



Stability evaluation of juçara, banana and strawberry pasteurized smoothie during storage

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Abstract

The objective of this work was to evaluate the effect of storage time and temperature on main characteristics of a juçara (20%), banana (40%) and strawberry (40%) pasteurized smoothie. The pasteurized smoothie was stored at 7 °C and 25 °C for 90 days. At both temperatures there was degradation of the smoothie anthocyanins, which, as expected, was more drastic in storage at room temperature, impacting also the instrumental color of the product. The microbiological quality of the product was assured at both storage temperatures, showing absence of *Salmonella* spp. and counting for thermotolerant coliforms <3 MPN.g⁻¹, which is in accordance to Brazilian legislation. Under refrigeration, the smoothie had good sensory acceptance with scores higher than 6 even after three months of storage. Taking into account the high preservation of the bioactive compounds, mainly of the anthocyanins, the juçara, banana and strawberry pasteurized smoothie can be stored for 90 days under refrigeration temperature (7 °C).

Keywords: *Euterpe edulis*; shelf life; bioactive compounds; instrumental color; sensory evaluation.

Practical Applications: The increase of chronic diseases related to inadequate dietary habits, such as the growing consumption of high density foods, leads to the need of making available to consumers healthier foods as a way of minimizing negative effects associated with the consumption of poor quality diets. Thus, this study may contribute to increase the availability of a healthy product, since the developed smoothie is rich in phenolic compounds and has no added sugar. In addition, may add value to the juçara agroindustrial-chain, a native fruit from Brazil, increasing the knowledge about development and storage of juçara-based smoothie.

1 Introduction

The consumption of ready-to-drink juices and nectars presented a significant growth in Brazil in the period from 2010 to 2015, according to Associação Brasileira das Indústrias de Refrigerantes e de Bebidas não Alcoólicas (2016). Globally, Priyadarshini & Priyadarshini (2018) estimate that this segment will grow about 18% in five years, comprising a production of 90 billion liters of fruit juices. This growth is due not only to the sensory characteristics of these products, but also because they are a source of vitamins, minerals and antioxidant compounds, important substances for human health, besides of being products practical and easy to consume (Crozier et al., 2009; Hurtado et al., 2015).

Mixed juices composed of fruits and vegetables without added water and sugar, also known as smoothies, represent a way of joining desirable sensory characteristics of different fruits in the same product, besides of the possible functional potential in the organism due to the combination of different bioactive compounds. Smoothies are also interesting since they contribute to the development of new flavors as well as to color and consistency enhancement of the final product. Furthermore, they contribute to increase the added value to the fruit productive chain (Bhardwaj & Pandey, 2011; Brasil, 2009).

Following the market trends, which demand practical and healthy foods and drinks, a juçara, banana and strawberry smoothie with good sensory acceptability and relevant phenolic compounds contents was developed. The juçara fruit has a high content of bioactive compounds, mainly anthocyanins, which contributes to the antioxidant capacity of the formulation (Ribeiro et al., 2018). The banana contains carbohydrates, vitamins, minerals and a small fraction of phenolic compounds, being widely used in the formulation of juices due to its sweet taste and consistency (Aurore et al., 2009). Strawberry is rich in minerals such as phosphorus, calcium, iron and magnesium and also contains anthocyanins and other bioactive compounds (Hossain et al., 2016; Tiwari et al., 2009), besides present slightly acidic taste (Oliveira et al., 2013), which contributes to the sweet/sour balance of the formulation.

