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Phenolic Compounds Stability of Grumixama (*Eugenia brasiliensis*) Juice during Processing and Storage

Elivaldo Nunes Modesto Junior [1], Rosane Patricia Ferreira Chaves [1], Mayara Galvão Martins [2], Gustavo Araujo Pereira [1,3], Renan Campos Chisté [1,3] and Rosinelson da Silva Pena [1,3,*]

[1] Graduate Program of Food Science and Technology (PPGCTA), Institute of Technology (ITEC), Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil; modesto.ufpa@outlook.com (E.N.M.J.); rosane.fchaves@hotmail.com (R.P.F.C.); gapereira@ufpa.br (G.A.P.); rcchiste@ufpa.br (R.C.C.)
[2] Innovation, Development and Adaptation of Sustainable Technologies Research Group (GPIDATS), Mamirauá Institute for Sustainable Development (IDSM), Tefé 69553-225, AM, Brazil; mayara.martins@mamiraua.org.br
[3] Faculty of Food Engineering (FEA), Institute of Technology (ITEC), Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil
* Correspondence: rspena@ufpa.br

Abstract: Grumixama is an anthocyanin-rich berry commonly found in South America, yet not widely consumed by Brazilians due to the lack of studies covering its technological properties. Therefore, the stability of anthocyanins and the activity of oxidoreductase enzymes in grumixama juice during thermal treatment (60 °C to 100 °C) and storage (at 25 °C and 7 °C) were evaluated. As a result, the pasteurization of grumixama juice at 80 °C for 60 s completely inactivated peroxidase (POD) and polyphenol oxidase (PPO), eliminated microorganisms, and induced low degradation of the total anthocyanins (40%) and minimal degradation of the total phenolic compounds. Four anthocyanins were monitored in the pasteurized juice, namely delphinidin 3-glucoside, cyanidin 3-glucoside, and two unidentified anthocyanins. Despite the temperature range, the qualitative chromatographic profile of the anthocyanins did not change, while the contents were affected. The binominal temperature/time of 80 °C/60 s effectively inactivated PPO and POD enzymes, promoted moderate alterations in the contents of total anthocyanins and total phenolic compounds, and ensured the microbiological quality of grumixama juice. Grumixama juice can be used as a health food due to its high content of antioxidant phenolic compounds, especially anthocyanins.

Keywords: bioactive compounds; food processing; enzymatic inactivation; thermal treatment; anthocyanins stability

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1. Introduction

Science and industry have been developing several studies about natural bioactive compounds that play an important role in oxidative stress and diseases. These compounds can protect against cardiovascular diseases, and they show several useful properties, such as antitumor, antimicrobial, anti-adhesion, and anti-inflammatory activities [1]. A daily intake of bioactive compounds can be achieved by consuming plant-based food, especially fruits and fruit juices containing diverse phytochemicals, such as vitamin C, phenolic compounds (flavonoids such as flavanols, flavones, flavonols, anthocyanins, and proanthocyanidins, as well as phenolic acids), and carotenoids [2].

In modern lifestyles, fruit beverages are a convenient and easy way of nutrient consumption, and they represent an appropriate means for dissolving functional components [3]. Hence, the beverage industry has grown, and there is a wide spectrum of functional beverages on the market such as sports performance aids, ready-to-drink tea infusions, vitamin-enhanced water, and plant-based beverages [4].

Berries are one of the most abundant sources of phenolic compounds and have been the subject of several types of research concerning their effects on human health [5]. In this

sense, *Eugenia brasiliensis* Lam. is a tree of the Brazilian coastal forests that belongs to the genus Eugenia, one of the largest in the family Myrtaceae [6,7]. *E. brasiliensis*, commonly known as grumixama, is a red-colored fruit due to its anthocyanin composition, especially cyanidin 3-glucoside and delphinidin 3-glucoside [8]. This fruit can be used to prepare food products, such as jelly, jam, and juice. Although it is cultivated in the south and southeast regions of Brazil, the processing of grumixama juice and its stability during processing have not yet been investigated. This gap delays the use of grumixama as a health food by Brazilian people, for example.

Thermal treatments, such as pasteurization, are used to deactivate microorganisms and enzymes that decrease the quality of fruit juices during food processing and storage. Despite these advantages, juices thermally treated at high temperatures can show variable losses of nutrients and bioactive compounds and can have altered colors and flavors [9]. Pasteurization can significantly decrease the content of ascorbic acid and anthocyanins, as well as the color of the product [10]. Additionally, thermal treatments can cause the degradation of anthocyanins, followed by the formation of brown-colored products, which are undesirable in fruit juices since consumers perceive them as indicators of low quality [11]. Even though pasteurization has advantages and limitations, food industries apply this thermal treatment extensively by modulating time and temperature parameters, which can preserve the food's quality and properties.

In this way, this study aimed to evaluate the stability of anthocyanins and phenolic compounds, antioxidant activity, and enzymatic activity of grumixama juice during thermal treatment (pasteurization) and storage. Data from this study may help food industries to process grumixama and market an innovative healthy product.

2. Material and Methods

2.1. Plant Material

Ripe grumixama fruits (*Eugenia brasiliensis* Lam.) (with a purple-colored peel and without injuries) (~2 kg) were manually collected in Salvaterra (Marajó, Pará, Brazil) (0°45′32″ S and 48°30′44″ W). The fruits were collected and identified by exsiccation in the Department of Botanical Identification of University Federal of Pará, by Herbarium HF Teacher Normélia Vasconcelos (Belém, Pará, Brazil), under the code HF4529. The ripe fruits were stored in an isothermic container and transported to the laboratory for up to 24 h. They were washed with water and sanitized in a sodium hypochlorite solution (100 mg/L) for 10 min, followed by rinsing with water to remove excess chlorine. After removing the seeds, the fruits (pulp and peel) were placed in a vacuum pack and stored at −18 °C until use.

