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Influence of preservation by heat and cold on the physicochemical and microbiological characteristics, bioactive compounds of pulp from sapota-do-Solimões (*Quararibea cordata*)

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**ABSTRACT**

The aim was to evaluate the influence of preservation by heat and cold on the physicochemical and microbiological characteristics and bioactive compounds of pulp from sapota-do-Solimões (*Quararibea cordata*) for 180 days of storage. The pulps were submitted to the following treatments: freezing; pasteurization + freezing; refrigeration; and pasteurization + refrigeration. The treatments affected the physicochemical parameters during storage. Of particular note was the reduction in water activity, the reduction in pH in the pulps stored under refrigeration, and the lightening in color of the pulps. Ascorbic acid remained stable during freezing, and the levels of total carotenoids were maintained in the pasteurization + freezing treatment. The total phenolics remained stable up to 150 days, and the antioxidant activity decreased during storage for all the treatments. The coliforms were less than 1 log CFU.g^-1^ and *Salmonella* spp. was absent. The pasteurization + freezing treatment, as well as the freezing treatment, maintained the quality of the pulp for 180 days of storage.

**Influencia de la preservación térmica sobre las características físico-químicas, microbiológicas y compuestos bioactivos de la pulpa de sapota-do-Solimões (*Quararibea cordata*)**

**RESUMEN**

El objetivo fue evaluar la influencia de la preservación por calor y frío sobre las características físico-químicas y microbiológicas y los compuestos bioactivos de la pulpa de sapota-do-Solimões (*Quararibea cordata*) durante 180 días de almacenamiento. Los frutos fueron despulpados y sometidos a los tratamientos: congelación; pasteurización + congelación; refrigeración; y pasteurización + refrigeración. Los tratamientos afectaron los parámetros físicoquímicos durante el almacenamiento. Destacan la reducción de la actividad de agua, la reducción del pH en las pulpas almacenadas bajo refrigeración y el aclaramiento en color de las pulpas. El ácido ascórbico se mantuvo estable durante la congelación y los niveles de carotenoides totales se mantuvieron en la pasteurización + congelación. Los fenoles totales permanecieron estables hasta 150 días y la actividad antioxidante disminuyó durante el almacenamiento para todos los tratamientos. Los coliformes fueron menores que 1 log CFU.g^-1^ y *Salmonella* spp. estuvo ausente. El tratamiento de pasteurización + congelación, así como el tratamiento de congelación, mantuvieron la calidad de la pulpa durante 180 días de almacenamiento.

**Introduction**

The northern region of Brazil is rich in fruit species which, although they are still not commercially exploited, are of great agro-industrial potential and may represent an important source of employment and income for the local population. The fruits of the sapota tree are globular and have sub-globular berries with a persistent calyx at the upper extremity. The epicarp is fleshy, with a thick consistency and a powdery surface that is brown in color. The flesh is juicy, orange in color, slightly fibrous, with low acidity, a pleasant taste, and considerable amounts of proteins, carotenoids, and minerals (Rabelo, 2012).

In recent decades, the demand for functional foods has led to increased interest in the properties of Amazonian fruits. Thus, the studies of these fruits, as well as the characterization of their bioactive compounds, are challenges that need to be overcome in order to ensure their effective use in agribusiness and the possibility of generating high-quality raw material. The major phenolic in the pulp extract was epicatechin (Berto, Ribeiro, de Souza, Fernandes, & Chisté, 2015).

Products derived from sapota-do-Solimões (*Quararibea cordata*) represent a great opportunity to reach niche markets for exotic products that are nutritious and rich in sources of substances that can maintain good health, as well properties that can prevent disease. Recent studies have demonstrated the antioxidant potential and bioactive compounds present in sapota-do-Solimões (Berto et al., 2015; Céron, Ng, El-Halwagi, & Cardona, 2014; Murillo et al., 2013; Murillo, Meléndez-Martinez, & Portugal, 2010).
The Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) (Brasil, 2000) defines fruit pulp as an unfermented, non-concentrated, undiluted product that is obtained by crushing fleshy fruits using an appropriate technical process with a minimum content of total solids from the edible part of the fruit, which is specific for each fruit pulp. Brazilian legislation sets out minimum microbiological standards regarding identity and physicochemical characteristics for some types of pulp; however, there are no defined parameters for sapota-do-Solimões pulp.

Several methods can be used to preserve fruit pulp, including those which apply or remove heat (pasteurization and freezing). These methods inhibit microbial growth and enzymatic activity and thereby increase shelf life and microbiological safety (Santos, Salles, Chagas Filho, & Rabelo, 2004). However, they can modify the content of some nutrients such as vitamins and carotenoids.

