Effects of thermal processing combined with sucrose on the vitamin C content, total phenolic content, antioxidant activity, and sensory characteristics of arazá (Eugenia stipitata McVaugh) purée during frozen storage

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ABSTRACT

The effects of a 80°C, 1 minute thermal treatment (H treatment) and of the same treatment combined with sucrose (SH treatment) on the chemical and sensory qualities of arazá purée were evaluated during 4 months of storage at -20°C. For the control, an untreated sample (C) was included. The chemical qualities evaluated were vitamin C content, total phenolic compounds (TPC), and antioxidant activity (AoA), which were measured with the ferric reducing ability of plasma (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 1,1-diphenyl-2-picrylhydrazyl (DPPH), while a trained panel was used for the sensory quality evaluation. After 4 months of frozen storage, SH was more effective than H at controlling the loss of chemical quality. SH provided a similar FRAP-AoA (89%) and vitamin C content (87%), as well as a higher TPC (145%), ABTS-AoA (272%), and DPPH-AoA (115%), when compared to C before frozen storage. The total sensory qualities in both the SH purée (15±1) and the H purée (16±1) after 4 months at -20°C were comparable to those of the C purée before the frozen storage (18±2). Therefore, using sucrose combined with a thermal treatment and subsequent frozen storage preserves the chemical and sensory qualities of arazá purée.

Key words: thermal treatment, tropical fruit, combined methods, Amazonian fruit.

Introduction

Arazá (Eugenia stipitata McVaugh) is an exotic, tropical fruit that is grown in Latin American countries, such as Colombia, Peru, and Brazil. The fruit is a fleshy berry (4-7 cm in diameter) with 6 to 16 seeds (1-4 cm in diameter) per fruit. It has an attractive flavor, a vitamin C content [8.9-26.6 mg/100 g fresh weight (FW)] (García-Reyes and Narváez-Cuenca, 2010; Contreras-Calderón et al., 2011) that is comparable to those of pineapple (Ananas comosus) and banana (Musa acuminata AAA) (Hernández et al., 2006), and a total phenolic content (TPC) (19.3-111.0 mg gallic acid equivalents/100 g FW) similar to those found in tropical fruits such as mango (Mangifera indica) and granadilla (Passifloraligularis) (García-Reyes and Narváez-Cuenca, 2010; Contreras-Calderón et al., 2011; Garzón et

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Attempts to delay the browning of the skin and excessive softening of fresh arazá fruits have been described in the literature (Narváez-Cuenca, 2003; Hernández et al., 2009; Carrillo et al., 2011). When stored at 20°C, green-mature fruits reach sensorial ripeness after one week with a subsequent decay of the sensory quality several days later (Hernández et al., 2009; Carrillo et al., 2011). The refrigeration of green-mature fruits at 12°C, with or without exposure to 1-methylcyclopropene, reduces the loss of organic acids and extends the sensory quality of arazá fruits for up to two weeks (Carrillo et al., 2011). Heat shock prior to refrigeration when using green-mature fruits was also useful in extending arazá fruit sensory quality for up to two weeks (Narváez-Cuenca, 2003).

The arazá fruit is predominantly used to prepare nectars, jams, and candies, and it is rarely consumed whole. Based on frozen storage experiments that were conducted over a period of 2 months (Millán et al., 2007; García-Reyes and Narváez-Cuenca, 2010), the preservation of arazá fruit as a frozen purée was promising. Millán et al. (2007) concluded that the combination of thermal treatments of arazá purée at 80°C for 1 min, quick freezing (by immersion in liquid nitrogen), and storage at -20°C preserved the sensorial and chemical [vitamin C, TPC, and antioxidant activity (AoA)] qualities of the arazá purée. These authors also indicated that the use of slow thawing yields purées with better textures than those prepared with fast thawing. Silva et al. (2011) tested the effect of the addition of sucrose (in concentrations ranging from 0 to 30%, w/w) to arazá purées on the sensorial quality of the stored frozen product and concluded that the presence of 20% (w/w) sucrose controlled the degradation of the sensory characteristics for the one-month storage period that was assessed. In the mentioned studies, the use of sucrose and slow freezing was comparable to the use of thermal processing in combination with quick freezing in terms of the sensorial texture stability of the arazá purée. Until now, sucrose has not been used in combination with thermal processing and frozen storage trials have not lasted longer than two months in the evaluation of arazá purée. Because there are up to three harvests of arazá fruit per year, the present research was conducted over a period of 4 months using frozen storage and evaluating the effect of thermal processing (at 80°C for 1 min) combined with sucrose (20% w/w) and the same thermal processing without sucrose on the chemical and sensorial qualities of arazá purée.

