

ORIGINAL ARTICLE

Camu-camu harvested with reddish-green peel preserves its physicochemical characteristics and antioxidant compounds during cold storage

Camu-camu colhido com a casca verde-avermelhada conserva seus compostos físico-químicos e antioxidantes durante o armazenamento a frio

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Abstract

Camu-camu (*Myrciaria dubia*), a fruit native to the Brazilian Amazon, is considered a source of antioxidant compounds. Due to its high perishability, postharvest studies aimed at increasing its shelf life are required. The aim of this study was to evaluate the influence of harvesting time on the conservation of the physicochemical characteristics and antioxidant compounds of camu-camu during cold storage. The fruits, harvested at different ripening stages (red and reddish-green peel), were stored in polyethylene terephthalate trays at 15 °C and 90% relative humidity. The following analyses were carried out on days 1, 2, 5, 7, 9 and 13 after harvest: luminosity, hue angle and chromaticity, pH, soluble solids content, titratable acidity, *SS/TA*, ascorbic acid content, total phenolic content, total anthocyanin content and free-radical-scavenging activity by the 1,1-diphenyl-2-picryl-hydrazil method. The data were submitted to a multivariate analysis. The fruits harvested at different ripening stages showed different postharvest characteristics, highlighting the parameters of colour, flavour and antioxidants. The reddish-green fruits, despite their low concentration of anthocyanins during storage, showed high levels of phenolic compounds, ascorbic acid and antioxidant activity, which were maintained for nine days of cold storage. Due to the flavour characteristics and antioxidant compounds, it is recommended that camu-camu be harvested in the reddish-green maturation stage to extend its shelf life.

Keywords: *Myrciaria dubia*; Plant physiology; Ripening stages; Amazon.



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Resumo

Camu-camu (*Myrciaria dubia*), uma fruta nativa da Amazônia brasileira, é considerada uma fonte de compostos antioxidantes. Devido à sua elevada perecibilidade, são necessários estudos em pós-colheita para aumentar a sua vida útil. O objetivo deste estudo foi avaliar o ponto de colheita do camu-camu na conservação de suas características físico-químicas e compostos antioxidantes durante o armazenamento a frio. Os frutos foram colhidos em diferentes estádios de maturação (casca vermelha e verde-avermelhada). Os frutos foram armazenados em bandejas de politereftalato de etileno a 15 °C e 90% de umidade relativa. As análises foram realizadas nos dias 1, 2, 5, 7, 9 e 13, após a colheita: luminosidade, °hue e cromaticidade, pH, conteúdo de sólidos solúveis, acidez titulável, conteúdo de ácido ascórbico, conteúdo fenólico total, conteúdo total de antocianina e atividade antioxidante. Os dados foram submetidos à análise multivariada. Os frutos colhidos em diferentes estádios de maturação apresentaram diferentes características pós-colheita, destacando-se os parâmetros de cor, sabor e antioxidantes. Os frutos de cor verde-avermelhada, apesar de sua baixa concentração de antocianinas durante o armazenamento, apresentaram altos níveis de compostos fenólicos, ácido ascórbico e atividade antioxidante, que foram mantidos até nove dias de armazenamento a frio. Devido às características de sabor e compostos antioxidantes, recomenda-se que a colheita de camu-camu seja no estágio de maturação verde-avermelhada, para prolongar assim a vida útil do fruto.

Palavras-chave: *Myrciaria dubia*; Fisiologia vegetal; Estágios de maturação; Amazônia.

1 Introduction

Camu-camu (*Myrciaria dubia*), a fruit native to the Brazilian Amazon, is becoming relevant in food since it is considered to be a promising source of antioxidant compounds, including ascorbic acid, anthocyanins, β -carotene and phenolic compounds (Chirinos et al., 2010). The benefits of these bioactive compounds range from reducing the risk of chronic diseases such as cancer and cardiovascular problems to stimulation of the immune system, decreasing platelet aggregation and blood pressure, as well as showing antibacterial and antiviral activities (Kuskoski et al., 2005).