The preparation of sapota-do-Solimões pulp can improve quality, as well as facilitating distribution and adding value. Consumers are increasingly demanding and have opted for products with a longer shelf life that maintain their sensory and nutritional characteristics during storage (Pinheiro, Cardoso, Chaves, Oliveira, & Rios, 2011). Thus, the aim of this study was to evaluate the influence of preservation using heat and cold on the physicochemical and microbiological characteristics, as well as the bioactive compounds of pulp from sapota-do-Solimões storage for 180 days.

**Materials and methods**

**Raw materials**

The fruits were collected from a private area in the community of Vila Vale, which is located near the experimental area of the Mamirauá Sustainable Development Institute. The geographical coordinates of the city of Tefé, Amazonas, Brazil, where the samples were collected, are latitude 03°21′15″S and longitude 64°42′41″W, with an altitude of approximately 75 m. According to the Köppen–Geiger classification, the climate is type Af, i.e. humid and equatorial tropical with an annual average temperature of 26.85°C and an average rainfall of 211.54 mm. The soil is classified as Plinthosol with reddish colors. The fruits were collected in an area that had 15 plants (individuals) of about 30 m in height which were approximately 50 years old. The fruits were evaluated in the mature stage in March 2015. A total of 105 fruits were collected and were initially transported via river and then air, totaling 36 h of travel, to the Federal University of Santa Maria and the Department of Technology and Food Science, where the experiments were conducted. Upon receipt, the fruits were selected for the absence of defects, pests, and diseases and then had their surfaces washed with mild detergent to remove dirt. They were then rinsed in running water.

**Obtaining the pulp and processing**

The following stages were performed to obtain the pulp and to process it (Figure 1).

The fruits were received, prewashed, washed, and then sanitized by immersion in chlorinated drinking water with a chlorine concentration of 50 mg.kg⁻¹ and immersion time of 30 min. The fruits were subsequently rinsed, selected, cut, and pulped. The seeds were removed, and the fruit was processed in a blender used to homogenize pulp. After processing, the pulp was packaged in 180 g polypropylene jars and separated according to the following treatments: (T1) freezing; (T2) pasteurization + freezing; (T3) refrigeration; and (T4) pasteurization + refrigeration. For the treatments involving pasteurization (T2 and T4), the method of Monteiro, Amaro, and Bonilha (2005) was used, with modifications. The treatments were subjected to heat treatment by immersing the jars containing pulp in a water bath at a

![Figure 1. Flowchart of the sequence of operational steps for processing and analyzing of pulp from sapota-do-Solimões (Quararibea cordata).](image-url)
temperature of 69–72°C. The internal temperature of the jars was measured with a thermometer at five different points in the water bath, and when the desired temperature was reached, it was maintained for 30 s. The pulps were then immediately stored in a freezer (−18°C) (T1 and T2) or refrigerated (+4°C) (T3 and T4). The samples were analyzed the day after the pulp was obtained and sequentially every 30 days over a period of 180 days of storage.

**Color analysis**

Color was measured using a Konica Minolta spectrophotometer CM-700d (Osaka, Japan) with color instrumentation technology that used the L*, a*, and b* parameters. The pulp samples were distributed in a sufficient quantity within the glass cell, and the readings were taken from the light-beam of the lens of the spectrophotometer and measured by reflectance. The numerical values of a* and b* were converted into the hue angle (h) and chroma color saturation (c). The results were expressed as L*, which represents the percentage of light ranging from black (0%) to white (100%); a*, where −a* represents direction to green, and +a* represents direction to red; b*, where −b* represents direction to blue, and +b* represents direction to yellow; c (saturation index) and h (hue angle).

**Water activity, pH, titratable acidity, and soluble solids**

AquaLab® (Series 4 TEV; Decagon Devices Inc., Pullman, USA) equipment was used to measure the water activity. The pH was measured using a Digimed® (Model DM-23, São Paulo, Brazil) digital potentiometer in accordance with the recommendations of the Association of Official Analytical Chemists (AOAC, 1998). The titratable acidity was determined by titration with 0.1 M NaOH to a pink color using 1% phenolphthalein as indicator (AOAC, 1998). The soluble solids were measured directly in an Atago® digital refractometer (Pocket PAL-3 model, Tokyo, Japan) using a scale of 0–93° Brix, with temperature compensation to 20°C, in accordance with the AOAC (1998).

**Total, reducing, and nonreducing sugars**

In order to determine the levels of total and reducing sugars in glucose, the Lane-Eynon method was employed using Fehling’s reagent, as described by the Instituto Adolfo Lutz (IAL, 1985). The nonreducing sugars in sucrose were calculated from the subtraction of the values found for the total and reducing sugars and then multiplied by 0.95. The results were expressed as a percentage (%).