Materials and methods

Materials

The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2’-azino-bis-(3-3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), dithiothreitol, 2,2-diphenyl-1-pi-crylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), and Folin Ciocalteu (FC) reagent were supplied by Sigma (St. Louis, MO). Sucrose was obtained from Ingenio Manuelita (Bogota, Colombia) and all of the other chemicals came from Panreac Química (Barcelona, Spain). Ripe arazá fruits (fully-yellow skin color) (Carrillo et al., 2011) were obtained from supermarkets in Bogota, Colombia. Vendors reported the fruit came from Cundinamarca, Colombia, but the exact location was unknown.

Preparation of samples and storage

The ripe arazá fruits were randomly split into nine lots (10-16 fruits per lot). Each lot was washed with tap water and peeled by hand. The seeds were removed and the pulp was puréed for 10 s in a domestic blender. Nine samples of purée (2 kg each) were obtained and immediately processed. Three samples of the purée were treated thermally in a kettle equipped with a stirrer. These samples were held at 80°C for 1 min once the temperature at the central part of the 2 kg-purée reached said temperature and immediately cooled to 15°C by water circulation (H treatment) (García-Reyes and Narváez-Cuenca, 2010). Three other samples were mixed with sucrose to obtain a final concentration of 20% (w/w) sucrose and then heated as described for the H treatment (SH treatment). Finally, three samples were used as the control (C), where neither sugar nor heating were included. All samples (H and SH treatments, and C) were packed in polyethylene bags (100 g of purée per bag), slowly frozen (0.2°C/min), and stored in darkness at -20°C for four months. After storage, the chemical and sensory quality parameters were evaluated. The following chemical analyses were performed on the H and SH treatments and the C samples: vitamin C content, TPC, and AoA (measured by ABTS, FRAP, and DPPH). The sensorial quality of the nectars prepared from the purée samples was measured by a trained panel. Measurements were performed before freezing and after 1, 2, 3, and 4 months of storage at -20°C. Except for day 0 (before freezing), the samples were thawed at room temperature and then analyzed.

Analysis of the chemical quality

Vitamin C. The vitamin C extraction and quantification were based on previous studies (Gökmen et al., 2000). To extract the vitamin C, 1 g of sample was added to 4 mL of deionized water. The mixture was vortexed for 2 min and the mixture was cooled to 15°C by water circulation (H treatment) (García-Reyes and Narváez-Cuenca, 2010). Three other samples were mixed with sucrose to obtain a final concentration of 20% (w/w) sucrose and then heated as described for the H treatment (SH treatment). Finally, three samples were used as the control (C), where neither sugar nor heating were included. All samples (H and SH treatments, and C) were packed in polyethylene bags (100 g of purée per bag), slowly frozen (0.2°C/min), and stored in darkness at -20°C for four months. After storage, the chemical and sensory quality parameters were evaluated. The following chemical analyses were performed on the H and SH treatments and the C samples: vitamin C content, TPC, and AoA (measured by ABTS, FRAP, and DPPH). The sensorial quality of the nectars prepared from the purée samples was measured by a trained panel. Measurements were performed before freezing and after 1, 2, 3, and 4 months of storage at -20°C. Except for day 0 (before freezing), the samples were thawed at room temperature and then analyzed.
filtered first through a black band paper filter and then through a 0.45 µm Millipore (Milford, MA) disposable filter. The filtrate was used for the vitamin C determination, which included the measurement of both ascorbic acid and dehydroascorbic acid in a single run. The dehydroascorbic acid present in the filtrate was reduced to ascorbic acid by treatment with 1 mg/mL dithiothreitol over a period of 2 h, in darkness and at room temperature. The vitamin C content was determined by reverse-phase high-performance liquid chromatography (RP-HPLC). The chromatographic system consisted of a HiChrom C18 stainless steel column (250 x 4 mm I.D.; 5 µm particle size; Reading, UK) operated at 18°C. The eluate was monitored at 254 nm using an UV-visible detector operating at 40°C. The eluent was 200 mM KH₂PO₄, adjusted to pH 2.4 with H₃PO₄. The flow rate was 0.5 mL min⁻¹. The sample volume for injection was 20 µL. A calibration curve was constructed for ascorbic acid solutions ranging from 10 to 50 mg mL⁻¹ (five data points, \( r^2 = 0.999 \)). The results were expressed as mg of vitamin C per 100 g of FW purée.