2.2. Juice Processing

The juice was formulated using the pulp and the peel (edible parts) of the fruit and mineral water in the proportion of 1:1 (*w*/*w*). Fruit and water were mixed for 60 s in a food mixer, which was followed by pasteurization without a filtration step. Ascorbic and citric acids were added to the juice, for enzymatic inactivation tests. These preliminary tests were carried out to evaluate which organic acid (ascorbic acid or citric acid) could be used to ensure enzyme inactivation and better preserve the total anthocyanin contents of grumixama juice before pasteurization. Concentrations ranging from 0.5 to 1.5% (*w*/*v*) of the organic acids were tested, and the total anthocyanin content and activities of polyphenol oxidase and peroxidase were evaluated. Citric acid (1%) was selected to be added to the juice, to avoid the enzymatic activity of oxidoreductase enzymes.

2.3. Pasteurization Process

Pasteurization was carried out by loading 10 mL of juice in stainless steel tubes, fixed in a thermal bath with a temperature controller. The pasteurization tests were carried out at 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C for 60 s. After the pasteurization time, the tubes were immediately cooled to 10 ± 2 °C in an ice bath (Figure 1). The total soluble solids

(°Brix), pH, total titratable acidity, total sugars, polyphenol oxidase, and peroxidase activity, and the total anthocyanin and total phenolic compound content in treated and non-treated juices, were evaluated according to the methods presented in Sections 2.5–2.8.

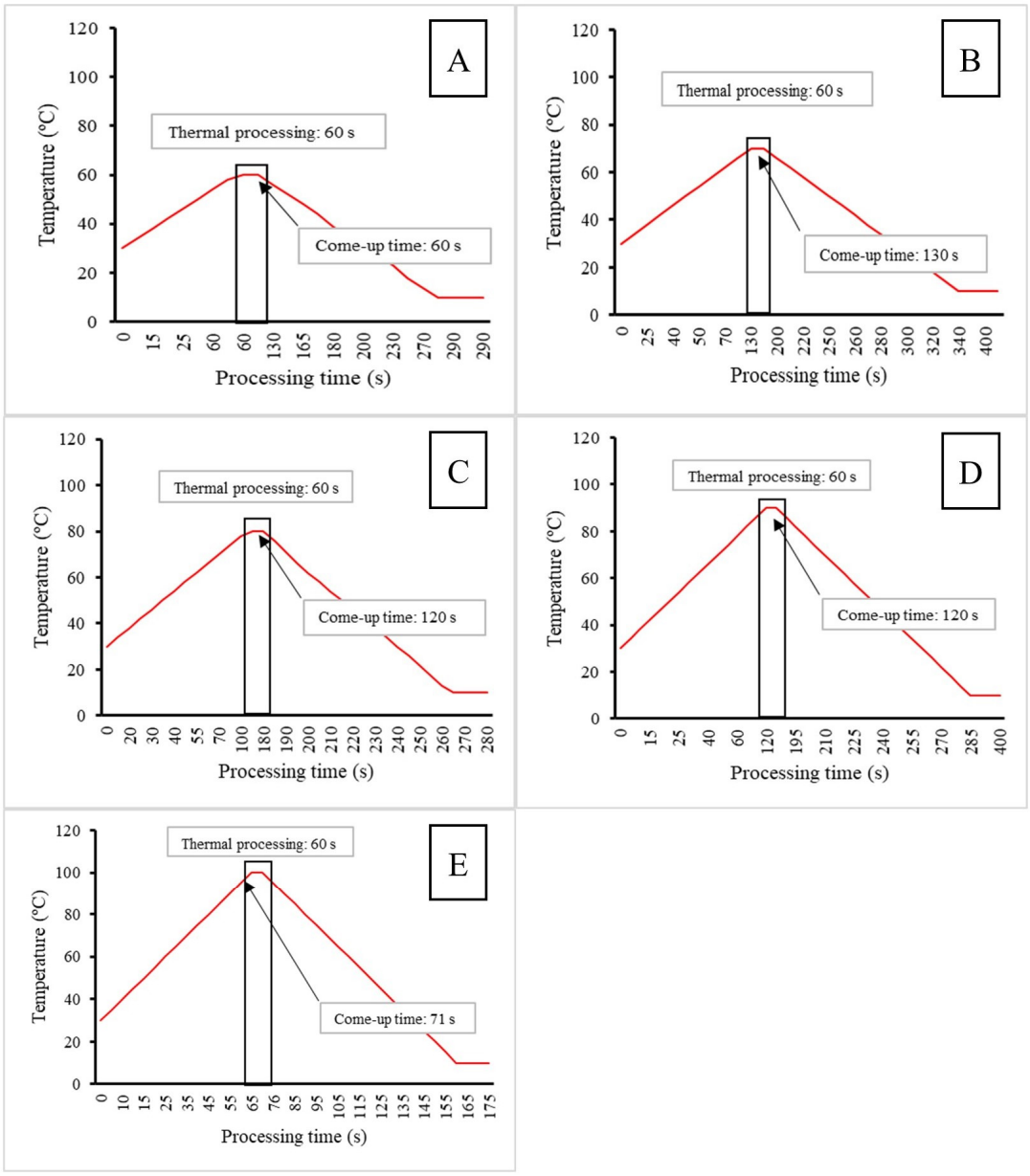

Figure 1. Temperature profile (red line) of the grumixama juice for each thermal treatment applied. (**A**) 60 °C; (**B**) 70 °C; (**C**) 80 °C; (**D**) 90 °C; and (**E**) 100 °C.

2.4. Pasteurized Juice Stability during Storage

The stability of total anthocyanins and total phenolic compounds, enzymatic activity, and microbial counts of the pasteurized grumixama juice were evaluated during 30 days of storage at 25 °C and 7 °C (±1 °C). The juice samples (10 mL) were placed in high-density polyethylene (HDPE) tubes with a capacity of 15 mL, protected from light with aluminum foil, and monitored at the beginning (zero time), and after 15 and 30 days of storage.