However, as the smoothie is a nutrient-rich product, which favors microbial growth, it is necessary that it is submitted to a preservation process, such as the thermal treatment, aiming to reduce the initial microbial load and to eliminate pathogenic microorganisms, contributing to the product stability during storage. Although some studies highlight the negative effects of heat on the sensory and nutritional qualities of the products, this technology still stands out due to the significant reduction of initial

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microbial load in liquid products such as fruit juices compared to non-thermal stabilization technologies (Keenan et al., 2011; Tomadoni et al., 2017). According to Walkling-Ribeiro et al. (2010), the combination of short time and medium and high temperatures for pasteurization of a pineapple, banana, apple, orange and coconut milk smoothie promoted a significant reduction of the microbial load without a significant difference in the overall sensory acceptance of the product, as compared to the control, showing that under suitable operational conditions the effects of heat can be minimized.

This work had as objective to evaluate the stability of the bioactive compounds and of the sensory, physical, chemical and microbiological parameters of a juçara (20%), banana (40%) and strawberry (40%) pasteurized smoothie stored for 90 days at temperatures of 7 °C and 25 °C.

2 Material and methods

2.1 Material

Strawberry and juçara pulps, both frozen and unpasteurized, were obtained from local market at Rio de Janeiro city and from the processing industry, in Espírito Santo state, respectively. The juçara pulp was pretreated in a basket centrifuge with 150 µm nylon sieve mesh (Centrifugal IEC - Model K7165, USA) in order to remove suspended solids and to reduce the lipid fraction, once it contributes to oxidation, affecting the product sensory quality.

The banana pulp was obtained by depulping of fruits from nanica variety, purchased from the local market in Rio de Janeiro, in a horizontal depulper (Itametal, Brazil) comprising 1.5 mm stainless steel sieve and polyethylene blades. The bananas were used at maturity stage 6, with fully yellow peels, according to the maturation scale described by Aurore et al. (2009).

2.2 Smoothie processing and storage

The pulps were combined taking into account the composition and impact of each fruit for the formulation of a product without the addition of sugar, acids or preservatives, as reported by Ribeiro et al. (2018). All pulps were stored at -18 °C until the experiments were performed.

The smoothie, obtained by mixing juçara (20%), banana (40%), and strawberry (40%) pulps in a pilot blender and homogenized in an APV homogenizer (Gaulin, USA) at 60 MPa, was then pasteurized.

The pasteurization was performed in a scraped surface heat exchanger (FT25D, Armfield, England) at 90 °C for 35 s. Hot and cold fill was carried out in an ultra-clean chamber. In the hot fill process, the product was filled at about 90 °C into 300 mL glass bottles closed with twist-type metal caps, cooled in an ice bath and stored at 25 °C. In the cold fill, the product was pre-cooled in the final pasteurization step (40 °C) and filled in 300 mL PET bottles closed with plastic screw caps. These bottles were cooled in an ice bath and stored at 7 °C. All bottles were previously sanitized with 50 mL.kg⁻¹ sodium hypochlorite and, after filling, they were stored in B.O.D. incubators under controlled temperature for 90 days.

Smoothie samples were evaluated every 15 days for phenolic compounds and anthocyanins content, antioxidant capacity, pH, soluble solids, titratable acidity and instrumental color. The same period was adopted to evaluate the microbiological quality of the smoothie. Samples were tested for the detection of molds and yeasts, and mesophilic and lactic acid bacteria. In order to attend the legislation, determinations of *Salmonella* spp. and thermotolerant coliforms were carried out at day zero (after pasteurization) and 90 days of storage, as well as to confirm the adoption of good manufacturing practices during product development and to validate them in the final period of stability study.

2.3 Analytical methods

Total monomeric anthocyanins

Total monomeric anthocyanins were quantified by differential pH method (Giusti & Wrolstad, 2001), using cyanidin-3-glucoside as reference, with a molar extinction coefficient of 26900 M⁻¹cm⁻¹ and a molecular mass of 449.2 g.gmol⁻¹. The obtained extracts were diluted in buffers, at pH 1.0 and pH 4.5, separately, and after 30 minutes of stabilization, absorbances were determined at 510 and 700 nm.