**Total phenolic content and antioxidant activity.** For the TPC and AoA, an extraction was performed following previous studies (García-Reyes and Narváez-Cuenca, 2010). A 2.5 g of purée were mixed with 5 mL of 80% (v/v) aqueous methanol and shaken for 1 h at 200 rpm. The resulting mixture was centrifuged (4,000 xg, 5 min at 4°C) and the supernatant was kept at 4°C. A second extraction was performed. The two supernatants were mixed, and the volume was brought to 10 mL in a volumetric flask, topped off with distilled water, and used for the TPC and AoA analyses. The undiluted extracts were analyzed for the TPC and the extracts were diluted from 1:1 to 1:5 (extract: 80% aqueous methanol, v/v) for the AoA measurements.

The TPC was determined using the FC method described by Velioglu et al. (1998). A 100 µL aliquot of the aqueous methanol extract (80% v/v) was mixed with 750 µL of FC reagent (10% v/v). After 5 min, 750 µL of a NaHCO₃ (6% w/w) solution were added. After 90 min, the absorbance of the reaction mixture was measured at 725 nm. The results were expressed as gallic acid equivalents (mg GAE/100 g FW purée). The calibration curve for gallic acid was constructed in concentrations ranging from 0.01 to 0.30 mg mL⁻¹ (five data points, \( r^2 = 0.998 \)).

The AoA of the extracts was measured with three methods: ABTS, FRAP, and DPPH. The ABTS assay was based on Re et al. (1999). A mixture of 7.0 mM ABTS and 2.45 mM potassium persulfate (final concentration) was prepared and stored in darkness for 16 h. The resulting ABTS⁺ solution was diluted with ethanol to reach an absorbance of 0.70 at 734 nm. Aliquots (10 µL) of the diluted extracts were incubated with 1,000 µL of the ABTS⁺ solution for 60 min at 30°C and the absorbance was monitored at 734 nm every 5 s.

The FRAP assay was carried out according to the method described by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 25.0 mL of sodium acetate buffer (300 mM, pH 3.6) with 2.5 mL of TPTZ (10 mM) in HCl (40 mM) and 2.5 mL of ferric chloride (20 mM). The FRAP reagent (900 µL) was mixed with 90 µL of distilled water and 30 µL of diluted extract. The mixture was incubated at 37°C and the absorbance was monitored at 593 nm every 5 s for 45 min.

The DPPH assay was performed following Sánchez-Moreno et al. (1998). A methanolic stock solution of 50 mM DPPH was diluted with methanol to obtain a working DPPH solution with an absorbance of 1.10 at 515 nm. The diluted extract (25 µL) was mixed with 975 µL of a working methanolic DPPH solution. The mixture was incubated at 20°C and its absorbance was measured at 515 nm every 5 s for 1 h. The scavenging effect (as a percentage) was calculated with \( \left[ \frac{(A_1 - A_2)}{A_0} \right] \times 100 \), where \( A_0 \) was the absorbance of the control (without extract), \( A_1 \) was the absorbance in the presence of the extract once the plateau was reached, and \( A_2 \) was the absorbance without DPPH. In the three different assays, ABTS, FRAP, and DPPH standard curves were created with Trolox in concentrations ranging from 100 to 1,500 µM (five data points, \( r^2 = 0.999, 0.989, 0.975, \) respectively). In all of the cases, the absorbance reached a plateau during the given incubation time. The results were expressed as µmol of Trolox per g of FW purée.

