

## Stability of postpackage pasteurized camu-camu pulp

### ABSTRACT

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Camu-camu (*Myrciaria dubia*) is a wild Amazon native fruit, with great nutritional potential due to high ascorbic acid content. As transport reduces its shelf life and fresh consumption is limited because of high acidity, posing obstacles to commercialization, pulping and pasteurization can enhance its shelf life. This study aimed to verify camu-camu pulp stability after postpackage pasteurization, using laminated packaging, assessing physical and chemical alterations caused by refrigerated (5 °C), frozen (-18 °C), and room temperature (28 °C) storage for 45 days. Samples were pasteurized in tanks at 75 °C for 1 min and rapidly cooled to room temperature. Frozen pulp presented the best physical and chemical characteristics at the end of 45 days storage (about anthocyanins: 0.8 mg equivalent to cyanidine 3-glucoside 100 g<sup>-1</sup> pulp; 2.2 g ascorbic acid 100 g<sup>-1</sup> pulp and 7% of phenolic compounds), but refrigerated stored samples also showed promising results at 45 days, maintaining the initial characteristics throughout the study. Even the pulps at room-temperature storage are cheaper option to preserve the product, they were degraded at 30 days of storage.

**KEYWORDS:** *Myrciaria dubia*; amazon; vitamin C; heat treatment; anthocyanins.

## INTRODUCTION

Camu-camu (*Myrciaria dubia*), a wild fruit native to the Amazon, belongs to the family Myrtaceae and is found on seasonally flooded banks of Amazonian rivers and lakes. The importance of this fruit lies both on its high nutritional value, since it is a rich source of ascorbic acid (MAEDA *et al.*, 2007), it is a fruit with great economic and nutritional potential as high content of anthocyanins, evidenced by its intense dark purplish-red color. The anthocyanins are concentrated in the pericarp (skin) and transferred to the pulp only during processing, generating a product with attractive color. The interest on anthocyanins and ascorbic acid as functional food is one of the factors stimulating the development of camu-camu products (MAYODA *et al.*, 2010).

The greatest disadvantage of commercializing fresh camu-camu lies in its short shelf life, mainly because of damage to tissues and cells during handling and/or transport, leading to weight loss caused by desiccation, increased respiration rate, and metabolic activity, so that enzymatic activity is enhanced, degrading the fruits, which are then rejected by consumers (ARÉVALO & KIECKBUSCH, 2006).

Furthermore, high acidity and low pH limit camu-camu fresh consumption, although these very characteristics are desirable for pulp and juice processing (Maeda *et al.*, 2007). Consequently, one way to stimulate camu-camu consumption is aggregating value to the product, which can be achieved by pulping it and using this basic product to prepare juice, jam, and sweets in general.

Consumption of tropical fruits is growing in domestic market and internationally, due to the recognition of its therapeutic and nutritional values, since many are rich in bioactive compounds, as camu-camu recognition, it has interest of consumers, farmers and industries, in addition to attracting importers from Japan, Europe and the USA (MYODA *et al.* 2010; RUFINO *et al.*, 2010).

Fruit pulp and juice pasteurization aims to inactivate enzymes and destroy potentially harmful microorganisms. This heat process consists in expose the product to high temperature for some seconds or to a milder temperature for a long time, also heat processing applied is generally carried out in temperatures below 100°C, since microorganisms found in this product present low thermal resistance and are also inhibited by pulp natural acidity (LIMA *et al.*, 2010).

Food preservation methods involve physical and/or chemical treatments to maintain or increase shelf life. The usage of rigid, semi-rigid, or flexible containers is also highly important, because they work as a barrier between the external environment and the food without affecting the latter (SOARES *et al.*, 2005).

Fruit pulp had marketed in flexible containers (polyethylene bags) or in aseptic carton packaging due to their protection against oxidation as well as transport and handling practicality. Not only do containers avoid alterations in sensory characteristics of the product, but they should also meet the needs of marketing and present good cost-benefit ratio and availability (BRUNINI *et al.*, 2002).