Total phenolic compounds

This determination was performed by spectrophotometry using the Folin-Ciocalteu reagent (Merck®, Germany) according to the method described by Singleton & Rossi (1965). The sample extracts were obtained by extraction with acetone 70%, followed by filtration. These extracts were diluted 10 times with distilled water. The absorbance was measured at 760 nm. A calibration curve was made from gallic acid standard (Sigma-Aldrich®, Brazil) and the blank was prepared with distilled water. Total phenolic compounds content was expressed in mg GAE.100 g⁻¹(gallic acid equivalent).

Antioxidant capacity

The antioxidant capacity was determined by the reduction method of the ABTS⁺ radical (Sigma-Aldrich®, Brazil) according to Re et al. (1999). Extraction was conducted in two steps with methanol 50% and acetone 70% (Rufino et al., 2007) For the reaction, 30 µL of sample extract was added to 3 mL of ABTS⁺. Absorbance was then measured at 734 nm after 6 minutes of reaction. Results were expressed as micromoles Trolox equivalents per gram (µmol TE.g⁻¹).

Physicochemical characteristics

The physicochemical characteristics were carried out following the methodologies proposed by the Association of Official Analytical Chemists (2006). An adequately calibrated automatic titrator (785 DMP Titrimo, Metrohm) was used for the pH and titratable acidity measurements. Soluble solids determinations were performed using a portable digital refractometer (Pal-3, Atago).

Instrumental color

The instrumental color analysis was performed in a colorimeter (ColorQuest XE, Hunterlab) using the coordinate CIELab/CIELCH system of L^* , a^* , b^* , chroma (C^*) and Hue angle (H°) (Ferreira, 1981). In addition, total color difference (ΔE) was considered for evaluation of color changes during storage. This parameter was quantified by Equation 1.

$$\Delta E = [(a^* - a^*_0) + (b^* - b^*_0) + (C^* - C^*_0)]^{1/2} \quad (1)$$

Microbiological analysis

Detection of *Salmonella* spp. and thermotolerant coliforms, aerobic mesophilic bacteria, lactic acid bacteria and mold and yeast counts were carried out according to the methodologies recommended by the American Public Health Association (2001).

Sensory analysis

The acceptability of smoothie during storage was evaluated only for the samples stored under refrigeration as it was the condition that best preserved the product quality. The test was performed with 74 non-trained panelists, adults, from both genders, chosen among trainees and employees from Embrapa in Rio de Janeiro, Brazil. All panelists were potential consumers of fruit beverages.

For each smoothie sample, panelists evaluated the global acceptance, which is related to the first impression of the whole

product (appearance, flavor, color, taste) and consistency. Both attributes were measured by a structured 9 point hedonic scale (9 = extremely liked, 5 = did not like or dislike, 1 = extremely dislike) (Meilgaard et al., 1991).

Codified (three digits) samples were served monadically in 50 mL plastic cups, at refrigeration temperature (8-10 °C), in individual booths, under white light. This part of the project was approved by the Ethical Committee for Research with Human Beings/RJ linked to Hospital Universitário Clementino Fraga Filho/HUCFF/UFRJ (n° 17796813.0.00005257).

2.4 Statistical analysis

The data were statistically analyzed using Statistica software version 12 (Statsoft Inc., Tulsa, OK, USA), by means of variance analysis (ANOVA) and Fisher LSD test, for verifying difference among averages, in a 95% confidence interval. Experiments were performed in triplicate and values presented are the average ones in wet basis.