**Analysis of the sensory quality**

The nectars were prepared by blending the purée, water, and sucrose to obtain a mixture with 25% (w/w) arazá purée and 12°Brix. Quantitative descriptive analysis (QDA) of the prepared nectars was carried out by a trained panel of seven members. QDA was performed for color and general aspects, as well as aroma, taste, and texture of the arazá purée. The panelists were both male and female, non-smokers, and 40 to 55 years old; they were trained twice per week over a period of two months on the evaluation of the quality parameters (Tab. 1) by preparing nectars with fresh purée, purée boiled for 1-5 min, and purée submitted to 1-3 cycles of frozen storage for 1 d with subsequent thawing. The evaluation was performed in a room designed specifically for sensory evaluation with individual booths and controlled, white lighting. In each session, each panelist...
tested nine randomly codified samples. Each panelist was asked to rate the product using intermediate scores (Tab. 1). Each parameter had a different weight according to its importance, as defined in a previous study (Millán et al., 2007). The total quality was calculated as the sum of the different parameters.

### TABLE 1. Scores for the sensory evaluation of the nectar prepared from the arazá purée.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Brilliant yellow color and very homogeneous material</td>
</tr>
<tr>
<td>1</td>
<td>Slight yellow, some suspended material</td>
</tr>
<tr>
<td>0</td>
<td>Atypical color, bleached yellow, sediment</td>
</tr>
</tbody>
</table>

### Statistical analysis

The statistical analyses were performed using Statgraphics Plus® 5.1 for Windows (Manugistics, Rockville, MD). The mean values and their standard deviations were reported. A two-way analysis of variance was performed with factor A as the two treatments and the control, H, SH, and C, and factor B as the five periods of frozen storage, 0 (before freezing), 1, 2, 3, and 4 months. The comparisons among the means were performed with Tukey’s test at a significance level of $P \leq 0.05$.

### Results and discussion

#### Chemical quality of fresh arazá purée

At day 0, the chemical quality of the fresh, untreated arazá purée was assessed by quantifying the vitamin C content, TPC, and AoA (Fig. 1A-E). The vitamin C content (20.3 ±0.8 mg/100 g FW) (Fig. 1A) was within the range previously reported for fresh arazá purée (8.9-26.6 mg/100 g FW) (García-Reyes and Narváez-Cuenca, 2010; Contreras-Calderón et al., 2011).

The TPC (Fig. 1B), expressed as mg GAE/100 g FW, of the fresh, untreated purée (26.5±3.6) was lower than the previously reported values (35.0±8.6 and 111.0±3.6) when the extraction was performed with 80% (v/v) aqueous methanol (García-Reyes and Narváez-Cuenca, 2010). The AoA of the fresh arazá purée was higher when measured by the FRAP method (8.8±1.1 µmol Trolox/g FW of purée) (Fig. 1D) than when measured by the ABTS method (3.2±0.5 µmol Trolox/g FW of purée) (Fig. 1C) or by the DPPH method (3.4±0.3 µmol Trolox/g FW of purée) (Fig. 1E). This trend indicates that the antioxidants present in fresh arazá purée have a better reducing capacity (as measured by FRAP) than the free radical scavenging capacity (as measured by ABTS and DPPH). This trend might be related to the polyphenolic composition of the extract. Caffeic acid, reported in the skin and pulp of arazá (Cuellar et al., 2013) at a concentration of 1 mg L$^{-1}$, yielded a FRAP-AoA value that was 1.4-times higher than the value obtained by either DPPH-AoA or ABTS-AoA (Maqsood and Benjakul, 2010). While the FRAP-AoA found in the fresh arazá purée was within the range reported for 24 fruits from Colombia (ranging from 3.2 to 175 µmol Trolox/g FW), the ABTS-AoA was below the values found in the same 24 Colombian fruits (ranging from 6.3 to 144 µmol Trolox/g FW) (Contreras-Calderón et al., 2011). The fresh arazá purée had a DPPH-AoA value within the range of values reported in 19 tropical fruits from Ecuador (ranging from 0.3 to 76 µmol Trolox/g FW) (Vasco et al., 2008).