2.5. Physicochemical Properties and Enzymatic Activity

The total soluble solids (TSS, method nº 932.12, expressed in °Brix), pH (method nº 981.12), and total titratable acidity (TTA, method nº 942.15, expressed in g citric acid/100 g

of sample) were determined in the grumixama fruit and the juices (unpasteurized and pasteurized), according to the protocols described by the Association of Official Analytical Chemists [12]. The total sugars (TS, method nº 923.09) were determined according to the method of Lane and Eynon (method nº 923.09) [12].

Polyphenol oxidase and peroxidase activity was analyzed by spectrophotometry at 415 nm [13] and 435 nm [14], respectively. Units of enzymatic activity (U) were defined as an increase of 0.001 absorbance units per minute, and the results were expressed as U/mL.

2.6. Total Monomeric Anthocyanins and Total Phenolic Compounds

Approximately 0.1 g of juice and 50 mL of 95% ethanol/HCl 1.5 M (85:15, *v*/*v*) were homogenized for 90 s, followed by filtration (Whatman nº 1), and the solid residue was washed repeatedly with acidified ethanol until it became colorless [15]. This extract was used for the following evaluations.

The total monomeric anthocyanins (TMA) were quantified by spectrophotometry, according to the pH differential method described by Wrolstad et al. [16]. The results were expressed as mg of cyanidin 3-glucoside equivalent/L of grumixama juice.

The total phenolic compounds (TPC) were also determined by spectrophotometry at 765 nm, according to the methodology proposed by Singleton and Rossi [17] and modified by Pereira et al. [18], using the Folin–Ciocalteu reagent. Gallic acid was used for the external analytical curves ranging from 5 to 80 mg/L. The results were expressed as mg of gallic acid equivalent (GAE)/L of grumixama juice.

The retention of total anthocyanins (mg cyanidin 3-glucoside/L) and total phenolic compounds (mg GAE/L) was calculated using Equation (1):

$$\text{Retention } (\%) = \frac{(\text{TMA or TPC on pasteurized juice})}{(\text{TMA or TPC on unpasteurized})} \times 100 \quad (1)$$

2.7. HPLC-DAD Analysis of Anthocyanins

A high-performance liquid chromatography (HPLC) system (Agilent Technologies 1260 Infinity II, Germany), equipped with a diode array detector (DAD), was used for the acquisition and processing of data. Separation was carried out on a Synergy Hydro-RP 80A column (250 × 4.6 mm, 4 µm) (Torrance, CA, USA). The mobile phases A (1% aqueous formic acid) and B (acetonitrile) were applied as follows: flow rate: 1 mL/min; gradient: 95% A solvent for 10 min, from 95% to 90% after 6 min, from 90% to 85% after 10 min, and from 85% to 75% after 15 min [19]. The anthocyanins were detected at 520 nm, and the analytical curves of cyanidin 3-glucoside and delphinidin 3-glucoside were constructed with concentrations ranging from 5 to 100 µg/mL.

2.8. Microbial Analysis

The microbiological quality of grumixama juice (unpasteurized and pasteurized) was evaluated for Coliform parameters at 35 °C and 45 °C, for *Staphylococcus aureus*, mesophilic aerobic bacteria, fungi, and *Salmonella* spp. using plates of nutrient agar using Petrifilm™, 3M (St. Paul, MN, USA).

2.9. Statistical Analysis

Analytical procedures were performed at least three times, and the data were presented as mean and standard deviation. Data obtained were submitted to a one-way ANOVA, followed by Tukey's test ($p \leq 0.05$). Minitab® (version 18.1) was used for principal component analysis (PCA), performed using the covariance matrix considering the active variables (total anthocyanins and total phenolic compounds, polyphenol oxidase, peroxidase, total titratable acidity, °Brix, pH, and ratio of TTA/TSS).

3. Results and Discussion

3.1. Enzymatic Inactivation and Characterization of Grumixama Juice

The physicochemical properties of grumixama juice with and without the addition of acid are presented in Table 1. All investigated properties showed statistical differences ($p \leq 0.05$), except for the sugar content, which remained the same after the addition of citric acid (CA).

Table 1. Physicochemical, chemical, and enzymatic properties of grumixama juice treated with and without citric acid.

Property/Component	Treatment	
	Juice without CA	**Juice with 1% CA**
Physicochemical properties		
Total soluble solids, TSS (°Brix)	7.0 ± 0.01^{b}	8.0 ± 0.58^{a}
Total titratable acidity, TTA (g CA/100 mL)	0.13 ± 0.01^{b}	0.91 ± 0.02^{a}
pH	4.4 ± 0.06^{a}	3.2 ± 0.14^{b}
TSS/TTA	55.87 ± 0.14^{a}	8.74 ± 0.06^{b}
Total sugars (g/100 mL)	9.48 ± 0.26^{a}	9.45 ± 0.25^{a}
Bioactive compounds		
Total anthocyanins (mg cyanidin 3-glucoside/L)	13.69 ± 0.08^{b}	18.77 ± 1.62^{a}
Total phenolic compounds (mg GAE/L)	31.09 ± 1.45^{b}	39.14 ± 1.51^{a}
Enzymatic activity		
Peroxidase (U/mL)	6.0 ± 0.70^{a}	0.39 ± 0.04^{b}
Polyphenoloxidase (U/mL)	8.0 ± 0.70^{a}	5.21 ± 0.56^{b}

CA: citric acid; means (±standard deviations) with different superscript letters in the same row are statistically different ($p \leq 0.05$, Tukey's test).

The juice without the addition of CA showed higher pH values and TSS/TTA ratio than juice with AA. The pH decrease in the juice with CA addition can be seen as an effective alternative to maintain the microbiological stability of the juice (pH < 3.5) and reduce enzymatic activity. The low increase in TSS and significant increase in TTA, concerning the control, resulted in a reduction in the TSS/TTA ratio, which can be used as an evaluation criterion for flavor and, consequently, the quality and acceptance of the juice.