3 Results and discussion

3.1 Bioactive compounds

The results for the bioactive compounds and antioxidant capacity of the smoothie during storage, at the two temperatures evaluated, are shown in Figure 1. Data analysis showed that there was a significant difference in the anthocyanins and phenolic compounds content in relation to the initial time at

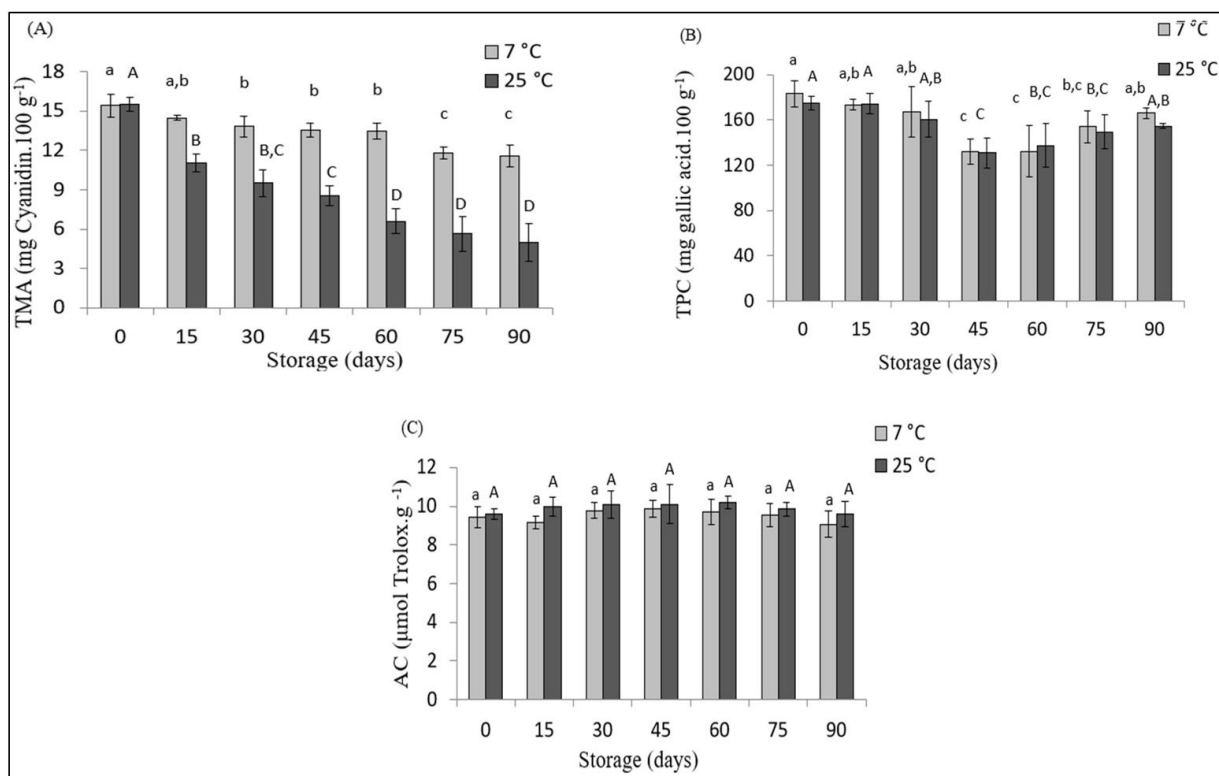


Figure 1. Total monomeric anthocyanins (TMA) (A), total phenolic compounds (TPC) (B) and antioxidant capacity (AC) (C) of juçara, banana and strawberry smoothie during storage at 7 °C and 25 °C. Lowercase letters indicate significant difference at 95% for the smoothie stored at 7 °C. Uppercase letters indicate significant difference at 95% for the smoothie stored at 25 °C.

both temperatures. At refrigeration temperature (7 °C) there were losses of 25% for anthocyanins and 9% for phenolic compounds at 90 days of storage. The smoothie stored at room temperature (25 °C) presented higher losses, being 66% for anthocyanins and 11% for phenolic compounds. Degradation of the phenolic compounds is probably due to oxidation and it was higher, as expected, at the highest temperature, which catalyzes the degradation of these compounds as reported by Laorko et al. (2013). These authors evaluated the degradation of phenolic compounds of clarified pineapple juice stored for six months at 4 °C, 27 °C and 37 °C, observing higher phenolic compounds content when product was stored at 4 °C.