Several studies have indicated large amounts of bioactive compounds in camu-camu such as ascorbic acid – with concentrations higher than those found in fruits such as cherry, blackberry and acerola (*Malpighia emarginata*) (Kuskoski et al., 2005; Yuyama 2002; Ferreira et al., 2010; Neves et al., 2017; Neves et al., 2015b). This feature is evidence of its great economic potential, putting it on the same level of importance as other traditional fruits of the Amazon region, such as açai (*Euterpe oleracea*) and cupuaçu (*Theobroma grandiflorum*) (Vieira et al., 2011).

However, the commercialization of fresh camu-camu fruit is limited by its short shelf life, due mainly to physical damage to the skin and tissues during the steps of handling and transport, resulting in weight loss leading to dryness and increases in the respiratory and metabolic rates. Visual changes related to perishability such as discolouration, loss of aroma, softening of the pulp, fruit browning and fungal growth can be observed up to the 4th day after harvest, negatively influencing the consumers at purchase time (Arévalo & Kieckbusch, 2006).

Studies to determine the optimal harvest time, as well as to evaluate ways of conserving and storing the fruit during the post-harvest period are essential to increase the shelf life, contributing significantly to its domestic and international markets (Chitarra & Chitarra, 2005). Thus the aim of this study was to evaluate the influence of harvest time on the conservation of the physicochemical characteristics and antioxidant compounds of camu-camu during cold storage.

2 Material and methods

2.1 Raw material

The fruits were harvested in July 2014 in the early hours of the morning, in *Sete Barras*, São Paulo, Brazil (24°23'16''S, 47°55'33''W), considering the colour of the peel: red (RR) and reddish-green (RG). The fruits were stored in plastic boxes and transported in an air-conditioned vehicle. Selection was carried out according to the aspects of size, peel colour, damage to the external parts, insects and other contaminants. The fruits were stored in polyethylene terephthalate trays (PET) (12 cm width \times 19 cm length \times 6 cm height \times 0.6 mm thickness) in cold storage (Pró-Frio Industrial Refrigeration, São Paulo, SP, Brazil) at 15°C and 90% Relative Humidity (RH) controlled by a digital thermo-hygrometer (Testo, 608-H1, São Paulo, SP, Testo do Brasil). Each tray contained 0.150 kg of fruits (approximately 18 units) and each treatment (RR and RG) included 18 trays.

2.2 Physicochemical analyses

The physicochemical analyses were carried out on days 1, 2, 5, 7, 9 and 13 after harvest for each treatment (RG1, RG3, RG5, RG7, RG9, RG13, RR1, RR3, RR5, RR7, RR9 and RR13) with 3 repetitions for each day. The seeds were removed manually and the peel and pulp homogenized using a mixer (Black & Decker – SB40). The peel colour was determined using a colorimeter (Minolta Chroma Meter CR-400) considering the parameters of lightness (L^*), hue angle ($^\circ$ Hue) and chromaticity (Chroma), with 16 measurements per fruit. The pH was determined using a potentiometer (Marconi - MA-522), the soluble solids content (SSC) using a digital refractometer (Krüss Optronic - DR 201-95) and the titratable acidity (TA) by titration. The *ratio* was considered as the ratio between SSC and TA (Association of Official Analytical Chemists, 2015).

The ascorbic acid content was determined by titration using a sodium indolfenol-2,6-dichlorophenol solution (DCFI) and 1.0% oxalic acid to dilute the samples. The results were expressed in g AA kg⁻¹ of camu-camu (Association of Official Analytical Chemists, 2015).

The total phenolic content was determined using the Folin-Ciocalteu reagent (Singleton et al., 1999). 3 g of camu-camu extracts (1 mg mL⁻¹) were mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 4% (w/v sodium carbonate solution). After incubation in the dark at room temperature for 2 hours, the absorbance was measured at 740 nm using a UV-visible spectrophotometer (Femto[®] Plus, São Paulo, Brazil). The TPC was expressed as gallic acid (% w/w) equivalents (GAE) according to the gallic acid standard curve.