This study aimed to verify the stability of postpackage pasteurized camu-camu pulp, using laminated packaging, assessing physical and chemical alterations caused by refrigerated (5°C), frozen (-18°C), and room temperature (28°C) storage for 45 days.

## MATERIALS AND METHODS

The ripe fruits of camu-camu were obtained from Vale do Ribeira region, county of Sete Barras, São Paulo (latitude 24°23'16 " South and longitude 47°55'32" West). The fruits were immerse in chlorinated solution (sodium dichloro-s-triazinetrione dihydrate – 200 ppm) for 15 min, and washed in tap water. Fruit pulping was performed using a stainless steel pulper with blades, model Bonina Compacta (NPC Metalúrgica Indústria e Comércio Ltda., Itabuna, BA, Brazil), and the pulp was manually packed into 100-mL high barrier polypropylene bags that were immediately sealed and immersed in a stainless steel tank made to order for pasteurization (75°C/1 min.).

The laminated packaging used in our study had: PET 15  $\mu$  – ethylene polytereftalate (polyester); OPA 15  $\mu$  – bi-oriented polyamide (nylon) and CPP 70  $\mu$  – (or PP Cast) co-extruded cast polypropylene. Therefore, they are resistant to temperatures up to 121 °C for 45 min, at pH 3.5, and under vacuum conditions.

The process of postpackaging pasteurization was performed through the immersion of bags in stainless steel tank (Mecamau "São José" Ltda., Espírito Santo do Pinhal, São Paulo, Brazil) with hot water, and maintained for 1 minute after the internal temperature the bag have reached 75 °C. The cooling of the product was carry out in basins with ice water until the internal temperature reaches values below 30 °C. Then, the product was stored at 28 °C, 5 °C and -18 °C (treatments).

Temperature control of pasteurization was carry out with assistance of equipment Thermocouple (Novus brand - My PC lab, T type, Class 1) plus four sensors connected via USB cable to a computer and, through the program of the equipment, the internal temperature of bags and water was recorded every 30 seconds. Two sensors were place in bags containing the product and the other two in the water during the pasteurization and cooling.

Pasteurized pulp samples stored at three different temperatures were assess at days 1, 15, 30, and 45 of storage, in triplicate samples (three bags for each treatment), using the following analyses:

a. Instrumental color: the luminosity values ( $L^*$ ), hue angle (color tone in degrees) and chromaticity (color saturation) were measured using the colorimeter CR-400 Chroma Meter 8 mm in diameter and standard illuminant CIE C, brand Konica Minolta Sensing (Tokyo, Japan). The instrument was calibrated on white surface according to the International Commission on Illumination (CIE 1976  $L^*$ ,  $a^*$ ,  $b^*$  - CIELAB) using the CIE standard illuminant C (KONICA MINOLTA, 1998).

b. pH: determined using potentiometer MA-522 (Marconi, Piracicaba, SP, Brazil) according to method 981.12, described in AOAC (2005).

c. Titratable acidity (g citric acid 100  $g^{-1}$  pulp): determined by titration based on the volume (mL) of 0.1 M NaOH and potentiometer according to method 942.15, described in AOAC (2005).

d. Soluble solid content (°Brix): quantified using a refractometer Reichert-Jung Auto Abbe, model 10500/10501 (Leica Microsystems Inc., Buffalo, NY, USA) according to method 932.12, described in AOAC (2005).

e. Ascorbic acid content (g ascorbic acid 100  $g^{-1}$  pulp): determined according to method 967.21 – 45.1.14, described in AOAC (2005).

f. Total anthocyanins (mg equivalent to cyanidine 3-glucoside 100 g<sup>-1</sup> pulp): determined through the pH difference technique according to the method n° 2005.02 of AOAC (2005).

g. Total phenolic compounds (mg of gallic acid equivalent 100 g<sup>-1</sup> pulp): determined spectrophotometrically using the Folin-Ciocalteu method, described by Singleton e Rossi (1965).