The antioxidant capacity of the smoothie did not differ significantly during storage at both temperatures (Figure 1). The antioxidant potential of the smoothie is mainly due to the presence of different phenolic compounds, primarily represented by anthocyanins and ellagic acid, which are present in high amounts in juçara and strawberry, respectively (Karacam et al., 2015; Giampieri et al., 2012; Schulz et al., 2016). Thus, the degradation of these compounds can reduce the antioxidant capacity of the product. As antioxidant capacity did not vary, it is suggested that the thermal degradation of anthocyanins may lead to the formation of other phenolic compounds of equal or even higher antioxidant potential (Patras et al., 2010).

Inada et al. (2018) verified the increase in total phenolic compounds and antioxidant capacity of jaboticaba juice obtained

by steam extraction and stored at room temperature for 90 days, in spite of the degradation of anthocyanins such as cyanidin and delphinidin. Since the authors determined the phenolic compounds profile of the product during storage, they suggested that the depolymerization of ellagitannins to ellagic acid and the cleavage of anthocyanins, which degradation product is gallic acid, were the responsible phenomena for this behavior, since the concentration of ellagic and gallic acids increased significantly throughout the study. Due to the similarity of the phenolic composition, their findings support the results obtained in the present work.

Regarding the physicochemical characteristics of the smoothie (Figure 2), few changes were observed during storage under refrigeration and room temperature. The pH of the smoothie at both temperatures showed a slight reduction after 75 days of storage. The soluble solids content and the acidity of the product stored at 7 °C did not vary significantly. The soluble solids content of the product stored at 25 °C presented a slight decrease in 15 days but remained constant until 90 days. The acidity also presented a small variation over the same period. However, due to the low analytical error of these physicochemical determinations, small variations in absolute values become statistically significant, which, in practical terms, will not cause relevant physicochemical changes at industrial level. Slight or no variations in physicochemical parameters of stored fruit drinks were also observed by Inada et al. (2018) and