The monomeric anthocyanin content was determined by the pH differential method according to AOAC (Association of Official Analytical Chemists, 2015), and expressed as cyanidin-3-glucoside. A 3.0 g portion of camu-camu was macerated with 27.0 mL of alcohol (80%). Two macerated pulp portions of 1.2 mL each were weighed. One portion was mixed with 3.8 mL potassium chloride buffer (pH 1.0) and the other with 3.8 mL sodium acetate buffer (pH 4.5). After 20 minutes of extraction at 4°C, the samples were filtered through Whatman N° 1 filter paper, and the absorbance measured at 520 and 700 nm, for solutions at pH 1.0 and pH 4.5, respectively, using a UV-visible spectrophotometer (Femto[®] Plus, São Paulo, Brazil). The TAC was expressed as cyanidin-3-glucoside (mg 100g⁻¹) equivalents.

The free-radical-scavenging activity of camu-camu was measured using the 1,1-diphenyl-2-picrylhydrazil (DPPH) method according to Mensor et al. (2001). Briefly, a 0.5 mM solution of DPPH in ethanol was prepared and 0.03 mL of this solution and 3.0 mL of ethanol added to 0.5 mL of the fruit extract. The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. The absorbance was then determined at 575 nm using a UV-visible spectrophotometer (Femto[®] Plus, São Paulo, Brazil), lower absorbance values of reaction mixture indicating higher free-radical-scavenging activity. The results were expressed in μ mol TEAC mg⁻¹ of camu-camu.

2.3 Statistical analysis

The results were submitted to the multivariate analysis of variance (MANOVA), the correlation analysis (CORR), the principal component analysis with a biplot graph (PCA) and the cluster analysis (CA). MANOVA was applied to compare the averages and to define the significance between the observations (ripening stages and storage days). CORR was carried out to evaluate the interdependence between the variables, calculating the correlation matrix. The PCA was used to characterize and establish the relationship between the ripening stages and storage days. For the CA, the cutoff was obtained using the average method with a euclidean distance, according to a similarity coefficient with a cutoff of 0.40. The statistical software used for the tests was the SAS 9.3 (Statistical Analysis System Institute, 2010).

3. Results and discussion

MANOVA was carried out to test the null hypothesis in order to certify there was no significant difference between the pairs for the variables analysed (Table 1). The null hypothesis was rejected in almost all cases, only being accepted for the Reddish-green/day 3 x Reddish-green/day 5 and Ripe/day 5 x Ripe/day 5 pairs. This test showed that (1) fruits harvested at different points in time have different post-harvest characteristics; and (2) these characteristics modified during cold storage. The total data group and the differences between the samples and variables were explained by the CORR and ACP analyses.

For the CORR of the set of physicochemical data and antioxidant variables, 15 correlations above 0.70 were obtained, since Sounis (1985) considers accented indexes values above $|0.70|$ (Table 2). The variable TAC presented a negative correlation with Luminosity and °Hue (Table 2). The anthocyanins are coloured pigments ranging from red to purple, and an increase in the °Hue indicates yellow fruits and reduced levels of TAC. The same applies to the L* parameter, which is higher the closer it is to white, indicating a smaller amount of anthocyanin in the fruit. In addition to these correlations, the TAC variable was positively correlated to the SSC variable, since they are directly proportional. This can be explained by the degradation of the anthocyanins which is related to the reduction of the soluble solids content, as represented by the organic acids, salts, vitamins and pectin compounds. The TA variable was positively correlated to the AA and DPPH variables, since it represents the organic acids of the camu-camu, including ascorbic acid. The same was observed for the DPPH variable, which was mostly represented by organic acids.

Table 1. MANOVA test to compare the variance between the pairs of samples by the Wilks's lambda test.