Table 1 also shows the total content of monomeric anthocyanins (TMA), phenolic compounds (TPC), and the enzymatic activity. The contents of TMA and TPC in grumixama juice increased after the acidification. According to Qi et al. [20], the expected color-rendering effect of anthocyanidin is achieved under acidic conditions, and the color can be stabilized through reactions with metal ions, copigmentation, or self-association processes. In summary, acidified grumixama juice showed suitable physicochemical properties for juices and remarkable contents of the bioactive compounds evaluated. The results of enzymatic activities showed that the addition of 1% CA to grumixama juice was effective for pre-enzymatic inactivation before pasteurization, as there was a significant reduction in enzymatic activity, and peroxidase was much more susceptible to this process than polyphenol oxidase. CA effectively inactivates oxidoreductases due to its ability to bind to the structural center of the enzymes [21]. On the other hand, it is an antagonist agent of anthocyanins, promoting color loss in the juice [22].

3.2. Pasteurization Process

Table 2 shows the physicochemical and chemical properties of grumixama juices, unpasteurized and pasteurized at different temperatures. The sugar content expressed in °Brix did not show significant variation ($p > 0.05$) with the applied thermal treatments, although slightly higher °Brix values were observed for the pasteurized juice compared to unpasteurized juice. The TTA increased in the pasteurized juices ($p \leq 0.05$) but exhibited a small variation as the pasteurization temperature increased. The observed behavior can be

attributed to the juice concentration during thermal treatments. The TMA decreased with the increased pasteurization temperature, varying from 18.77 mg cyanidin 3-glucoside/L of unpasteurized juice to 9.05 mg cyanidin 3-glucoside/L of pasteurized juice, at 100 °C. The values represented a retention of total anthocyanins from 70% at 60 °C to 50% at 100 °C.

Table 2. Physicochemical properties of grumixama juice, unpasteurized and pasteurized at different temperatures.

Property	Unpasteurized (Control)	Pasteurization Temperature				
		60 °C	70 °C	80 °C	90 °C	100 °C
Total soluble solids, TSS (°Brix)	8.0 ± 0.58 ^{a}	8.10 ± <0.01 ^{a}	8.7 ± 0.12 ^{a}	8.5 ± <0.01 ^{a}	8.4 ± <0.01 ^{a}	8.4 ± <0.01 ^{a}
Total titratable acidity, TTA (g CA/100 mL)	0.91 ± 0.02 ^{c}	1.77 ± 0.01 ^{b}	2.09 ± 0.02 ^{a}	1.65 ± 0.07 ^{b}	1.65 ± <0.01 ^{b}	1.83 ± 0.07 ^{b}
pH	3.2 ± 0.14 ^{a}	3.3 ± <0.01 ^{a}	3.2 ± 0.06 ^{a}	3.3 ± 0.06 ^{a}	3.3 ± <0.01 ^{a}	3.3 ± 0.06 ^{a}
TSS/TTA	8.74 ± 0.06 ^{a}	4.57 ± 0.21 ^{c}	4.16 ± 0.08 ^{d}	5.13 ± 0.21 ^{b}	5.10 ± 0.01 ^{b}	4.71 ± 0.18 ^{c}
Total anthocyanins (mg cyanidin 3-glucoside/L)	18.77 ± 1.62 ^{a}	13.20 ± 0.86 ^{b}	12.47 ± 1.05 ^{b}	10.93 ± 0.75 ^{bc}	11.72 ± 0.13 ^{bc}	9.05 ± 0.02 ^{c}
Total phenolic compounds (mg GAE/L)	39.14 ± 1.51 ^{a}	36.84 ± 4.21 ^{a}	41.41 ± 0.54 ^{a}	41.09 ± 1.30 ^{a}	39.73 ± 0.64 ^{a}	36.96 ± 0.47 ^{a}
Peroxidase, POD (U/mL)	0.39 ± 0.04 ^{b}	0.30 ± <0.01 ^{b}	1.0 ± <0.01 ^{a}	nd	nd	nd
Polyphenol oxidase, PPO (U/mL)	5.21 ± 0.56 ^{a}	2.0 ± <0.01 ^{b}	2.0 ± <0.01 ^{b}	nd	nd	nd

Means (±standard deviations) with different superscript letters in the same row are statistically different ($p \leq 0.05$, Tukey's test); nd: not detected.

According to Shi et al. [23], the degradation rate of anthocyanins increases during processing and storage as the temperature increases, mainly through the formation of pseudo bases and chalcones. Thermal degradation of anthocyanins initiates with the opening of the central ring followed by hydrolysis of the molecule, resulting in colorless products [24]. Modesto Júnior et al. [25] showed that the anthocyanins from grumixama should not be exposed to temperatures above 50 °C, yet they evaluated the stability of anthocyanin-rich extract in ethanol. Grumixama juice contains mono- and oligo- polymers (e.g., hydrocolloids) that may interact with anthocyanins, increasing their stability during thermal processes. Hou et al. [26] reported that the stability of anthocyanins may increase via intermolecular interactions, such as copigmentation and self-association. Thus, the stability of anthocyanins in grumixama juice during thermal treatment could be attributed to the presence of a high flavonoid content (e.g., copigmentation) or due to a high anthocyanin content (self-association).

The total phenolic compounds (TPC), in turn, did not show a significant difference ($p > 0.05$) in all the conditions of pasteurization, as well as in the unpasteurized juice. The thermal degradation of anthocyanins, in particular at pH 3.5, follows the pathway of intermediate chalcone and the final derivatives of aldehyde and benzoic acid, being structurally differentiated based on the corresponding anthocyanidin [27,28]. It could influence the TPC because the reactive medium used is the Folin–Ciocalteu, which binds to agents with reducing potential, as the degradation products of anthocyanins. At the temperature range used, the enzymes peroxidase (POD) and polyphenol oxidase (PPO) were completely inactivated, which are antagonistic to the class of some bioactive compounds. According to Chisari et al. [29], the enzyme PPO oxidizes phenolic compounds to *o*-quinones, which can subsequently polymerize, yielding dark pigments (polymers).