The experimental design was complete randomized, with three triplicates, being the pulp analyzed in four periods, 1, 15, 30 and 45 days. The pulps were storage in three temperatures (-18 °C, 5 °C and 28 °C), the factorial scheme was 1 x 4 x 3. The results of physicochemical analyzes of camu-camu pulp were evaluated by the F-test for analyses of variance (ANOVA) and mean comparison by the Tukey's test (5%) using the Statistical Analysis System software (SAS, 1996). All the storage periods and treatments were taken into consideration in the statistical analyses.

## RESULTS AND DISCUSSION

### INSTRUMENTAL COLOR

The color of the product stored under room temperature was no significant difference at 30 and 45 days of storage. Frozen pulp maintained stable value L\* after 15 days at storage. Refrigerated pulp became lighter in color during storage. At day 1 of storage, both the samples under room temperature and refrigerated stored presented similar value L\* and were darker than the pulp under refrigerated storage. At days 15 and 30, the samples stored under room temperature were lighter than the others. At day 45 of storage, the results were similar to those obtained at day 1 of storage (Table 1).

However, there are no studies involving camu-camu pulp, only about camu-camu in post-harvest conditions, so our study compared with other pulp fruits in the same conditions. Fontes (2002) analyzed mango pulp and observed a decrease in value L\* as a response of heat processing. The author attributed this fact to the presence of reactions sensitive to high temperature associated to the degradation of thermolabile pigments, resulting in the formation of dark compounds that can reduce value L\*. A similar result was find by Della Modesta *et al.* (2002) when evaluating pineapple juice pasteurized at 95 °C for 30 s and packed in laminated carton packaging. The authors observed darkening, loss of green color, as well as increase in yellow color and turbidity during storage at 32°C for 90 days.

In this study, chromaticity values for camu-camu pasteurized pulp ranged from 8.74 to 28.67 and decreased in all treatments from day 15 of storage on, indicating alteration of the peculiar color of this fruit pulp, which became more opaque. Frozen pulp had the color stabilized from day 15 of storage on, since freezing helped maintain the pigments, turning the color more vivid. Refrigerated pulp presented a decrease in color intensity during the periods under study and the same occurred to the samples stored under room temperature (Table 1).

The color of frozen and refrigerated camu-camu pulp samples was pinkish at day 1 of storage and, after that, changed to orange (Table 1). The color of the pulp stored under room temperature became orange at day 15 of storage and remained stable until the end of the experiment. This may have happened due to the high

temperature of storage, which degraded the antocianic pigments responsible for the attractive pink color of this product.

**Table 1.** Value L\*, Chromaticity and Hue angle detected in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values,  $\pm$  SD, n=6).

Value L*				
Treatment	Period of storage (days)			
	1	15	30	45
28 °C	37.30 $\pm$ 0.68Cb	41.83 $\pm$ 0.17 Aa	40.27 $\pm$ 0.96 Ba	39.47 $\pm$ 1.00 Bb
5 °C	37.58 $\pm$ 0.96Cb	39.25 $\pm$ 0.78 Bb	39.25 $\pm$ 0.47 Bab	41.34 $\pm$ 0.42 Aa
-18 °C	39.07 $\pm$ 0.50ABa	38.53 $\pm$ 0.37 Bb	37.95 $\pm$ 0.70 Bb	39.87 $\pm$ 0.59 Ab
Chromaticity				
Treatment	Period of storage (days)			
	1	15	30	45
28 °C	28.67 $\pm$ 0.67Aa	11.29 $\pm$ 0.23Cb	13.12 $\pm$ 0.38BCb	14.09 $\pm$ 0.31Ba
5 °C	26.29 $\pm$ 1.08Ab	15.10 $\pm$ 1.42Ba	12.11 $\pm$ 1.93Cb	8.74 $\pm$ 0.60Db
-18 °C	26.20 $\pm$ 0.93Ac	14.81 $\pm$ 0.68Ba	15.21 $\pm$ 1.06Ba	15.16 $\pm$ 0.70Ba
Hue angle				
Treatment	Period of storage (days)			
	1	15	30	45
28 °C	16.88 $\pm$ 2.61Da	94.16 $\pm$ 1.73Aa	86.93 $\pm$ 1.04Ba	81.23 $\pm$ 1.23Ca
5 °C	17.53 $\pm$ 2.43Da	28.96 $\pm$ 0.79Cb	34.15 $\pm$ 1.34Bb	45.74 $\pm$ 3.89Ab
-18 °C	17.83 $\pm$ 2.47Ca	28.02 $\pm$ 0.50Bb	39.65 $\pm$ 0.99ABc	33.84 $\pm$ 1.03Ac