The higher values for the vitamin C content, TPC, and ABTS-AoA previously reported for fresh arazá purée (García-Reyes and Narváez-Cuenca, 2010), when a similar extraction method was used, might be the result of differences in the year, harvest location, and storage conditions, as shown in other fruits (Al-Turki et al., 2010; Guzmán-Maldonado et al., 2010). The lower TPC, FRAP-AoA, ABTS-AoA, and DPPH-AoA values found in the arazá purée when the extraction was performed with 100% (v/v) acetone (Garzón et al., 2012) as compared to the values found by us when the extract was prepared in 80% (v/v) aqueous methanol suggest a more important contribution from the hydrophilic antioxidants, such as hydroxybenzoic and phenolic acids, rather than from the hydrophobic antioxidants, such as carotenoids, which are present in arazá fruits (Garzón et al., 2012; Cuellar et al., 2013).

#### Chemical quality of treated arazá purée before frozen storage

The effect of thermal treatments or sucrose with thermal treatments before storage at -20°C on the chemical quality...
of arazá purée (Fig. 1A-E). Compared to the C sample, there was no significant effect of the H treatment on the vitamin C content, TPC, ABTS-AoA, or FRAP-AoA. The SH treatment did not significantly change the TPC or FRAP-AoA. In contrast, at time 0, while the H treatment yielded a statistically higher DPPH-AoA (4.0±0.2 µmol Trolox/g FW of purée) as compared to the control (3.2±0.5 µmol Trolox/g FW of purée as measured by DPPH), the SH treatment resulted in a statistically higher vitamin C content (23.0±0.4 mg/100 g FW), ABTS-AoA (5.1±0.7 µmol Trolox/g FW of purée), and DPPH-AoA (4.0±0.2 µmol Trolox/g FW of purée) as compared to the control (20.3±0.8 mg vitamin C/100 g FW, 3.2±0.5 µmol Trolox/g FW of purée as measured by ABTS, and 3.4±0.3 µmol Trolox/g FW of purée as measured by DPPH).

The increase observed for the chemical quality of the fresh arazá purée after application of the H or SH treatments
suggests a positive role for both the thermal treatments and the sucrose additives. The stability of the vitamin C content observed in the thermal treatment of the arazá purée is in agreement with the lack of significant vitamin C loss found after thermal treatments on guava (*Psidium guajava* L. cv. Chung shan) purée (Yen and Lin, 1996) or watercress (*Nasturtium officinale* R. Br.) leaves (Gonçalves et al., 2009), but contrasts the degradation of this compound after infrared thermal treatments on fresh-cut mango (Sogi et al., 2012). During the thermal treatment, phenolics and other compounds contributing to the AoA that were part of the insoluble bound fraction must have been released from the purée matrix, as previously shown by Sogi et al. (2012) in infrared heated fresh-cut mango. These authors found an increase in the TPC and AoA, as measured by DPPH, FRAP, and ORAC assays, as a result of the infrared treatment. The stabilizing effect of sucrose on the chemical qualities of arazá purée corroborates a previous report from Kopjar et al. (2008). These authors reported that the addition of trehalose to strawberry cream improves the stability of the anthocyanins in the product prepared by evaporation at 80°C.

**Chemical quality of treated arazá purée during frozen storage**

During frozen storage of the C sample, there was a decrease ($P \leq 0.05$) in the vitamin C content, TPC, FRAP-AoA, and DPPH-AoA, and an increase ($P \leq 0.05$) in the ABTS-AoA (Fig. 1A-E). Interestingly, the $H$ and $SH$ treatments yielded greater values ($P \leq 0.05$) than the $C$ sample for each chemical quality parameter evaluated during frozen storage (Fig. 1A-E).