The inactivation of PPO and PPO was effective for the grumixama juice at 80 °C. Therefore, in our study, grumixama juice can be considered an enzymatically stable product if pasteurized at 80 °C for 60 s. Although the use of high temperatures can affect the sensory and nutritional aspects of juices, this enzymatic inactivation is needed. Residual enzymatic activity is still commonly detected after the pasteurization, but this was not observed in this study. Bhagat and Chakraborty [30] observed that the pasteurization of pomegranate juice at 95 °C promoted the loss of bioactive compounds as a function of time, but it was effective regarding enzymatic inactivation. Etzbach et al. [31] evaluated the enzymatic

inactivation of POD in goldenberry (*Physalis peruviana* L.) puree at different conditions and demonstrated that 75 °C for 2 min was effective.

3.2.1. Individual Anthocyanins by HPLC-DAD

Figure 2 shows the chromatogram of anthocyanins identified by HPLC-DAD in unpasteurized grumixama juice, and Table 3 shows the individual values of the anthocyanins identified, according to the thermal treatments. The anthocyanin content changed during the thermal treatments (ramping down and ramping up) and the individual profile data were different. Plant cell walls may be partially broken during heating, which releases phenolic compounds and anthocyanins that are bound to cell wall components [32]. It is a complex system in which release, degradation, and interactions occur and consequently increase the anthocyanin's stability or its availability, yet degradation still occurs. The total anthocyanins, determined by HPLC, showed stability of these compounds during pasteurization, but the individual profile demonstrated dynamic behaviors of anthocyanins in the different thermal treatments, since grumixama juice is a complex system.

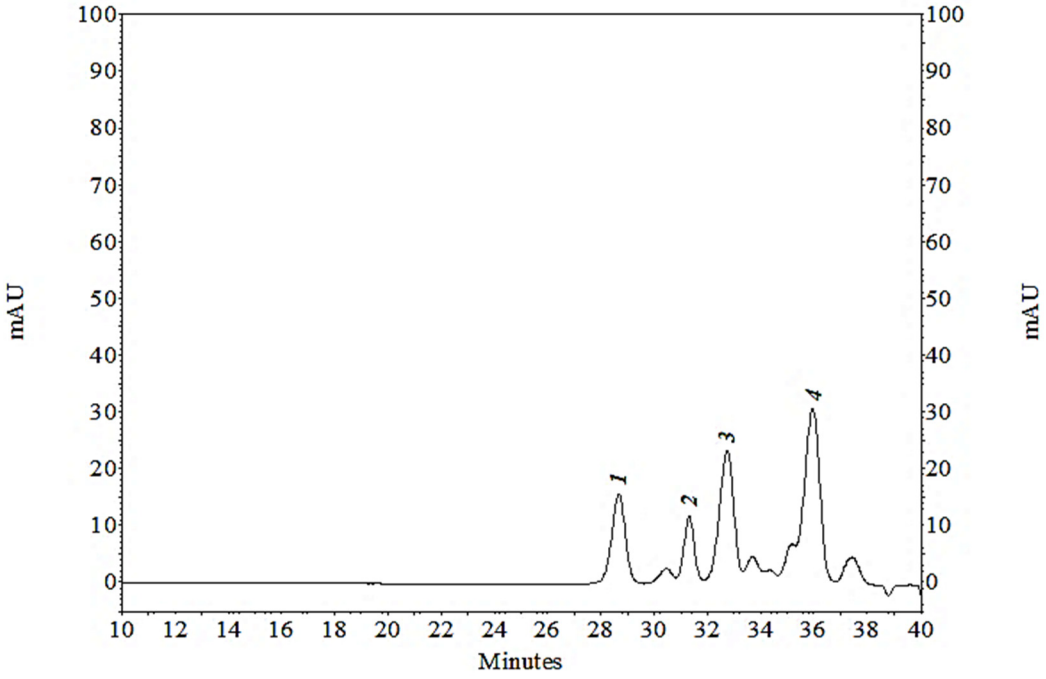

Figure 2. Anthocyanins identified by HPLC-DAD in the unpasteurized grumixama juice: Peak 1—delphinidin 3-glucoside; Peak 3—cyanidin 3-glucoside; and Peaks 2 and 4—unidentified anthocyanins.

Table 3. Contents of individual anthocyanins of grumixama juice, unpasteurized and pasteurized at different temperatures.

Anthocyanins	Unpasteurized (Control)	Pasteurization Temperature				
		60 °C	70 °C	80 °C	90 °C	100 °C
Peak 1—delphinidin 3-glucoside (mg/L)	10.68 ± 0.60 ^{a}	11.72 ± 0.29 ^{a}	7.40 ± 0.07 ^{b}	11.22 ± 0.08 ^{a}	8.65 ± 0.32 ^{b}	6.77 ± 0.72 ^{c}
Peak 2—Unidentified (mg cy-3-glu/L)	4.51 ± 0.06 ^{b}	5.43 ± 0.06 ^{a}	3.35 ± 0.08 ^{c}	4.94 ± 0.39 ^{a}	4.08 ± 0.47 ^{b}	3.42 ± 0.14 ^{c}
Peak 3—cyanidin 3-glucoside (mg/L)	13.67 ± 0.04 ^{a}	9.81 ± 0.08 ^{c}	14.37 ± 0.09 ^{a}	10.80 ± 0.18 ^{b}	8.77 ± 0.28 ^{c}	11.96 ± 0.84 ^{b}
Peak 4—Unidentified (mg cy-3-glu/L)	17.53 ± 0.25 ^{b}	12.77 ± 0.24 ^{c}	24.76 ± 0.05 ^{a}	14.99 ± 0.66 ^{c}	17.85 ± 1.89 ^{b}	27.68 ± 0.12 ^{a}
Total anthocyanins (mg cy-3-glu/L)	46.39 ± 1.01 ^{b}	39.73 ± 0.62 ^{c}	49.88 ± 0.52 ^{a}	41.95 ± 1.54 ^{c}	39.35 ± 2.85 ^{c}	49.83 ± 2.76 ^{ab}

Means (±standard deviations) with different superscript letters in the same row are statistically different ($p \leq 0.05$, Tukey's test).