	RG1	RG3	RG5	RG7	RG9	RG13	RR1	RR3	RR5	RR7	RR9	RR 13
RG1	-	*	*	*	*	*	*	*	*	*	*	*
RG3		-	n.s.	*	*	*	*	*	*	*	*	*
RG5			-	*	*	*	*	*	*	*	*	*
RG7				-	*	*	*	*	*	*	*	*
RG9					-	*	*	*	*	*	*	*
RG13						-	*	*	*	*	*	*
RR1							-	*	*	*	*	*
RR3								-	*	*	*	*
RR5									-	n.s.	*	*
RR7										-	*	*
RR9											-	*
RR13												-

RG1 = Reddish-green/day 1, RG3 = Reddish-green/day 3, RG5 = Reddish-green/day 5, RG7 = Reddish-green/day 7, RG9 = Reddish-green/day 9, RG13 = Reddish-green/day 13, RR1 = Ripe/day 1, RR3 = Ripe/day 3, RR5 = Ripe/day 5, RR7 = Ripe/day 7, RR9 = Ripe/day 9, RR13 = Ripe/day 13. *significant, n.s. = not significant, Wilks's lambda=0.937, $p < 0.05$.

Table 2. Correlation coefficients observed for the post-harvest variables of camu-camu.

Variables	Correlation coefficients
Titrateable acidity – Ascorbic acid	0.7830
Soluble solids content – Anthocyanins	0.7434
Titrateable acidity – DPPH	0.7397
Chroma – Luminosity	0.8155
°Hue – Luminosity	0.7535
Chroma – °Hue	0.9283
Ratio – Soluble solids content	0.7379
Titrateable acidity – DPPH	0.7397
Ascorbic acid – SSC/TA ratio	-0.7593
Anthocyanins – Luminosity	-0.8361
Anthocyanins – °Hue	-0.7731
Chroma – Soluble solids content	-0.7651
Chroma – SSC/TA ratio	-0.7076
°Hue – Soluble solids content	-0.7884
Titrateable acidity – SSC/TA ratio	-0.7777

The PCA was represented by the projections of the variables (Figure 1a) and observations (Figure 1b). In this study, two PCs were obtained from the total data group (Table 3), explaining 70.89% of the variance. The first principal component (PC1) explained 44.69% of the statistical variance and was positively correlated with the colour variables (L^* , Chroma and °Hue), and negatively with the variables related to taste (SSC and SSC/TA ratio). The second principal component (PC2) explained 26.20% of the statistical variance and was positively correlate with the variables related to the antioxidant compounds (AA, TAC, DPPH and TA).

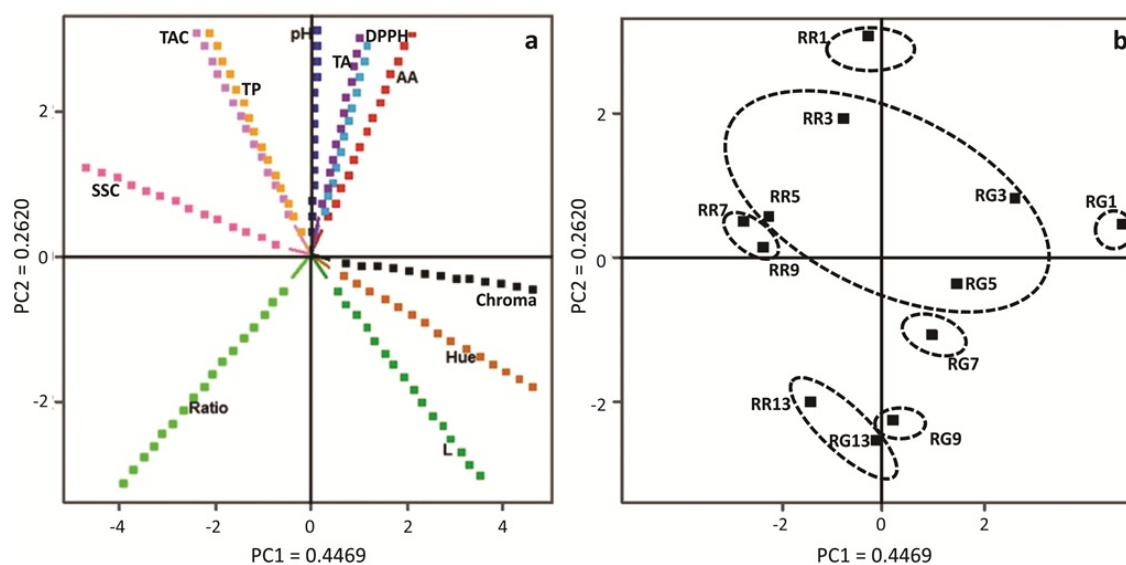


Figure 1. The PCA as represented by the projection of the variables (a) and observations (b).