The vitamin C content decreased in the $C$ sample during the frozen storage, with a calculated half-life time of 93 d after applying a first order kinetic model. The degradation of the vitamin C was retarded with the $H$ and $SH$ treatments. The half-life of the vitamin C was extended to 116 d with the $H$ treatment and 278 d with the $SH$ treatment. After 4 months of frozen storage, the vitamin C content in the $C$, $H$ treated, and $SH$ treated purée samples represented 40, 64, and 87%, respectively, as compared to the vitamin C content of the fresh, untreated purée sample. The ratio values (expressed as a percentage) between the TPC after four months of frozen storage of the $C$, $H$, and $SH$ treatments and the TPC of the fresh, untreated purée sample were calculated. The ratio values for ABTS-AoA, FRAP-AoA, and DPPH-AoA were calculated as well. The $SH$ treated purée samples had the highest ratio values (145 for TPC, 272 for ABTS-AoA, 89 for FRAP-AoA, and 115% for DPPH-AoA), followed by the $H$ treated purée samples (114, 178, 91, and 88%, respectively) and then the $C$ purée samples (66, 116, 67, and 76%).

In the present research, we found that both the $H$ and $SH$ treatments controlled the degradation of vitamin C, TPC, and AoA, as compared to the control. Furthermore, the $SH$ treatment exhibited better control of chemical quality losses during frozen storage, as compared to the $H$ treatment. The observed protective effect of sucrose on phenolic compounds and vitamin C upholds previous results for frozen fruits (Oszmiański et al., 2009; Rincon and Kerr, 2010). The use of sugars, e.g., glucose, fructose and sucrose, to coat whole fruits or to mix with fruit purées prior to freeze-drying, freezing, or evaporation at 80°C has positive effects on the chemical quality (Kopjar et al., 2008; Galmarini et al., 2009; Kopjar et al., 2009; Loncaric et al., 2014). These sugars effectively increase both aroma and color retention and reduce the loss of TPC, anthocyanins, and AoA during storage at either room temperature or freezing temperatures. The positive effect of sugars on the chemical quality stability has also been related to an increase in the transition glass temperature, which is associated with low shrinkage, collapse, and crystallization of freeze-dried products (Roos, 1995). The increase in the FRAP-AoA of the solutions containing sucrose has been attributed to the production of non-enzymatic browning reaction products during heating at a low pH (Tsai et al., 2005). The potential susceptibility of phenolic compounds to a thermal treatment was overcome by the higher stability of these compounds during frozen storage of the arazá purée. The stabilizing effect of thermal treatments on individual phenolic compounds was previously shown in grapefruit juice during frozen storage (Igual et al., 2011).

**Sensory quality of treated arazá purée during frozen storage**

The trained panelists indicated that the $H$ and $SH$ treatments generated a boiled taste (day 0); nevertheless, the effect of the treatments was not significant when the total quality of the nectars was evaluated (Fig. 1F). The total sensory quality of the $C$ sample was negatively affected during frozen storage, moving from an initial value (before frozen storage) of 18±2 to 8±3 after 4 months. No statistically significant differences were obtained between the $H$ and the $SH$ treated samples at any storage time when evaluating the total sensory quality. The total sensory quality of the $H$ and $SH$ treated samples was greater than the quality of the $C$ sample during frozen storage. After 4 months, the $H$ (16±1) and $SH$ (15±1) treatments produced total quality levels that were comparable to that of the fresh, untreated purée (18±2). The use of sugars has been reported to have
a positive effect on the retention of sensory qualities, such as aroma, color, and texture in whole fruits, fruit purées or fruit juice during storage at room temperature or at -18°C (Kopjar et al., 2008; Kopjar et al., 2009; Kopjar et al., 2012). Furthermore, thermal processing has been reported to enhance sensory quality by eliminating throat irritation, as compared to fresh purées (Ledeker et al., 2014).

Conclusions

After frozen storage of the HS treated purée (20% (w/w) sucrose plus thermal treatment) for four months, the vitamin C content, TPC, AoA, and sensorial quality were comparable to or higher than those of the control purée before frozen storage. The use of 20% (w/w) sucrose with a thermal treatment in combination with frozen storage is, therefore, useful in extending both the chemical and sensory qualities of arazá purée. Further studies to describe the changes in the phenolic composition during frozen storage might be of interest when the biological activity of such compounds is taken into account.

Literature cited