**Ascorbic acid and total carotenoids**

The ascorbic acid content was determined by the spectrophotometric method at a wavelength equal to 450 nm (Biospectro, model SP-220, São Paulo, Brazil), as described by Cox and Pearson (1976). The results were expressed as mg.100 g⁻¹ of pulp. The total carotenoids were determined by the Higby method (1962). The readings were taken using a spectrophotometer (Biospectro, SP-220 model) at a wavelength equal to 450 nm, and the results were expressed in µg.g⁻¹ of pulp.

**Obtaining the extracts**

The extracts for the determination of total phenolics and antioxidant activity were obtained according to the methodology described by Larrauri, Rupérez, and Saura-Calixto (1997), with modifications. The extraction was performed at room temperature (24°C) by taking 5.0 g of each pulp; then 20 mL of 50% ethanol solution was added (first extraction solution); and the mixture that was obtained was homogenized and allowed to stand for 1 h for extraction. After this period, the mixture was centrifuged at 3000 rpm for 10 min and the supernatant that was obtained was filtered and placed in a 50 mL flask protected from light. The precipitate obtained by centrifugation was dissolved in 20 mL of 70% acetone (second extraction solution). This mixture was left to stand for 1 hour and then centrifuged at 3000 rpm for 10 min. The second supernatant that was obtained was mixed with the first, in the same flask, which was completed with distilled water.

**Total phenolic compounds**

The total phenolics were determined following the method described by Larrauri et al. (1997). The absorbance was read with a spectrophotometer (Biospectro, model SP-220) at 700 nm, using as reference the standard curve of gallic acid y = 0.0101x−0.0266, R² = 0.9963, which was constructed with concentrations ranging from 5 to 70 mg.L⁻¹. The results were expressed as mg gallic acid equivalent (GAE) per 100 g of extract.

**Antioxidant activity by DPPH method**

The methodology used was as described by Brand-Williams, Cuvelier and Berset (1995). The following different concentrations of each extract in mg.mL⁻¹ were used (100; 50; 25; 12.5; 6.2; 3.1; 1.6; 0.8; 0.4; and 0.2). The readings of the samples were performed using a spectrophotometer (Biospectro, SP-220 model) at a wavelength of 517 nm. The percentage of antioxidant activity (AA%) was calculated by the percentage of uptake of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, and the results were expressed in the extract concentration of 0.2 mg.mL⁻¹.

**Microbiological analyses**

The microbiological analyses were as follows: counts of coliforms, Salmonella sp., molds, yeasts, and mesophilic aerobic bacteria at all storage times and methodologies in accordance with the proposals of the American Public Health Association (APHA, 2001).

**Statistical analysis**

The experiments were conducted using a completely randomized design in split plots with three repetitions. The heat treatments (both with and without pasteurization) represented the plots and the storage temperatures (−18°C and + 4°C) were the sub-plots. Each repetition of the treatments was analyzed at seven storage times (1, 30, 60, 90, 120, 150, and 180 days) in duplicate. The data were subjected to the Shapiro–Wilks test in order to test the normality and Levene’s test in order to test the homogeneity of variance that was presupposed by ANOVA (analysis of variance). Not all the variables showed normal distribution and homogeneity of variance. In this case, when some
variables did not present normal distribution and others did, then nonparametric analysis was performed. Consequently, the data were analyzed using the Kruskal–Wallis test to evaluate the difference between the treatments, and Dunn’s test was performed to compare the averages of the treatments. The Wilcoxon test was performed to analyze the difference between the times of 1 day and 180 days of storage. All the statistical analyses were performed using Statistica version 7.0 software (StatSoft Inc., Tulsa, USA, 2004). The results were expressed as mean ± standard deviation. The criterion for the selection of the model for the displayed graphics was the F test significance (p < 0.05) and by the highest value for the coefficient of determination (R²); a minimum of 0.70 was used (Pimentel-Gomes, 2000). Furthermore, an exploratory analysis of the data using principal component analysis (PCA) was performed to visualize the correlation between the variables and possible groupings between the samples. The Pirouette 3:11 (Woodinville, USA, 2003) statistical program was used. The data matrix consisted of 28 samples and 13 independent variables, which contained the results of the quality parameters, bioactive compounds, and microbiological analyses of pulp from sapota-do-Solimões in relation to the different treatments and storage times. The data for each variable were autoscaled in order to assume the same weight during analysis.

Results and discussion

Table 1 shows that on the first day of storage, the different treatments did not affect the characteristics of water activity, acidity, soluble solids, total sugars, reducing sugars, nonreducing sugars, ascorbic acid, and total phenolics of the pulps. However, the parameters of color, pH, total carotenoids, and antioxidant activity were affected. The differences can be justified by the method of obtaining the pulp because the treatments that were submitted to pasteurization showed changes in color, a reduction in pH, which also affects the color of the product, as well as may have contributed to the oxidation of the carotenoids that resulted in a loss of antioxidant activity (Freire et al., 2009; Rodríguez-Amaya & Kimura, 2004; Santos, Neto, & Donzeli, 2016).