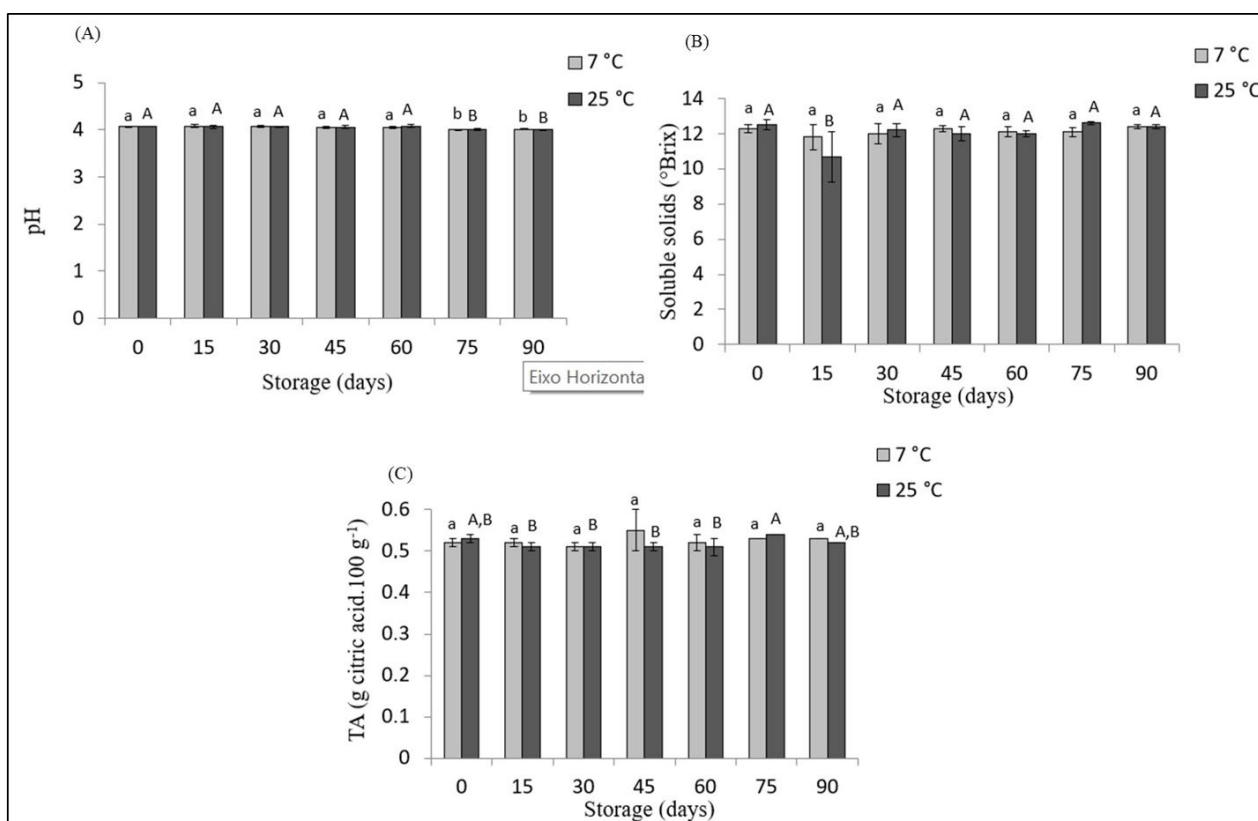


Figure 2. pH (A), soluble solids (B) and titrable acidity (TA) (C) of juçara, banana and strawberry smoothie during storage at 7 °C and 25 °C. Lowercase letters indicate significant difference at 95% for the smoothie stored at 7 °C. Uppercase letters indicate significant difference at 95% for the smoothie stored at 25 °C.

Tomadoni et al. (2017), during the storage of jaboticaba juice obtained by steam-extraction and strawberry juice pasteurized and sonicated, respectively.

3.2 Instrumental color

Regarding instrumental color, it was verified that the luminosity did not change at both temperatures. The other color parameters were significantly affected by storage time and temperature, and the more drastic changes were observed in the smoothie stored at room temperature (Table 1).

The parameter a^* , which represents the intensity of red in the sample, varied mainly in the product stored at 25 °C, corroborating with the degradation of the anthocyanins observed in the previously discussed results.

Comparing the changes observed in the values of b^* and Hue angle (H°), which correspond to the intensity of yellow and the color of the product that is visualized by naked eyes, higher stability of instrumental color was observed when the smoothie was stored under refrigeration. In the smoothie stored at room temperature the parameters b^* and H° varied drastically, with an increase of 11° in the Hue angle. These changes are due to

the chemical reactions that can occur between the product own components, catalyzed by temperature during the exposure time. Anthocyanin-rich products may undergo polymerization and/or cleavage during processing and storage, as reported in the scientific literature (Weber & Larsen, 2017; Silva et al., 2017). These reactions cause undesirable changes in the color of the product, which can, in many cases, decrease its sensory acceptance.

According to Choi et al. (2002), total color difference (ΔE) values higher than 2 indicate visible color changes in products relating to a standard. Thus, only the smoothie stored under refrigeration presented no visible changes in relation to the initial sample color (zero day storage time) up to 90 days. Regarding the product stored at room temperature, visible color changes to the consumer were observed after 75 days of storage (Table 1).

Microbiological quality

In Table 2 it is showed the results of microbiological analysis of the smoothie stored both under refrigeration and at room temperature. The absence of *Salmonella* spp. in 25 g and < 3 MPN/g counting for thermotolerant coliforms at 0 and 90 days were

Table 1. Instrumental color parameters of juçara, banana and strawberry smoothie during storage at 7 and 25 °C.