Delphinidin 3-glucoside (del-3-glu) and cyanidin 3-glucoside (cy-3-glu) showed similar stability in thermal processes, with 80 °C being the best point of stability of the pigment, yet del-3-glu has more hydroxyl groups in its chemical structure than cy-3-glu, which is

reported to decrease stability (Table 3). According to Patras et al. [33], the main thermal degradation pathway of glycosylated anthocyanins such as cy-3-glu is deglycosylation, which removes the 3-bond sugar and cleaves the B or A ring, forming phloroglucinol aldehyde or 4-hydroxybenzoic acid. Das et al. [34] reported that during the heating of an aqueous solution of anthocyanins of purple rice bran, cy-3-glu undergoes hydrolysis, producing cyanidin aglycone by releasing a glucose molecule, becoming less stable.

Among the unidentified peaks, peak 2 showed the lowest anthocyanin contents, varying from 3.35 to 5.43 mg/L, expressed in cy-3-glu; peak 4 showed the highest anthocyanin values, varying from 12.77 to 27.68 mg/L, expressed in Cy-3-glu. Peak 4 showed the highest rate of anthocyanin release at 100 °C, and it was the peak with more anthocyanins released during thermal treatments. One possible explanation is the ability of anthocyanins to bind to other structures; during heating, the cellular structure is broken, generating a greater release of the compound [35]. Some studies demonstrated that anthocyanins possess strong binding affinities towards proteins via non-covalent interactions, resulting in the formation of copigmentation complexes and more resistance to thermal treatments [35,36].

3.2.2. Multivariate Analysis for Pasteurization Processes

Principal component analysis (PCA) for the pasteurization process of grumixama juice (Figure 3) showed 86% cumulative variability, characterized by two principal components (PC1 and PC2). According to Rencher [37], at least 70% of the total variance should be explained by the first and second major components. PC1 explains 56% of the variation, whereas PC2 accommodates 30% of the variability in the dataset. The score graph (Figure 3A) shows that the unpasteurized juice (control) and juice pasteurized at 70 °C are outside the quadrant of juice pasteurized at 60 °C, 80 °C, 90 °C and 100 °C. In contrast, the loading chart (Figure 3B) shows the variables that contributed to the division of groups. The unpasteurized juice showed the highest values of TMA content, PPO activity, and TSS/TTA ratio, and it shows that the thermal treatment modified the characteristics of the grumixama juice, even with a greater or lesser impact on the studied properties. For the group formed by the higher temperatures (mainly 80 °C to 100 °C), pH was the property that generated similarity. Finally, the group formed at 70 °C is linked to the highest values of TPC, POD, TSS, and TTA.

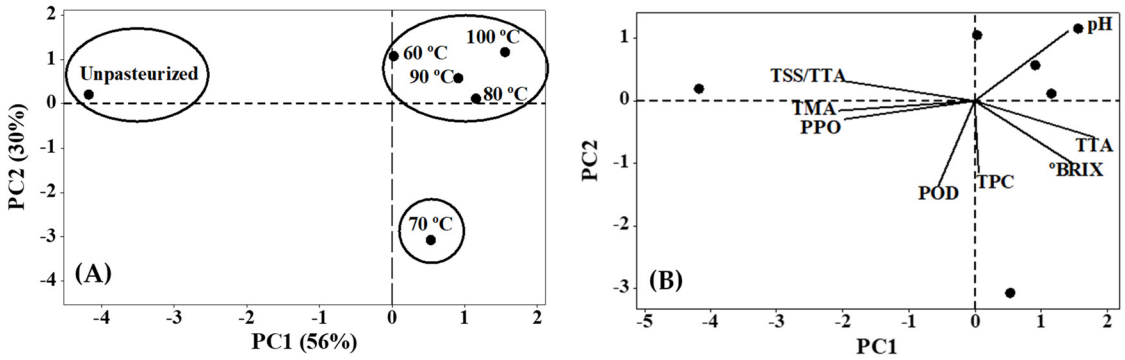

Figure 3. Principal component analysis (PCA) with scores for the respective temperatures analyzed (**A**) and loadings with the respective properties evaluated (**B**).

3.2.3. Effectiveness of the Selected Binomial Time/Temperature

Considering that the thermal treatment at 80 °C was the one that promoted the inactivation of POD and PPO enzymes and the preservation of TMA and TPC in grumixama juice, other pasteurization tests were conducted at 80 °C, with durations ranging from 60 to 120 s (Table 4).

Table 4. Effectiveness of the selected binomial time/temperature: pasteurization of grumixama juice at 80 °C for 60 to 120 s.

Properties	Time (s)		
	60	**90**	**120**
Total anthocyanins (mg cy 3-glu/L)	11.27 ± 0.75 [a]	9.34 ± 0.29 [b]	11.56 ± 0.82 [a]
Total phenolic compounds (mg GAE/L)	41.09 ± 1.30 [a]	36.68 ± 0.83 [a]	30.97 ± 1.47 [b]
Peroxidase (U/mL)	nd	nd	nd
Polyphenol oxidase (U/mL)	nd	nd	nd

Means (±standard deviations) with different superscript letters in the same row are statistically different ($p \leq 0.05$, Tukey's test); nd: not detected.