![Image of Table 1](image-url)

For the color parameters, there was a significant variation from 44.39 to 45.74 between treatments in relation to L°, which indicate a lightening trend. Positive values for a° (17.88–20.20) and b° (26.42–31.54) were observed, and they were attributed to the presence of carotenoids in the pulp; the variations for a° and b° were significant. Carvalho, Damiani, and Asqueri (2014) found values for fresh fruit of 44.90, 18.27, and 43.06 for L°, a°, and b°, respectively. The intensity of color of the pulps represented by Chroma (c) varied significantly from 31.91 to 37.46. The lowest average, which presented the lowest intensity of orange color, color tonality, and hue angle (h), varied significantly from 55.75 to 57.30, indicating the yellow coloring of the fruit.

The physicochemical analyses showed that the sapota pulp had high water activity (0.9919–0.9924); pH that varied significantly from 6.76 to 6.86; and low acidity (0.11–0.13%). Therefore, this type of pulp must be properly processed and stored so that it does not deteriorate quickly. The soluble solids ranged from 12.02% to 12.87% but showed no significant difference, demonstrating that the sapota-do-Solimões pulp was harvested and used at the same degree of ripeness. Total sugars (7.06–7.33%), reducing sugars (2.28–2.35%), and nonreducing sugars (4.54–4.73%) also varied, but did not differ between the treatments. Another study found values of 6.83 for pH, 0.11% for total acidity, 12.20% for soluble solids, 7.06% for total sugars, 2.88% for reducing sugars, and 4.18% for sucrose in the pulp of fresh sapota (Carvalho et al., 2014). Céron et al. (2014) found pH of 7.5 in the pulp of Colombian sapota and 15.9° Brix, while Alegria, Hoyos, and Prado (2007) reported pH of 6.8 and 6.5 for Cauca and Ecuadorian sapota, respectively, as well as 10.9% and 9.0% for soluble solids. In general, the sapota can be considered to be a good source of soluble solids, with low acidity and a high level of total soluble solids and titratable acidity, which characterize it as a fruit with a sweet and mild flavor and potential for agro-industrial processing. It is abundant in water and

Table 1. Physicochemical characteristics and bioactive compounds of pulp from sapota-do-Solimões (Quararibea cordata) on day 1 of storage.

| Treatments                  | T1                  | T2                  | T3                  | T4                  |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|
| Analyses                     |                     |                     |                     |                     |
| Lightness (L°)               | 44.65 ± 1.44        | 45.74 ± 1.09        | 45.66 ± 1.99        | 44.39 ± 2.29        |
| a° parameter                 | 20.07 ± 0.76        | 20.20 ± 1.18        | 19.21 ± 1.51        | 17.88 ± 2.09        |
| b° parameter                 | 31.22 ± 2.19        | 31.54 ± 2.83        | 29.53 ± 3.33        | 26.42 ± 4.12        |
| Chroma (c)                   | 37.12 ± 2.21        | 37.46 ± 2.97        | 35.24 ± 3.58        | 31.91 ± 4.57        |
| Hue angle (h)                | 57.21 ± 1.03        | 57.30 ± 1.20        | 56.83 ± 1.21        | 55.75 ± 1.26        |
| Water activity               | 0.992 ± 0.001       | 0.992 ± 0.001       | 0.992 ± 0.001       | 0.992 ± 0.001       |
| pH                           | 6.86 ± 0.02         | 6.78 ± 0.02         | 6.81ab ± 0.03       | 6.76 ± 0.02         |
| Titratable acidity            | 0.11 ± 0.02         | 0.13 ± 0.02         | 0.12 ± 0.02         | 0.13 ± 0.02         |
| Soluble solids               | 12.6 ± 0.25         | 12.0 ± 0.67         | 12.9 ± 0.51         | 12.6 ± 0.38         |
| Total sugars                 | 7.30 ± 0.36         | 7.33 ± 0.34         | 7.06 ± 0.25         | 7.13 ± 0.46         |
| Reducing sugars              | 2.34 ± 0.06         | 2.35 ± 0.10         | 2.28 ± 0.07         | 2.33 ± 0.16         |
| Nonreducing sugars           | 4.72 ± 0.29         | 4.73 ± 0.25         | 4.54 ± 0.18         | 4.56 ± 0.30         |
| Ascorbic acid                | 10.72 ± 0.60        | 10.68 ± 0.56        | 10.25 ± 0.61        | 10.19 ± 0.69        |
| Total carotenoids            | 1.50 ± 0.20         | 1.96 ± 0.11         | 1.29ab ± 0.04       | 1.01 ± 0.18         |
| Total phenolics              | 9.52 ± 0.91         | 9.11 ± 0.61         | 9.37 ± 1.36         | 8.42 ± 0.59         |
| Antioxidant activity         | 15.32a ± 0.17       | 13.79ab ± 0.55      | 15.39 ± 0.77        | 13.45b ± 0.23       |

T1: freezing; T2: pasteurization + freezing; T3: refrigeration; T4: pasteurization + refrigeration.