Table 3. Total data group (mean, \pm SD, n = 4).

Treatments	Storage days					
	1	2	5	7	9	13
Luminosity						
RR	27.3 \pm 1.22	28.5 \pm 1.49	26.0 \pm 0.67	25.5 \pm 1.40	25.4 \pm 0.37	30.3 \pm 3.14
RG	42.2 \pm 1.84	33.5 \pm 4.25	37.2 \pm 0.89	41.1 \pm 2.51	42.5 \pm 1.54	35.4 \pm 2.67
$^{\circ}$Hue						
RR	29.6 \pm 1.31	26.2 \pm 1.75	20.2 \pm 1.01	19.3 \pm 1.91	18.5 \pm 0.78	29.1 \pm 1.17
RG	45.6 \pm 1.45	38.1 \pm 0.01	38.1 \pm 1.64	38.9 \pm 2.18	36.7 \pm 0.01	34.9 \pm 3.22
Chromaticity						
RR	20.5 \pm 2.02	22.3 \pm 3.33	20.2 \pm 2.29	17.5 \pm 3.57	16.3 \pm 2.76	20.3 \pm 3.80
RG	29.5 \pm 2.38	26.8 \pm 1.48	25.5 \pm 1.25	25.4 \pm 0.62	23.6 \pm 1.03	21.7 \pm 1.17
pH						
RR	2.40 \pm 0.01	2.54 \pm 0.01	2.55 \pm 0.03	2.56 \pm 0.03	2.48 \pm 0.01	2.39 \pm 0.01
RG	2.45 \pm 0.03	2.59 \pm 0.02	2.56 \pm 0.01	2.53 \pm 0.01	2.52 \pm 0.01	2.43 \pm 0.01
Soluble solids content ($^{\circ}$Brix)						
RR	7.30 \pm 0.44	7.53 \pm 0.15	7.53 \pm 0.15	7.50 \pm 0.20	7.03 \pm 0.12	7.10 \pm 1.17
RG	5.90 \pm 0.17	6.23 \pm 0.29	6.70 \pm 0.10	6.10 \pm 0.26	6.90 \pm 0.10	6.86 \pm 0.35
Titrateable acidity (%)						
RR	2.29 \pm 0.01	2.24 \pm 0.10	2.06 \pm 0.12	2.06 \pm 0.12	1.98 \pm 0.06	1.88 \pm 0.06
RG	2.28 \pm 0.13	2.18 \pm 0.11	2.03 \pm 0.07	2.03 \pm 0.07	1.87 \pm 0.17	1.87 \pm 0.17
Ratio						
RR	3.18 \pm 0.19	3.36 \pm 0.14	3.65 \pm 0.26	3.63 \pm 0.27	3.54 \pm 0.09	3.74 \pm 0.14
RG	2.59 \pm 0.09	2.86 \pm 0.20	3.30 \pm 0.08	3.49 \pm 0.10	3.69 \pm 0.36	3.67 \pm 0.36
Ascorbic acid content (mg 100 g⁻¹)						
RR	3417 \pm 0.18	2248 \pm 0.07	2274 \pm 0.21	2119 \pm 0.09	2274 \pm 0.23	1989 \pm 0.17
RG	3449 \pm 0.08	2416 \pm 0.08	2196 \pm 0.11	2429 \pm 0.03	1963 \pm 0.11	2132 \pm 0.17
Phenolic compounds content (mg 100 g⁻¹)						
RR	1986 \pm 0.05	1950 \pm 0.03	2025 \pm 0.03	2321 \pm 0.13	2249 \pm 0.06	1614 \pm 0.26
RG	1842 \pm 0.09	2042 \pm 0.08	2065 \pm 0.05	2421 \pm 0.13	2238 \pm 0.03	1469 \pm 0.14
Total anthocyanins content (mg 100 g⁻¹)						
RR	45.5 \pm 5.36	35.9 \pm 3.68	35 \pm 4.74	32.6 \pm 4.03	30.4 \pm 3.82	26.9 \pm 2.68
RG	11.8 \pm 3.25	14.1 \pm 2.14	16 \pm 3.14	13.6 \pm 2.95	16.3 \pm 4.20	11.3 \pm 4.57
Free-radical-scavenging DPPH (μmol TEAC mg⁻¹)						
RR	0.17 \pm 0.02	0.19 \pm 0.02	0.09 \pm 0.00	0.07 \pm 0.00	0.08 \pm 0.00	0.04 \pm 0.00
RG	0.11 \pm 0.00	0.18 \pm 0.00	0.17 \pm 0.00	0.09 \pm 0.00	0.07 \pm 0.00	0.05 \pm 0.00