Parameter	Storage temperature	Storage (days)						
		0	15	30	45	60	75	90
L^*	7 °C	32.5 ± 1.1 ^a	33.2 ± 1.2 ^a	33.6 ± 0.6 ^a	32.1 ± 2.0 ^a	31.9 ± 2.5 ^a	32.0 ± 2.6 ^a	32.1 ± 2.6 ^a
	25 °C	32.5 ± 1.1 ^a	32.6 ± 2.1 ^a	32.9 ± 2.1 ^a	32.9 ± 1.9 ^a	32.6 ± 2.2 ^a	32.7 ± 2.2 ^a	33.3 ± 1.8 ^a
a^*	7 °C	10.4 ± 0.1 ^a	10.2 ± 0.2 ^a	9.9 ± 0.3 ^a	10.9 ± 0.6 ^a	10.9 ± 0.5 ^a	10.9 ± 0.5 ^a	10.8 ± 0.5 ^a
	25 °C	10.4 ± 0.1 ^{ab}	10.7 ± 0.3 ^a	10.3 ± 0.6 ^{ab}	9.9 ± 0.5 ^{ab}	9.5 ± 0.5 ^{ab}	9.4 ± 0.2 ^b	9.2 ± 0.4 ^b
b^*	7 °C	1.6 ± 0.1 ^a	1.5 ± 0.2 ^{ab}	1.1 ± 0.0 ^b	1.3 ± 0.2 ^{ab}	1.4 ± 0.2 ^{ab}	1.6 ± 0.2 ^{ab}	1.5 ± 0.2 ^{ab}
	25 °C	1.6 ± 0.1 ^d	1.9 ± 0.3 ^{cd}	2.3 ± 0.3 ^{bcd}	2.6 ± 0.5 ^{ab,cd}	2.9 ± 0.4 ^{ab,c}	3.2 ± 0.4 ^{ab}	3.4 ± 0.3 ^a
C^*	7 °C	10.5 ± 0.1 ^a	10.3 ± 0.2 ^a	9.9 ± 0.3 ^a	11.0 ± 0.6 ^a	11.0 ± 0.5 ^a	11.0 ± 0.5 ^a	11.0 ± 0.5 ^a
	25 °C	10.5 ± 0.1 ^a	10.9 ± 0.4 ^a	10.5 ± 0.7 ^a	10.2 ± 0.6 ^a	10.0 ± 0.6 ^a	9.9 ± 0.4 ^a	9.8 ± 0.5 ^a
H°	7 °C	9.0 ± 0.8 ^a	8.3 ± 1.4 ^{ab}	6.3 ± 0.3 ^b	7.0 ± 0.6 ^{ab}	7.1 ± 0.9 ^{ab}	8.1 ± 0.7 ^{ab}	8.0 ± 0.7 ^{ab}
	25 °C	9.0 ± 0.8 ^d	10.3 ± 1.2 ^{cd}	12.4 ± 1.1 ^{cd}	14.4 ± 2.2 ^{bc}	16.7 ± 1.8 ^{ab}	18.8 ± 1.7 ^a	20.0 ± 1.1 ^a
ΔE	7 °C	-	0.7	1.3	1.2	1.5	1.6	1.6
	25 °C	-	1.2	1.4	1.5	1.9	2.2	2.3

Results expressed as mean ± standard deviation. L^* - luminosity. a^* - red intensity. b^* - yellow intensity. C^* - Chroma. H° - Hue angle. ΔE - total color difference. Averages on the same line followed by equal letters did not differ statistically from each other ($p < 0.05$).

Table 2. Microbiological quality of juçara, banana and strawberry smoothie during storage at 7 °C and 25 °C.