1Expressed as %; 2Expressed as mg.100.q−1 of pulp; 3Expressed as μg.g−1 of pulp; 4Expressed as mg GAE per 100 g of extract. Values expressed as mean ± standard deviation. Different small letters in the same line indicate 5% level of significance by Dunn’s test.

1Expressed como %; 2Expressado como mg.100.q−1 de pulpa; 3Expressado como μg.g−1 de pulpa; 4Expressado en mg GAE por 100 g de extracto. Valores expresados como media ± desviación estándar. Diferentes letras pequeñas en la misma línea indican el nivel de significación del 5% por la prueba de Dunn.
In the present study, the ascorbic acid content ranged from 10.19 to 10.72 mg·100 g−1 of pulp at the beginning of storage. These values were higher than those found by the Instituto Nacional de Salud Perú (INSP, 2009), where 8.90 mg of ascorbic acid was found in 100 g of pulp from sapota-do-Solimões. There was a significant variation in relation to total carotenoids which varied from 1.01 to 1.50 μg·g−1 of pulp (Table 1). Berto et al. (2015) studied the carotenoid content in alcoholic extracts obtained from sapota-do-Solimões pulp acquired in Manaus, Amazonas, Brazil. In relation to the pulp, their study found a total of 6 μg·g−1 of extract on a dry basis. The main equivalents of zeaxanthin and β-carotene were 2.5 and 2.2 μg·g−1 of extract on a dry basis, respectively. Murillo et al. (2013) detected the presence of 22 carotenoids, including β-carotene (23.3%), and taking into account the large amount of β-carotene detected in sapota-do-Solimões, it presents provitamin A potential. Therefore, this fruit should be considered as an important addition in the diet for those who need to combat hypovitaminosis A.

The total phenolics showed no difference between the means of the treatments at the beginning of storage. There was a variation from 8.42 to 9.52 mg of gallic acid per 100 g of extract. Carvalho et al. (2014) quantified the phenolic content in sapota pulp and found values of 6.31 mg GAE·100 g−1 in the alcoholic extract and 15.06 mg GAE·100 g−1 in the aqueous extract. Interest in phenolic compounds has increased because of their antioxidant and anti-inflammatory properties. In a recent study, Berto et al. (2015) identified and quantified 10 phenolic compounds in alcoholic extracts obtained from sapota-do-Solimões pulp.

The total antioxidant activity varied significantly from 13.45% to 15.39% in the extract, with a concentration of 0.2 mg·mL−1. In a study by Carvalho, Damiani, Asquieri, Orsi, and Nishi (2012), the antioxidant potential was also determined by the scavenging ability in relation to DPPH. In the former study, the total antioxidant activity was 27.85% (10.65% in the alcoholic extract and 16.27% in the aqueous extract).

Table 2 provides a comparison of the means for the sapota-do-Solimões pulps at day 1 and day 180 of storage in relation to the color parameters. There were significant changes in color during storage; the pulps became lighter, with an increase in L* values, except for the pasteurization + refrigeration treatment, and in relation to the a* and b* parameters, there was a significant tendency for the pulps to become redder and yellower, respectively. There was an increase in the intensity of the orange color represented by Chroma (c). The tonality of the frozen pulps did not change significantly, and the refrigerated pulps were closer to yellow, which is the color itself by the hue angle (h). The increase in these values for the a* and b* parameters in the sapota-do-Solimões pulps can be explained by the stability of the carotenoids throughout the storage period, which was due to the method of storage because storage at low temperatures, especially freezing, usually favors the preservation of carotenoids in foods (Rodriguez-Amaya & Kimura, 2004). Furthermore, the decrease in water activity during storage protected the carotenoids from degradation (Zielinski et al., 2014).