SD: standard deviation; n: number of replicates; RR: red camu-camu; RG: reddish-green camu-camu.

For the CA, the observations were separated into seven groups by the cutoff fixed at 0.40, as shown by the dotted line (Figure 2). The same cutoff was used to separate the groups (dotted circles), as shown in the projection of the observations (Figure 1b).

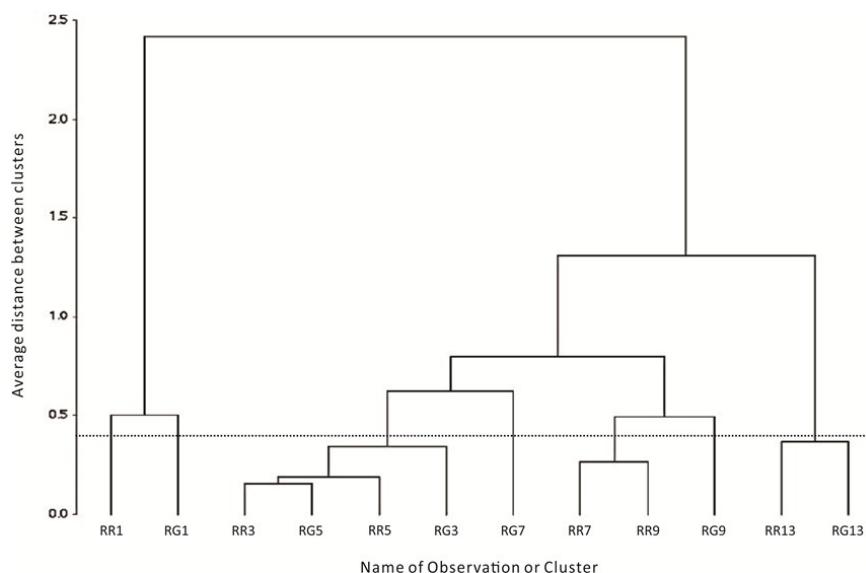


Figure 2. Observations separated into seven groups by fixing the cutoff at 0.40, as shown by the dotted line.

The Reddish-green/day 7 and Reddish-green/day 9 reddish-green camu-camu fruits were characterized by high L^* values, i.e., brighter (41.14 and 42.52, respectively). However, the Ripe/day 5, Ripe/day 7 and Ripe/day 9 fruits presented low L^* values (26.03, 25.53 and 25.46, respectively) and were considered darker due to their anthocyanin contents. Regarding the $^{\circ}$ Hue, the Reddish-green/day 5 and Reddish-green/day 7 fruits indicated a yellowish tonality (38.15° and 38.99° , respectively), whereas Ripe/day 5, Ripe/day 7 and Ripe/day 9 were characterized by a tonality closer to red (20.23° , 19.32° and 18.56° , respectively), also due to the presence of anthocyanins. Reddish-green/day 5, Ripe/day 5 and Ripe/day 7 presented values for chromaticity near the average value and Ripe/day 9 exhibited a diffuse colour (16.36) (Figure 1, Table 3).