The total anthocyanin content showed a variation of approximately 11% ($p \leq 0.05$), depending on the pasteurization time. The TPC values, on the other hand, decreased by 11% at 90 s and 25% at 120 s. POD and PPO showed no activity in all the tested time/temperature combinations, indicating the effectiveness of the pasteurization processes. These results indicate that the thermal treatment at 80 °C for 60 s to 120 s can be used in the pasteurization of grumixama juice. However, the thermal treatment at 80 °C for 60 s was effective in inactivating the PPO and POD enzymes, in addition to promoting less degradation of TMA and TPC. Therefore, this time/temperature combination was subsequently used in the procedures.

The microbiological data (Table 5) showed that aerobic mesophilic bacteria (1.84×10^3 CFU/mL) and *Staphylococcus aureus* (1.80×10^2 CFU/mL) were observed in the unpasteurized juice. However, minimum and acceptable levels for coliforms at 35 °C, coliforms at 45 °C, aerobic mesophilic bacteria, fungi, and *Staphylococcus aureus*, as well as the absence of *Salmonella* sp., were observed in the grumixama juice after pasteurization at 80 °C for 60 s.

Table 5. Microbiological standard for grumixama juice, unpasteurized and pasteurized at 80 °C for 60 s.

Microorganism Group	Grumixama Juice	
	Unpasteurized	**Pasteurized**
Coliforms at 35 °C (CFU/mL)	<3.0	<3.0
Coliforms at 45 °C (CFU/mL)	<3.0	<3.0
Aerobic mesophiles (CFU/mL)	$1.84 \times 10^3 \pm$ <0.01	<1.0
Fungi (CFU/mL)	<1.0	<1.0
Staphylococcus aureus (log CFU/L)	$1.80 \times 10^2 \pm$ <0.01	<1.0
Salmonella sp.	Absence	Absence

CFU: colony forming unit.

In addition to pasteurization, the pH of grumixama juice with 1% CA (pH = 3.2) was also an important factor in the control of microbiological growth, reducing the count of aerobic mesophilic bacteria, fungi, and *Staphylococcus aureus* [38]. These results ensure the microbiological stability of the grumixama juice pasteurized at 80 °C for 60 s.

3.2.4. Juice Storage under Different Conditions

The properties analyzed during the storage of pasteurized grumixama juice at room temperature (≈25 °C) and chilling temperature (≈7 °C) are presented in Table 6. During storage at room temperature, there was a reduction in the levels of TSS (9.6 to 4.8 °Brix) and TTA (2.42 to 1.17 g CA/100 mL), which may have occurred due to progressive sugar consumption and the formation of organic acids. However, in the juice stored at the refrigeration temperature, the variation in TSS and TTA was minimal ($p > 0.05$), indicating that this condition ensures greater stability during storage.

Table 6. Shelf-life of pasteurized grumixama juice at room temperature and chilling temperature.

Properties	Room Temperature (25 ± 1 °C)			Chilling Temperature (7 ± 1 °C)		
	Time (Days)			Time (Days)		
	0	15	30	0	15	30
Physicochemical and chemical properties						
Total soluble solids, TSS (°Brix)	9.60 ± <0.01 ^{a}	5.1 ± 0.06 ^{b}	4.8 ± <0.01 ^{c}	8.9 ± 0.06 ^{a}	8.2 ± <0.01 ^{c}	8.6 ± 0.06 ^{b}
Total titratable acidity, TTA (g CA/100 mL)	2.42 ± 0.07 ^{a}	1.38 ± 0.17 ^{b}	1.17 ± 0.12 ^{b}	0.89 ± 0.02 ^{b}	1.19 ± 0.09 ^{a}	0.96 ± 0.06 ^{ab}
pH	3.3 ± 0.06 ^{b}	4.0 ± 0.06 ^{ab}	3.9 ± 0.29 ^{a}	3.7 ± 0.06 ^{a}	3.4 ± <0.01 ^{b}	3.2 ± 0.02 ^{c}
Retention of total anthocyanins (%)	100 ± 0.62 ^{a}	60.31 ± 2.69 ^{b}	57.51 ± 2.13 ^{b}	100 ± 1.88 ^{a}	114 ± 1.37 ^{a}	62.14 ± 0.02 ^{b}
Retention of total phenolic compounds (%)	100 ± 0.97 ^{a}	84.69 ± 3.46 ^{b}	69.47 ± 1.38 ^{c}	100 ± 0.90 ^{a}	91.28 ± 1.44 ^{a}	71.49 ± 2.19 ^{b}
Enzymatic activity						
Peroxidase (U/mL)	nd	nd	nd	nd	nd	nd
Polyphenol oxidase (U/mL)	nd	nd	nd	nd	nd	nd
Microbiological standard						
Aerobic mesophiles (CFU/mL)	<1.0	<1.0	2.6×10^{2} ± <0.01	<1.0	<1.0	<1.0
Fungi (CFU/mL)	<1.0	1.86×10^{4} ± 1.12	1.30×10^{5} ± 1.02	<1.0	<1.0	<1.0

Means (±standard deviations) with different superscript letters in the same row (for room or chilling temperature separately) are statistically different ($p \leq 0.05$, Tukey's test); CFU: colony forming units; nd: not detected.

The retention of anthocyanins in the juice significantly decreased during the 30 days of storage at both storage conditions ($p \leq 0.05$). At room temperature, a reduction of approximately 43% was observed, while under refrigeration the reduction was approximately 48%. Thus, both storage conditions were able to avoid anthocyanin loss (>50%) after 30 days of storage, which was more pronounced in refrigerated storage. Similar behavior was observed for the retention of TPC, in which losses of approximately 30% for both storage conditions after 30 days were observed. Yang et al. [39] stored the juice (with anthocyanins used as a colorant in white currant juice) at room temperature (24 °C) and refrigeration (4 °C), and they reported a greater decrease in anthocyanin in the juice stored at refrigeration. Campos et al. [40] observed that anthocyanins of a jambolan (*Syzygium cumini*) and camu-camu (*Myrciaria dubia*) juice blend had a significant decrease after 35 days of storage at room temperature (25 °C), with a retention level of 28% for the pasteurized juice blend.