In a study of the pulp from acerola fruit, the L* values remained stable throughout storage, and the pasteurized pulps were superior to the non-pasteurized pulps. In terms of the a* parameter, there was a decrease in values at the end of 360 days of storage under freezing. The values for the b* parameter of the acerola pulp showed a small increase. The Chroma (c) values decreased, and the values of the hue

| Analyses       | Time 1 | Time 180 |
|----------------|--------|----------|
| Lightness (L*) | 46.12ab ± 2.24 | 45.74ab ± 1.09 |
| a* parameter   | 20.07ab ± 0.76 | 19.21ab ± 1.51 |
| b* parameter   | 22.61ab ± 1.27 | 21.21ab ± 1.52 |
| Time 180       | 31.23ab ± 2.19 | 29.53ab ± 3.33 |
| Chroma (c)     | 37.12ab ± 2.21 | 35.24ab ± 3.38 |
| Hue angle (h)  | 41.30ab ± 3.94 | 42.45ab ± 5.19 |

Values expressed as mean ± standard deviation. Different small letters in the same line indicate 5% level of significance by Dunn’s test. Different capital letters in the same column for each color parameter indicate 5% level of significance by the Wilcoxon test.

T1: freezing; T2: pasteurization + freezing; T3: refrigeration; T4: pasteurization + refrigeration.
angle (h) showed no significant interaction between the storage time and the treatment (Lima et al., 2012).

The quality parameters of sapota-do-Solimões pulp stored for 180 days are shown in Figure 2.

In terms of water activity, there was no difference between the averages for the treatments (Figure 2(a)) and there was a decrease in values for all treatments. According to Gava, Silva, and Frias (2008), water activity influences changes in foods because it is related to the growth and metabolic activity of microorganisms; during hydrolytic reactions, fruit pulps tend to have a value for water activity exceeding 0.98. da Silva et al. (2010) studied the stability of bacuri pulp that was frozen for 12 months and found water activity values that ranged from 0.987 to 0.994, which did not differ statistically over time.

The pH values in the frozen pulps showed no significant differences between the initial and final time of storage; however, in the refrigerated pulps, there was a significant decrease. From a technological point of view, the pH values of the sapota pulp that was stored under freezing in the present study showed low counts of yeasts and molds, this possibly explains the small changes that occurred in the pH in the frozen treatments.

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Figure 2. Graphs of the means found for the variables of water activity (a), pH (b), titratable acidity (c), soluble solids (d), total sugars (e), reducing sugars (f), and nonreducing sugars (g) in relation to time of storage in days.

Figura 2. Gráficos de las medias encontradas para las variables de actividad del agua (a), pH (b), acidez titulable (c), sólidos solubles (d), azúcares totales (e) y azúcares reductores (f), azúcares no reductores (g) en relación con el tiempo de almacenamiento en días.
Acidity is an important parameter in assessing the state of preservation of food products. The acidity was stable during storage for all the treatments (Figure 2(c)). This behavior also occurred in another study of pasteurized and unpasteurized acerola pulp during storage (Lima et al., 2012). The acidity in camu-camu pulp remained stable during 4 months of storage for all treatments (freezing, pasteurization, high hydrostatic pressure, and lyophilization) (Moraes-de-Souza, 2011). da Silva et al. (2010) argued that acidity results can be important because this variable is determinative of the quality of fruit for fresh consumption and for industrial processing. Furthermore, acidity values may indicate the deterioration of foods by bacteria that produce acid, so it is important to monitor the stability of foods.

In terms of soluble solids, no significant interactions were detected between the treatments and storage time; a decrease was observed in all treatments throughout the storage period (Figure 2(d)). da Silva et al. (2010) studied soluble solids in bacuri pulp and found that they differed significantly during 12 months of storage. In a study by Moraes-de-Souza (2011) of camu-camú pulp, soluble solids were stable for 4 months of storage, and in relation to total sugars, reducing sugars, and nonreducing sugars, no significant interactions were detected between treatments and storage time. The total sugars, reducing sugars, and nonreducing sugars (Figures 2(e–g), respectively, were directly related to the total soluble solids content; therefore, they varied according to the maturation stage of the fruits.

The figures for bioactive compounds and antioxidant activity of sapota-do-Solimões pulp stored for 180 days are shown in Figure 3.

In relation to ascorbic acid, there was no significant difference between the treatments for each storage period; however, there was significant difference between the time of 1 day and 180 days. The freezing treatment showed no loss of ascorbic acid during storage, and this resulted in better stability (Figure 3(a)). The ascorbic acid content of passion fruit pulp pasteurized at different temperature ranges (69–72°C, 73–76°C, and 77–82°C) declined during a storage period of 180 days under refrigeration (Monteiro et al., 2005). Silva, Júnior, and Ferreira (2008) studied the stability of vitamin C in cagaita pulp frozen for 4 months and observed a gradual reduction of approximately 30% in the first month and 50% in the third month compared with the initial concentration. Lee and Kader (2000) reported that a gradual reduction of ascorbic acid content in fruits occurs in line with an increase in temperature and storage time. In the present study, the pulps stored under refrigeration had lower values for ascorbic acid compared with the frozen pulps.

The total carotenoid content decreased during storage for all the storage times. There was no significant difference in the pasteurization + freezing treatment, which remained stable, but the treatments stored at +4°C showed lower values compared to the treatments stored under freezing (Figure 3(b)). Lopes, Mattietto, and de Menezes (2005) studied the stability of pitanga pulp that was frozen for 90 days, and they observed that there was a significant drop of about 13.76% in the total carotenoid content in the first 30 days of storage; however, at 45, 60, and 90 days of storage, no significant decrease was observed. Carotenoids are naturally protected in plant tissues; however, when fruit and vegetables are or cut or disintegrate, there is an increase in the exposure of the carotenoids to oxygen and contact with enzymes, which catalyzes the oxidation procedure (Rodriguez-Amaya & Kimura, 2004). In the pasteurization + freezing treatment of the sapota pulp, there was greater protection of carotenoids due to the process of the inactivation of the enzymes, combined with the low temperatures provide by freezing.

The total phenolic content remained stable between the treatments until 150 days of storage; however, the content decreased from 150 to 180 days of storage. Among the storage times, there was a decline in relation to each treatment. There was stability until 30 days of storage, but from that time, a decrease in t was observed (Figure 3(c)).

Figure 3. Graphs of the means found for the variables of ascorbic acid (a), total carotenoids (b), total phenolics (c), and antioxidant activity (d) in relation to time of storage in days.

Figura 3. Gráficos de las medias encontradas para las variables de ácido ascórbico (a), carotenoides totales (b), compuestos fenólicos totales (c) y actividad antioxidante (d) en relación con el tiempo de almacenamiento en días.
point, the values decreased greatly, especially at 180 days (Figure 3(c)). Processing and storage can affect the content and bioavailability of fruit pulps, resulting in losses, because they contain components that are susceptible to oxidation processes and are highly unstable (Melo, Maciel, de Lima, & de Araújo, 2008). Lower levels of total phenolics may be a result of a reaction with oxygen (oxidation) during the homogenization of the pulp, which continues to occur during storage, as well as the high temperature used in pasteurization (Damiani et al., 2013).

The antioxidant activity decreased during storage in all the treatments but remained stable up to 90 days. In the freezing treatment, the antioxidant activity was stable up to 150 days, and therefore, pasteurization seems to have influenced the reduction in antioxidant activity of the sapota-do-Solimões pulp (Figure 3(d)). Kaur and Kapoor (2001) argue that natural antioxidants can be significantly lost as a consequence of processing and storage, thereby affecting the antioxidant capacity of food. In general, fruits contain several compounds with antioxidant properties including ascorbic acid, phenolic compounds, and carotenoids (Damodoran, Parkin, & Fennema, 2010). These bioactive compounds are susceptible to oxidation reactions that occur during the processing and storage of food, and the incorporation of air during processing aids aerobic degradation reactions by oxidation (Lima, Mélo, & Lima, 2000) or thermal degradation during pasteurization (Maia et al., 2007). According to Hassimotto, Genovese, and Lajolo (2005), the correlation between phenolics and antioxidant activity of a product is very small and is mainly linked with the difference in the phenolic composition of plant extracts.

Table 3 shows the microbiological results regarding the counts of molds, yeasts, and aerobic mesophilic bacteria in the sapota-do-Solimões pulp. Brasil (2001) defines the microbiological standards for each food. Fruit pulp that is concentrated (or not) and with or without heat treatment, refrigeration or freezing only has parameters for coliforms at 76°C, and 77°C ± 0.22 (2005) for fresh or frozen pulp and 2 × 10² CFU.g⁻¹ for chemically conserved pulp and/or pulp which has undergone heat treatment. Brazilian legislation does not set specific limits for the total count of aerobic mesophilic bacteria in fruit pulp.

Yeast and molds are sensitive to the temperatures used in pasteurization, and therefore, it was possible to observe the effectiveness of the process, given that treatments T2 and T4 showed lower counts than T1 and T3. The pulp that was refrigerated for 150 days of storage showed a count for yeasts and molds that was higher than that permitted by law.

The total count of aerobic mesophilic bacteria was also lower in the treatments that used pasteurization, and the treatments that used refrigeration showed higher values than the pulps that were stored frozen. As there is no legislation to limit values for the count of mesophilic aerobic bacteria in fruit pulp, it was not possible to come to a conclusion about the degree of contamination of the pulps, but the presence of mesophilic bacteria in large numbers can indicate factors such as raw material that is excessively contaminated, inadequate cleaning and sanitizing of surfaces, insufficient hygiene in the production or storage of food, inadequate conditions of time/temperature during the production or storage of food, or a combination of these circumstances (Franco & Landgraf, 2005). Monteiro et al. (2005) evaluated the quality of pasteurized passion fruit pulp at three temperature ranges (69–72°C, 73–76°C, and 77–82°C) for 30 s and observed that the pulps showed similar behavior in relation to the growth of mesophilic aerobic microorganisms, independent of heat treatment.