SD = Standard deviation of the mean, n = number of replicates used. Means followed by the same capital letter within the same line and by the same lower case letter within the same column are not significantly different at the 5% level by the Tukey's test.

### pH, TITRATABLE ACIDITY AND SOLUBLE SOLID CONTENT

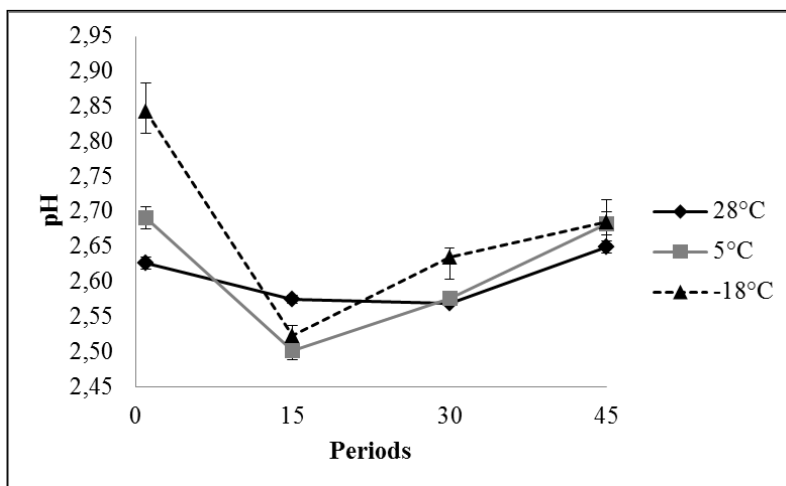
After first day of evaluation, camu-camu pasteurized samples presented no statistically significant differences in pH among treatments and periods of storage at the 5% significance level (Figure 1). Maeda *et al.* (2007) analyzed camu-camu pulp and reported pH 2.64, similar to the value we found in this study.

Titratable acidity of pasteurized camu-camu pulp remained stable in all treatments and periods of storage (Figure 2).

The values found for titratable acidity in our study characterize camu-camu as a fruit with sour taste. Similar results were reported by Bueno *et al.* (2002) when analyzing acerola (1.40 g 100 mL<sup>-1</sup>) and cupuaçu (1,90 g 100 mL<sup>-1</sup>) pulp. At 30 days, acidity increased for refrigerated pulp (5 °C), possibly by an amostral variation, because microbial growth in one of the samples.

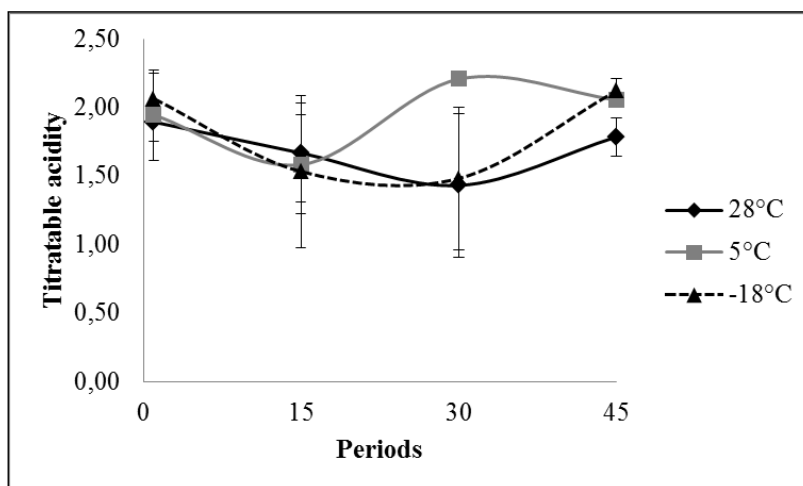
No statistically significant differences were observe for soluble solids among treatments and periods of storage at the 5% significance level, indicating that this parameter did not present variation during storage and was not influenced by the treatments (Figure 3). This may have occurred because the package protected the product against humidity and gas exchange with the surroundings.

Maeda *et al.* (2007) found 6.20 °Brix in camu-camu pulp, so the study carried out a similar value in our study.



**Figure 1.** pH, values measured in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values, n=6).

\* Vertical bars indicate the standard deviation of the mean, n = number of replicates used.



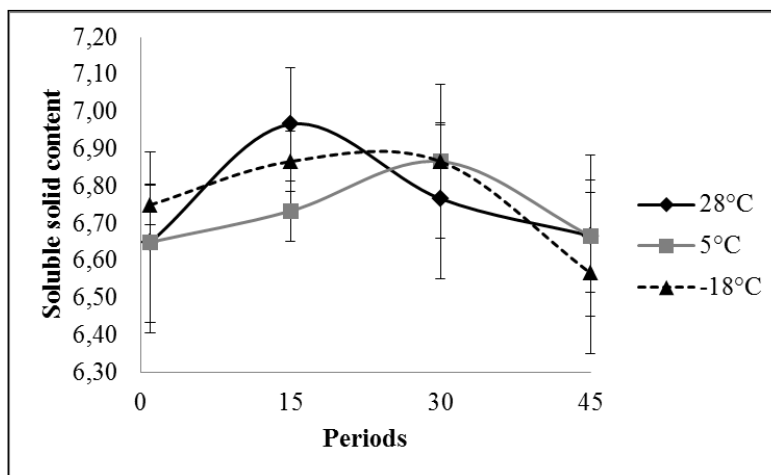
**Figure 2.** Titratable acidity (g citric acid 100 g<sup>-1</sup> pulp), values measured in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values, n=6).

\* Vertical bars indicate the standard deviation of the mean, n = number of replicates used.

### ASCORBIC ACID CONTENT

At days 1, 30, and 45 of storage, no statistically significant differences were detected in ascorbic acid content among treatments at the 5% significance level, but the values had reduction in all treatment applied. At day 15 of storage, refrigerated pulp presented the lowest ascorbic acid content, statistically differing from the other samples tested. Camu-camu pulp samples stored under room temperature and in the freezer presented no statistically significant differences at days 30 and 45 of storage, indicating stabilization of ascorbic acid content. On the

other hand, at day 1 of storage, refrigerated pulp presented higher ascorbic acid content, statistically differing from the other treatments and periods (Table 2).



**Figure 3.** Soluble solid content (°Brix), values measured in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values, n=6).

\* Vertical bars indicate the standard deviation of the mean, n = number of replicates used.

**Table 2.** Ascorbic acid content (g ascorbic acid 100 g<sup>-1</sup> pulp) determined in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values, ± SD, n=6).

Treatment	Period of storage (days)			
	1	15	30	45
28 °C	3.20 ± 0.001Aa	3.04 ± 0.08Aa	1.92 ± 0.05Ba	1.96 ± 0.08Ba
5 °C	3.18 ± 0.02Ba	2.23 ± 0.07Ab	2.12 ± 0.09Aa	2.32 ± 0.25Aa
-18 °C	3.11 ± 0.02Aa	2.86 ± 0.03Aa	1.93 ± 0.05Ba	2.20 ± 0.01Ba

SD = Standard deviation of the mean, n = number of replicates used. Means followed by the same capital letter within the same line and by the same lower case letter within the same column are not significantly different at the 5% level by the Tukey's test.

Franco (1998) showed that ascorbic acid content of processed fruits may be reduced due to degradation caused by heat, oxidation, desiccation, storage, low temperature, and alkalinity, but for this work, the parameters that influenced the reduce were heat, oxidation, storage and low temperature.

Yamashita *et al.* (2003) studied stability of vitamin C in acerola (*Malpighia sp.*) products stored for 4 months and observed that this parameter depends both on the type of processing and on the storage temperature. Brunini *et al.* (2002) assessed alterations in frozen mango pulp and reported that vitamin C content decreased during storage, similarly to the results of the present study.

### TOTAL ANTHOCYANINS

At days 1 and 15 of storage, no statistically significant differences in total anthocyanins among treatments were recorded. Nevertheless, after day 15 of

storage, total anthocyanin values decreased approximately by 60% in refrigerated pulp and by 40% in frozen pulp and remained constant at the other periods of storage. At day 30 of storage, the sample stored at room temperature presented the lowest detectable total anthocyanin content, which dropped drastically after that and could not be detected at day 45 of storage (Table 3).

**Table 3.** Total anthocyanins (mg equivalent to cyanidine 3-glucoside 100 g<sup>-1</sup> pulp) detected in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values,  $\pm$  SD, n=6).

Treatment	Period of storage (days)			
	1	15	30	45
28 °C	1.51 $\pm$ 0.45Aa	0.42 $\pm$ 0.03Ba	0.01 $\pm$ 0.01Cb	Not detected
5 °C	1.72 $\pm$ 0.36Aa	0.70 $\pm$ 0.11Ba	0.49 $\pm$ 0.09Ba	0.29 $\pm$ 0.10Bb
-18 °C	1.47 $\pm$ 0.51Aa	0.62 $\pm$ 0.15Ba	0.65 $\pm$ 0.16Ba	0.80 $\pm$ 0.19Ba

SD = Standard deviation of the mean, n = number of replicates used. Means followed by the same capital letter within the same line and by the same lower case letter within the same column are not significantly different at the 5% level by the Tukey's test.

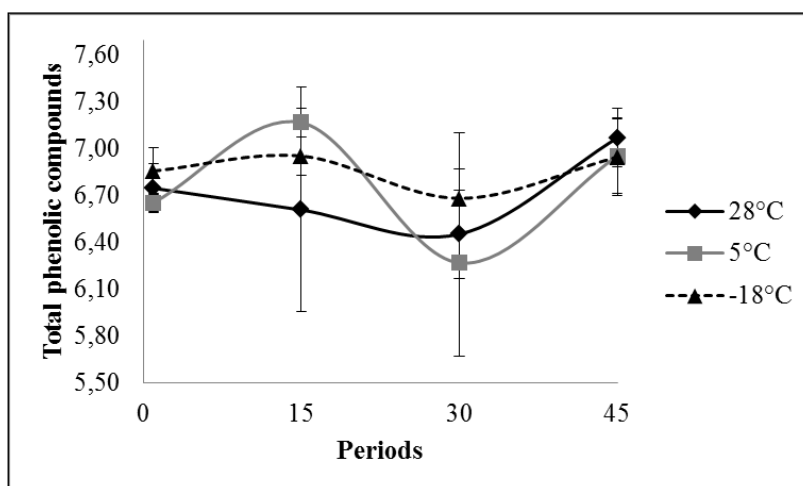
According to Bobbio e Bobbio (1992), anthocyanins interact with ascorbic acid, metals, sugars, oxygen, light, temperature, and enzymes to produce polymeric degradation products that decrease their stability. Jurd (1972) reported a condensation reaction between ascorbic acid and anthocyanins and affirmed that the higher the vitamin C content in the system, the higher is the degradation rate of the antocianic pigments. Camu-camu fruits have high ascorbic acid content, which intensifies anthocyanin degradation. Kirca *et al.* (2006) detected a great effect of storage temperature on anthocyanin stability in all the fruit juices and nectars tested in their study, and observed that degradation was much faster during storage at higher temperatures.

### TOTAL PHENOLIC COMPOUNDS

No statistically significant differences were observed for total phenolic compounds among treatments and periods of storage at the 5% significance level (Figure 4). Storage temperature did not affect phenolic compound content of the samples. The heat processing together with the storage temperature did not alter this parameter, which is very interesting. Although camu-camu fruit is known to present high total phenolic compound content, Silva & Andrade (1997) found values ranging from 1.37% to 2.11%, much lower than our result, since we registered an average of 7% in this study.

Similar results were obtained by Verbeyst *et al.* (2012) that evaluated the influence of temperature (50-140°C/20 minutes) on the concentration of bioactive compounds in strawberries and raspberries purees. In these products, treatments showed little effect on the total phenolic content; only the data for heat treatment suggested a slight increase of phenolic compounds with increasing temperature, but is not significant ( $p < 0.05$ ).





**Figure 4.** Total phenolic compounds (mg of gallic acid equivalent 100 g<sup>-1</sup> pulp) found in pasteurized camu-camu pulp samples stored at three different temperatures for four different periods (mean values,  $\pm$  SD, n=6).

\* Vertical bars indicate the standard deviation of the mean, n = number of replicates used.

## CONCLUSIONS

Even pasteurized camu-camu pulp stored at room temperature is a cheaper technology and it does not require cold chain, the results obtained in this study are good just until 30 days of storage, after that the pulps were degraded.

The frozen pulp had the best results, this is an expensive process that can be replaced by refrigerated storage, since this one presented promising results in our study, maintaining camu-camu pulp physical and chemical characteristics. Furthermore, refrigerated storage is cheaper and sustainable because it requires less energy than freezing.

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## Estabilidade da polpa de camu-camu pasteurizada pós-embalagem

### RESUMO

O camu-camu (*Myrciaria dubia*) é uma fruta nativa da Amazônia e que possui grande potencial nutricional devido ao seu alto teor de ácido ascórbico. O transporte reduz a vida útil do fruto e o consumo fresco é limitado devido à alta acidez, o que coloca obstáculos à comercialização, sendo assim, o despulpamento e a pasteurização são importantes ferramentas para aumentar sua vida útil e mercado. Este estudo teve como objetivo verificar a estabilidade da polpa de camu-camu após a pasteurização pós-embalagem, foram utilizadas embalagens laminadas, avaliando alterações físicas e químicas causadas pelo armazenamento refrigerado (5 °C), congelado (-18 °C) e temperatura ambiente (28 °C) durante 45 dias. As amostras foram pasteurizadas em tanques a 75 °C por 1 min e rapidamente resfriadas à temperatura ambiente. A polpa congelada apresentou as melhores características físicas e químicas ao final de 45 dias de armazenamento (antocianinas: 0,8 mg equivalente a cianidina 3-glucosídeo 100 g<sup>-1</sup> polpa; 2,2 g ácido ascórbico 100 g<sup>-1</sup> polpa e 7% de compostos fenólicos), mas as amostras armazenadas refrigeradas também apresentaram resultados promissores aos 45 dias, mantendo as características iniciais ao longo do estudo. Mesmo as polpas armazenadas à temperatura ambiente são uma opção mais barata para preservar o produto, pois elas se degradaram apenas aos 30 dias de armazenamento.

**PALAVRAS-CHAVE:** *Myrciaria dubia*; amazônia; vitamina C; tratamento térmico; anthocianinas.

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