In both storage conditions, the reactivation of POD and PPO during the 30 days of storage was not observed. Although both storage systems showed similar behavior, the storage at the chilling temperature showed a lower oscillation in the properties studied, and therefore is the most highly recommended for the grumixama juice.

3.2.5. Multivariate Analysis for Juice Storage

A principal component analysis (PCA) of pasteurized grumixama juice, stored at room temperature and refrigeration temperature, showed that 80% of cumulative variability in the dataset can be elucidated by a set of two principal components (PC1 and PC2). PC1 explained 60% of the variation whereas PC2 accommodated 20% of the variability in the dataset (Figure 4A). In the score graph, the formation of four groups can be observed, among room temperature (RT) and chilling temperature (CT). In the loadings chart (Figure 4B), the properties that contributed to the formation of these groups are observed. The treatment RT0 formed an isolated group, which is assigned to the highest TTA value. The second group consisted of CT0 and CT15 treatments, whose similarity is attributed to the highest TMA and TPC values. This result indicates that after 15 days of storage, the juice still maintained the levels of TMA and TPC observed in the initial time point (CT0). The third group was composed of RT15 and RT30 treatments, in which the highest pH values were observed, as well as variation between the values of TMA, TPC, and TSS. This indicates that, under room temperature after 15 days, the product was already altered and different from the initial time point (RT0), and it remained that way until the end of the 30 days of

storage. The last group was also an isolated group, formed by CT30 treatment and assigned a high value of TSS, and had the lowest values of TTA, pH, and TMA. The data show that, when stored under refrigeration, significant changes in the product occur after 30 days of storage. For a pasteurized juice, this is a short time, but considering our goal of maintaining physicochemical and nutritional stability, it is possible to observe the need for the use of food stabilizers such as ascorbic acid, BHT (butylated hydroxytoluene), or TBA (trenbolone acetate), commonly used in the food industry.

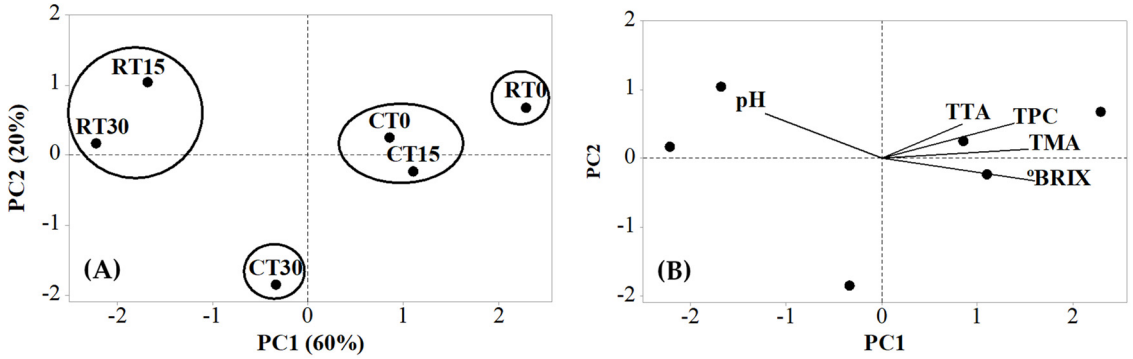

Figure 4. Principal component analysis (PCA) with scores for storage of pasteurized grumixama juice (**A**) and loadings with the respective properties evaluated (**B**).

3.2.6. Microbiological Evaluation of Pasteurized Juice during Storage

The count of aerobic mesophilic bacteria increased after 30 days of storage (2.6×10^2 CFU/mL) at room temperature (Table 6). According to De Carvalho [41], counts greater than 10^6 CFU/mL (6.0 log CFU/mL) for this bacteria class are indicative that the food is under some sanitary alteration. After 30 days of storage at room temperature, this count was not reached by pasteurized grumixama juice. For the juice storage under refrigeration, this microorganism did not grow.

Regarding fungi in pasteurized juice, the counts increased to 1.86×10^4 CFU/mL in 15 days and to 1.30×10^5 CFU/mL in 30 days of storage at room temperature. These values were higher than the maximum value (2.0×10^3 CFU/mL) recommended by Brazilian legislation [42] for this microorganism class in fruit juice. Lacerda de Oliveira et al. [43] observed that after 12 days of storage at 35 °C, the pasteurized pulp of *Passiflora setacea* presented a growth of fungi (3.30 log CFU/g). On the other hand, fungi did not grow in pasteurized juice storage under refrigeration. The growth of the molds that can produce mycotoxins in fruit juices should be prevented for public health, e.g., patulin is a mycotoxin that can be produced by various species of molds found in fruits. Additionally, fermentation caused by yeasts can be a problem in the juice industry [44]. Based on the microbiological results, the storage of pasteurized grumixama juice at room temperature (25 °C) should not exceed 15 days. However, the grumixama juice pasteurized at 80 °C for 60 s remained microbiologically safe for 30 days if stored at refrigeration.

4. Conclusions

This was the first time that pasteurization conditions to produce stable grumixama juices have been investigated. The binominal temperature/time of 80 °C/60 s effectively inactivated PPO and POD enzymes, induced a few alterations in the contents of total anthocyanins and total phenolic compounds, and ensured the microbiological quality of grumixama juice. The results indicated that anthocyanins from grumixama berries are stable to conventional pasteurization. The microbiological pattern and enzymatic activities showed that grumixama juice can be stored at 7 °C for 30 days, while this time decreases to 15 days if the juice is stored at room temperature (25 °C). Additionally, the results showed that the grumixama juice kept at a chilling temperature presented high retention rates of anthocyanins and phenolic compounds. Furthermore, additional studies are necessary to

investigate the use of food preservatives and evaluate different packaging options targeting high stability over the storage of grumixama juice.

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