The same was observed for the CORR results, where the fruits harvested ripe were characterized by a redder and darker peel colour than those harvested in the preceding stage. The difference in the anthocyanin contents observed between the ripening stages studied was related to the increase in the concentration of the red pigment during the ripening process, which happens concurrently with the change in colour of the peel from green to red (Genovese et al., 2008; Andrade et al., 2010). Higher anthocyanin contents were observed in ripe camu-camu by Carrillo et al., (2011) and Pinto et al., (2013); in ripe acerola by Araújo et al., (2009); and in ripe blackberry by Siriwoham & Wrolstad (2004). In addition, the anthocyanin levels observed in the ripe camu-camu were high and it must be emphasized that these high anthocyanin contents in the ripe camu-camu were maintained during cold storage.

Regarding flavour, Ripe/day 5, Ripe/day 7 and Ripe/day 9 fruits were characterized by high levels of SSC (7.53, 7.50 and 7.03 $^{\circ}$ Brix, respectively) due to the increase in sugar concentration during the ripening process. The opposite was observed in the Reddish-green/day 7 and Reddish-green/day 5 fruits (6.10 and 6.70 $^{\circ}$ Brix, respectively), harvested in the preceding stage. Ripe/day 13 fruits presented the highest value for the *Ratio* (3.74), high SSC values (7.10 $^{\circ}$ Brix) and low TA (1.88%). On the other hand, Reddish-green/day 13 fruits showed the lowest SSC/TA ratio (3.67). Fruits with red peel have large amounts of anthocyanins and SSC, due to the increase in reducing sugars concomitantly with the changes in peel colour, when it becomes completely red (Alves et al., 2002; Rossetto et al., 2004). This same relationship between the anthocyanin content and SSC in camu-camu was found by Neves et al. (2015c) and Prasanna et al., (2007).

During the ripening process, the TA decreased due to the use of organic acids in fruit respiration (Prasanna et al., 2007). However, despite this reduction, the TA values remained high, resulting in a low ratio value (Neves et al., 2015c).

The Ripe/day 1 fruits presented the highest AA level (3417 mg 100 g⁻¹), but by the end of storage (Ripe/day 13) they showed the lowest value (1989 mg 100 g⁻¹) due to the degradation of ascorbic acid, which is highly sensitive to parameters such as light, heat and storage for long periods, amongst others (Silva et al., 2006). The Ripe/day 1 and ripe/day 3 fruits were characterized by high TAC values (45.53 and 35.96%, respectively), and all Reddish-green/day fruits presented much lower levels of this compound. The Ripe/day 1 fruits showed higher levels of free-radical-scavenging activity (DPPH variable) as compared to Ripe/day 13 fruits (0.17 and 0.04 μmol TEAC mg⁻¹, respectively) indicating the sensitivity of these compounds, decreasing during storage. Moreover, these fruits presented the highest values for DPPH and AA in comparison with the Ripe/day 13 fruits, evidencing the sensitivity of these compounds during storage.

The Reddish-green/day fruits showed high values for antioxidant compounds such as PC and AA, and high antioxidant activity, despite showing low concentrations of TAC during storage. These compounds were preserved during cold storage in the Reddish-green/day fruits, but not in the ripe fruits. High values of antioxidants are associated with the exposure to biotic and abiotic stress during the fruit ripening process. Furthermore, maintenance of the phenolic compounds and antioxidant activity made possible by the presence of ascorbic acid in the fruit (Neves et al., 2015a).

The AA peak occurred when the camu-camu reached physiological maturity or at an intermediate ripening stage, which justifies the maintenance of the values of this compound in the Reddish-green/day fruits (Bardales et al., 2008). In the ripe camu-camu fruits, the antioxidant compounds were degraded by the action of enzymes such as ascorbate oxidase, phenolase and cytochrome oxidase (Correa et al., 2011). The same results reported in this study were found in camu-camu by Chirinos et al. (2010), Rufino et al. (2010) and Reynertson et al. (2008).

The camu-camu proved to be a rich source of antioxidant compounds in comparison with other native Brazilian tropical fruits (Neves et al., 2015b). This feature favours its marketing both in Brazil and abroad. In the dendrogram presented in Figure 2 a group was formed by the Ripe/day 13 and Reddish-green/day 13 fruits, which showed that at the end of cold storage, the ripe and reddish-green fruits presented similar characteristics, with degradation of the antioxidant compounds on the last day. