo, presenta pérdidas de alrededor del 30% en poscosecha. El objetivo del trabajo fue estudiar el efecto de la termosonicación (TS) sobre propiedades fisicoquímicas y la oxidación enzimática de la pulpa de guanábana. Los tratamientos evaluados fueron control (0 min), ultrasonido a 20 °C (US20), a 40 °C (US40), y a 60 °C (US60) durante 25 minutos. Se evaluaron principalmente la concentración de vitamina C, la inactivación de la peroxidasa (POD) y la polifeniloxidasa (PPO) en la pulpa de guanábana. Los resultados indican que se observó una pérdida de vitamina C en un 2,63; 13,16 y 28,95% para temperaturas a 20, 40 y 60 °C, respectivamente. Por otro lado, una inactivación parcial de las enzimas, POD, en 48,41; 69,83 y 74,24% y para PPO en 56,38; el 74,47 y el 81,91% se logró a temperaturas de 20, 40 y 60 °C, respectivamente. Finalmente, se demostró que el ultrasonido combinado con calor (TS) inactivan en un gran porcentaje las enzimas POD y PPO en la pulpa de guanábana logrando importantes niveles de retención de la vitamina C.

Palabras clave
pH • sólidos solubles totales • polifeniloxidasa • peroxidase • vitamina C

Introducción

Calidad de alimentos minimamente procesados es altamente valorada por los consumidores. Durante la elaboración, las enzimas Peroxidasa (POD) y Polifenoloxidasa (PPO) activan procesos enzimáticos que afectan la coloración natural del producto. Según Raimbault et al. (2010) estos dos enzimas producen quinonas después de la oxidación de compuestos decolorantes fenólicos. En el objetivo de aumentar la vida de exhibición y mejorar la apariencia del producto, la inactivación total o parcial (>70%) de estas dos enzimas es esencial. Según Soysal (2008) y Goyeneche et al. (2013) los productos de frutas y verduras sometidos a tratamientos térmicos a 60 y 95 °C detienen la enzimática de los alimentos, mientras que Castro et al. (2008) reportó que con inactivación, puede también degradar nutrientes como vitamina C. En este sentido, la demanda por productos frescos ha impulsado la investigación para encontrar tratamientos térmicos no-convencionales, tales como el ultrasonido (US), una onda acústica inaudible de frecuencia superior a 20kHz (33). Islam et al. (2014) y Abid et al. (2014) evaluaron los efectos de US en la inactivación enzimática y indicaron que las ondas acústicas alteran la estructura terciaria enzimática, lo que conduce a la pérdida de actividad biológica. En otro trabajo, Sulaiman et al. (2015) estudiaron el efecto del ultrasonido en la inactivación de PPO observando un incremento en la velocidad de inactivación después de 10 minutos de US. Jang and Moon (2011) estudiaron los efectos de US y ácido ascórbico en la actividad de PPO y POD en productos frescos durante el almacenamiento. Liu et al. (2017) estudiaron el efecto de US en la melón de jardín de cantalupo, informando que la alta intensidad US generó cambios conformacionales enzimáticos, causando inactivación.

Soursop es un fruto exótico con altos niveles de compuestos bioactivos, y gran potencial agroindustrial debido a su alta recuperación y excelente características sensoriales (3). Sin embargo, como se ha mencionado, este fruto es susceptible a la enzimática de los alimentos (6). En Colombia, la enzimática es un factor principal, generando pérdidas de alrededor del 30% en el post-cosecha de esta fruta. Por lo tanto, se requiere investigación en este tema. El objetivo de este trabajo es estudiar el efecto de la termosonicación (TS) sobre las propiedades fisicoquímicas y la oxidación enzimática de la pulpa de soursop.

Materiales y métodos

Químicos
Phosphate buffer (pH 7.5), polyvinylpolypyrrolidone (PVPP), triton X-100, potassium iodide [90%], and hydrochloric acid [37%] (V/V) were obtained from Merck. Guaiacol [98%], hydrogen peroxide [30%], catechol [99%] and Arsenic (III) oxide [99.995%] were purchased from Sigma-Aldrich.
Pulp preparation
Five kg of fresh ripen soursop (Annona muricata) were purchased from a local supermarket in the city of Cúcuta, Colombia. These fruits were immediately taken to the Research Laboratory of the Food Innovations Group of the University of Pamplona, Villa del Rosario, Colombia. After peeling, the seeds were manually removed. Then, the pulp was vacuum packed (95%) and stored under refrigeration (4 °C) until further processing or analysis.

Treatment with thermosonication (TS)
Fruit pulp was divided in four samples, control (0 min), ultrasonic at 20 °C (US20), ultrasonic at 40 °C (US40), and ultrasonic at 60 °C (US60). Pulp samples (150 g) were vacuum-packed at 95%, and treated in an ultrasonic bath system (Elmasonic E equipment Model: E60H), with 40 kHz frequency and maximum ultrasonic power of 100 W. Internal dimension: 300 mm x 151 mm x 150 mm. All treatments were carried out in the dark, immediately cooled by immersion in an ice water bath and stored at 4 °C until further analysis.

Physicochemical properties
Total soluble solids (SST) were measured by refractometry (ATC-FG113), and pH was measured using an Orion 210ª electronic pH meter previously calibrated following AOAC (1990). Vitamin C was quantified by titration of ascorbic acid according to Santos and Daghastanli (2001).

Peroxidase (POD) extraction and activity
Enzymatic extraction was carried out after Silva et al. (2015) modified. Twenty g of soursop pulp were mixed with 100 mL distilled water, and centrifuged at 10000 g, 7 °C, for 10 minutes. Then, 1.5 ml of the extract was mixed with 320 µL of guaiacol 5% (V / V) and 160 µL of H₂O₂ [0.147 M] (V / V). Absorbance was recorded using a spectrophotometer (ThermoSpectronic Genesys 20) at 470 nm, every 20 seconds for 4 minutes, at 25 °C. Absorbance was related as a function of time. Enzymatic activity was obtained by equation 1:

\[ AE/e = (M) / (Ke) \]

where:
AE / e = enzymatic activity of the treated or not treated sample,
M = rate of reaction progress (ΔAbs / Δtim), and Ke: extinction coefficient.
Residual POD activity (%), resulted from equation 2:

\[ AER (%) = ((ΔE/Ao) * 100) \]

where:
ΔE = enzymatic activity of the thermosonicated sample,
Ao = enzymatic activity of the control sample (untreated),
AER = residual enzymatic activity.

Oxidation caused by hydrogen peroxide reduces Guayacol to Tetraguayacol, generating brown coloration. According to Liburdi et al. (2019), the molar extinction coefficient of tetraguayacol is 26.6 mM⁻¹ cm⁻¹ for an absorbance of 470 nm. This value is used to calculate enzymatic activity of POD after treatments with TS using eq. 1 and 2.

Polyphenoloxidase (PPO) extraction and activity
Enzymatic extraction was carried out according to Campo and Gélvez (2011) with modifications. Ten g of soursop pulp were mixed with 25 mL of 0.2M phosphate buffer solution (pH 6.5) containing 4% polyvinylpolypyrrolidone (PVPP) and 1% Triton X-100, homogenized for 3 minutes and then centrifuged (10000 rpm / 30 min at 4 °C). Seventy-five µL of the extract were extracted and mixed with 3 mL of 0.007M catechol in 0.05M phosphate buffer (pH 6.5) as enzyme substrate. Absorbance was recorded using a spectrophotometer (ThermoSpectronic Genesys 20) at 420 nm, every 20 seconds, for 4 minutes, at 25 °C. Absorbance was related as a function of time. This value is used to calculate enzymatic activity of PPO after treatments with TS using eq. 1 and 2, calculating residual activity of PPO (%). According to Palmer (1963), Catecol is reduced to Benzoquinone by O₂ with an extinction coefficient of 2.6 M⁻¹ cm⁻¹ at 420 nm.
Statistical analysis

Results are expressed as means ± standard deviations. Treatment effects (control, US20, US40, and US60) on individual dependent variables (PPO and POD activity, Vitamin C, total solids and pH) were evaluated by one-way ANOVA. Significant differences between means were determined by the Tukey pairwise comparison test at a significance level of P < 0.05. Statistical analyses were conducted using the SPSS 18 program for Windows. All treatments were carried out in triplicate.

RESULTS AND DISCUSSION

Analysis of physicochemical properties

Figure 1 shows pH values of the treated and untreated samples. This variable increased significantly (p <0.05) after TS and temperature due to cavitation, after breaking pulp hydroxyl bonds (OH-). These results were also reported by Avalos et al. (2015) and Campo et al. (2011), indicating that with US in exposure time range of 15 and 30 minutes, a slight increase in cherimoya pulp pH occurred. In contrast, Porras et al. (2011), reported a decrease in pH in mango pulp for times greater than 30 minutes. In the present study, pH reduces PPO and POD enzymatic actions, allowing pulp stability. In addition to the inactivation of these enzymes, the OH- group is associated with antimicrobial and antioxidant activities that allow preserving and improving the shelf life of food (2). According to Avalos et al. (2015), this slight increase is related to the release of volatile aromatic substances given cavitation and temperature effects in the TS treatment, favoring organoleptic properties of soursop pulp.

Figure 1. Soursop pulp pH treated and untreated with thermosonication at different temperatures.