Parameter	Storage temperature	Storage (days)						
		0	15	30	45	60	75	90
Thermotolerant coliforms ¹	7 °C	<3	np	np	np	np	np	<3
	25 °C	<3	np	np	np	np	np	<3
CMAB (CFU.g ⁻¹)	7 °C	1.4 × 10 ³	2.8 × 10 ³	3.9 × 10 ³	4.8 × 10 ³	3.0 × 10 ³	5.6 × 10 ³	3.0 × 10 ³
	25 °C	6.4 × 10 ³	3.9 × 10 ³	1.7 × 10 ³	1.4 × 10 ³	1.3 × 10 ³	1.7 × 10 ³	1.1 × 10 ³
CLAB (CFU.g ⁻¹)	7 °C	<1.0 × 10 ¹	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	9.8 × 10 ²
	25 °C	<1.0 × 10 ¹	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	9.2 × 10 ²
MY (CFU.g ⁻¹)	7 °C	<1.0 × 10 ¹	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²
	25 °C	<1.0 × 10 ¹	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²	<1.0 × 10 ²
<i>Salmonella</i> spp. (in 25 g)	7 °C	absence	np	np	np	np	np	absence
	25 °C	absence	np	np	np	np	np	absence

¹Results expressed in MPN.g⁻¹. np - not performed. CMAB - Count of mesophilic aerobic bacteria. CLAB - Count of lactic acid bacteria. MY - Mold and yeasts.

Table 3. Scores of the sensory acceptability of the juçara, banana and strawberry smoothie stored for 90 days at 7 °C.

Storage (days)	Overall acceptance (scores)
0	6.5 ^b
29	7.0 ^a
60	7.0 ^a
75	6.8 ^{a,b}
90	6.9 ^{a,b}

Averages on the same column followed by equal letters did not differ statistically from each other ($p < 0.05$).

verified at both temperatures, proving the adoption of good manufacturing practices in the elaboration and storage of the product. The results are also in agreement with the Brazilian legislation, as described in RDC n° 12 of the National Health Surveillance Agency (Brasil, 2001).

Count of mold and yeast for both temperatures remained below 10^2 CFU.g⁻¹ during the storage period. According to the Ministry of Agriculture, Livestock and Food Supply, frozen fruit pulps can present a counting of mold and yeast up to 10^3 CFU.g⁻¹ (Brasil, 2002). Thus, the smoothie formulated attends the standards established, being considered safe for consumption up to 90 days, when processed and stored under the conditions of this study.

According to Franco & Landgraf (2003) the presence of lactic acid and mesophilic aerobic bacteria in high concentrations can affect the quality of the food, promoting changes in physicochemical parameters such as pH, acidity and soluble solids, for example. Since these changes were not observed, as already discussed (Figure 2), the maximum growth of 10^3 CFU.g⁻¹ observed in both groups did not affect the product quality. The count of aerobic mesophilic bacteria is used to indicate the sanitary quality of the food. Values higher than 10^6 CFU.g⁻¹ suggest the use of contaminated raw material or unsatisfactory processing (Franco & Landgraf, 2003).

3.3 Sensory evaluation

The scores of sensory acceptability are shown in Table 3, in which it is observed that the overall acceptance of the juçara, banana and strawberry smoothie was not affected during its storage for 90 days at 7 °C. It should be noted that all samples were accepted, once the scores ranged of 6 to 7, which represents the concept between “neither liked nor disliked and liked moderately”.

The overall acceptance refers to the first impression caused to the consumer by the product as a whole (appearance, aroma, color and flavor). In this sense, the storage under refrigeration preserved the sensory quality of the smoothie, which is related to the data previously shown, such as the physicochemical parameters and the retention of anthocyanins and instrumental color.

4 Conclusions

The results indicated that the developed juçara, banana and strawberry smoothie, preserved by pasteurization, under the conditions proposed in this work, can be stored for up to 90 days under refrigeration at 7 °C, ensuring its microbiological stability.

At these conditions, the product presented good stability of anthocyanins, physicochemical characteristics and instrumental color, preserving its antioxidant capacity and sensory acceptance.

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