Exploratory data analysis through the multivariate statistical technique PCA was performed. Figure 4 shows the graphs for the scores (samples) and the weights (variables) of the first and second components. The results show a trend towards the differences in the treatments, indicating that pasteurization has a more significant effect on the microbial composition of the pulps than refrigeration.

### Table 3.

| Treatments | T1       | T2       | T3       | T4       |
|------------|----------|----------|----------|----------|
| Yeast and molds¹ | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 1     | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 30    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 60    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 90    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 120   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 150   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 180   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Aerobic mesophilic count¹ | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 1     | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 30    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 60    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 90    | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 120   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 150   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |
| Time 180   | 1.0³ab  | 1.0³ab  | 1.0³ab  | 1.0³ab  |

¹Log CFU.g⁻¹ = colony forming units per gram. Different smaller letters in the same line indicate 5% level of significance by Dunn’s test. Different capital letters in the same column indicate 5% level of significance by Dunn’s test.

T1: freezing; T2: pasteurization + freezing; T3: refrigeration; T4: pasteurization + refrigeration.

1: Log CFU.g⁻¹ = unidades formadoras de colonias por gramo. Diferentes letras mayúsculas en la misma columna indican un nivel de significación del 5% por la prueba de Dunn. Diferentes letras mayúsculas en la misma columna indican un nivel de significación del 5% por la prueba de Dunn.

2: Log CFU.g⁻¹ = colony forming units per gram. Different small letters in the same line indicate 5% level of significance by Dunn’s test. Different capital letters in the same column indicate 5% level of significance by Dunn’s test.
of the first two principal components (PC) resulting from the PCA, which incorporated 68.59% of the total variance. In this analysis, it was possible to extract relevant information regarding the correlation between the variables to characterize the samples.

There was a partial separation of the samples in relation to the different treatments and storage times of the sapota-do-Solimões pulps so that the frozen samples were positioned in the negative quadrants and the refrigerated samples were in the positive quadrant of PC II. However, the treatment that involved freezing until 150 days of storage was located in the positive quadrant of PC I, as were the samples that were pasteurized + frozen until 90 days of storage. This behavior occurred in relation to the different types of preservation to which the samples were subjected, and consequently, they had different concentrations in relation to the physicochemical and microbiological characteristics and bioactive compounds that were analyzed.

The main difference between the groups regarding the physicochemical characteristics was in relation to the greater concentration of water and pH activity in the initial storage period in all the treatments of the sapota-do-Solimões pulps, while in the final stages of storage, the soluble solids, titratable acidity, total sugars, reducing sugars, and nonreducing sugars stood out. Regarding the microbiological parameters, the main difference between the groups was related to a greater concentration of molds, yeasts, and mesophilic aerobic bacteria count in the treatments that were refrigerated for 90 days of storage. For the bioactive compounds and antioxidant activity, it was observed that in all treatments, and especially in the initial storage times, the concentration of ascorbic acid, carotenoids, and total phenolics was higher, as well as antioxidant activity, which showed that the storage time had a negative influence, except for the treatment which was frozen for 150 days and the treatment which was pasteurized + frozen for 90 days.

**Figure 4.** Principal component analysis of the quality parameters, bioactive compounds, and microbiological analyses of sapota-do-Solimões (Quararibea cordata) pulp in the different treatments and storage times. (a) – score plots (samples), T1 = freezing, T2 = pasteurization + freezing, T3 = refrigeration, T4 = pasteurization + refrigeration. (b) – weight plots (variables).

**Figura 4.** Análisis de componentes principales de los parámetros de calidad, compuestos bioactivos y análisis microbiológicos de la pulpa de sapota-do-Solimões (Quararibea cordata) en los diferentes tratamientos y tiempos de almacenamiento. (a) – puntuación de parcelas (muestras), T1 = congelación, T2 = pasteurización+congelación, T3 = refrigeración, T4 = pasteurización+refrigeración. (b) – gráficos de pesos (variables).
Conclusions
The different treatments applied to the sapota-do-Solimões pulp affected the physicochemical parameters during storage. Of particular note was the reduction in water activity and pH in the pulps that were stored under refrigeration, as well as the lightening in color of the pulps.

In relation to the bioactive compounds, ascorbic acid remained stable during freezing; carotenoids were preserved in the pasteurization + freezing treatment; total phenolics remained stable up to 150 days of storage in all the treatments; and the antioxidant activity decreased during storage for all the treatments.

The pulps showed good stability during the 180 days of storage, except for the refrigerated pulp after 150 days. The pasteurization was effective because there were lower counts than in the unpasteurized pulps. The pasteurization + freezing treatment and the freezing treatment maintained pulp quality during the 180 days of storage.

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