Figure 2 (page 256) shows SST (°Brix) of the treated and untreated samples. SST increased significantly (p <0.05) after TS and temperature, as observed for mango pulp, apple juice and cherimoya pulp, (1, 5, 25). During ultrasound treatments, polysaccharide diffusion coefficient and solubility increased, mainly due to cavitation in the plant matrix, fracturing cell walls (32). An alternative explanation states that increasing SST (°Brix) is given by micro evaporation of water during ultrasonic cavitation (17, 25). According to Makino et al. (1983), cavitation causes a dissociation of water molecules into hydrogen ions (H+) and hydroxyl groups (OH-) increasing pulp ° Brix. Figure 3 (page 256) shows vitamin C values of the treated and untreated samples.
Significant differences \((p < 0.05)\) in vitamin C retention among control sample and treatments were found with temperatures around 40 and 60 °C. The lowest vitamin C retention value (71.05%) occurred for TS around 60 °C, followed by TS at 40 °C with 86.84%. On the contrary, no significant differences could be found for TS at 20 °C. Our results agree with those reported by Dabir and Ananthanarayan (2018) in custard apple. These authors found no significant effect between treated and untreated samples with US at room temperatures. However, Abid et al. (2014) indicated that under TS, when temperature raised above 40 °C, a significant loss of vitamin C occurred. The synergistic effect of radical formation (OH and H⁺) and temperature during thermosonication, triggers hydrolysis, decarboxylation, regrouping and cyclization reactions, initiating the formation of compounds like 2,3-diketogulonic, 3-hydroxy acids -2-pirone, and 2-furoic, losing nutritional value (23).
Effects of thermosonication on POD and PPO enzymatic activity in soursop

During fruit processing and storage, PPO and POD enzymes can affect product final quality. PPO is involved in the oxidation of polyphenols to o-quinones, which subsequently polymerize into undesirable brown, red, or black pigments in the presence of oxygen (18). These authors also comment that POD, an antioxidant enzyme, catalyzes the conversion of hydrogen peroxide to water using polyphenols as hydrogen donors. Figure 4 shows POD reaction before and after the treatment. Its reaction rate decreases with TS and temperature. POD residual enzymatic activity under TS around 20 °C, is 52.59% compared to the control sample, whereas when temperature is increased, only 30.17 and 25.86% are observed at 40 and 60 °C, respectively (figure 4A). Other studies also report a reduction in POD activity after ultrasound. In bayberry juice, residual activity levels resulted in between 9.49-73.14%, while in pear juice, inactivation levels reached between 4.30-43.2% (9, 27).

![Figure 4](image_url)

**Figure 4.** Progression curve of (Abs vs Time) soursop peroxidase reaction before and after TS treatments.

**Figure 4A.** Residual enzymatic activity of peroxidase in soursop after thermosonication at different temperatures.
On the other hand, figure 5 shows PPO behaviour before and after the treatment. PPO reaction rate also decreased with TS and temperature. Its residual enzymatic activity with TS around 20 °C, was 43.62% compared to the control sample, whereas when temperature is increased, only 25.53 and 18.09% were observed for 40 and 60 °C, respectively (figure 5A). Accordingly, in bayberry juice, registered residual activity levels reached between 0.08 and 53.23%, while in pear juice reported inactivation levels resulted between 1.91-89.33% (9, 27).

According to Ercan and Soysal (2011) ultrasonic generated heat, and exposure time leading to increased sample temperatures, allow the reduction of POD and PPO activities. When US is combined with temperature, a synergic effect generates enzymatic dissociation of the prosthetic group, significantly decreasing (p<0.05) the content of the structure of the helix/alpha and causing conformational changes in the secondary and tertiary structures of POD and PPO after cavitation effect. This finally leads to partial or total protein denaturation (14, 20, 22).
Anaya-Esparza et al. (2017) reported that US generates micro-streaming phenomena that can interrupt polypeptide Van der Waals interactions and hydrogen bonds. In addition, free radicals generated during US can react with disulfide bonds, destabilizing the enzymatic conformation, or oxidizing amino acids related to enzymatic catalytic activity and stability, such as tryptophan, tyrosine, histidine, and cysteine. According to Dabir and Ananthanarayan (2018), complete inactivation of POD and PPO is achieved at 5 minutes under an ultrasonic power of 85 W and 90 W respectively, followed by 21.6% loss of vitamin C. In this study, TS at 40 °C resulted the most efficient treatment inactivating POD in 69.83% and PPO, in 74.47%, while retaining 86.84% vitamin C. On the other hand, TS and its lower temperatures (<60 °C), favoured vitamin C retention, in comparison with conventional thermal treatments using temperatures higher than 70 °C. According to Burdurlu et al. (2006), greater temperature decreases vitamin C.

CONCLUSIONS

TS treatment in soursop pulp (*Annona muricata*) affects its physicochemical properties, increases pH and soluble solids, while decreases vitamin C retention. The evaluated TS treatments inactivated POD and PPO enzymes in a range of 48.41-74.24% and 56.38-81.91%, respectively. Maximum POD (69.83%) and PPO (74.47%) inactivation were reached with 40 kHz and 40 °C TS, keeping vitamin C at 86.84%. Further research on POD and PPO inactivation in soursop is needed. Studies demonstrating that TS could be more efficient than blanching and bleaching, should be developed. Given that lower temperatures do not need additional chemicals, this constitutes a product and environmentally friendly proposal.

REFERENCES

Effect of thermosonication on soursop pulp


ACKNOWLEDGEMENTS
This work was supported by Universidad de Pamplona, Norte de Santander, Colombia.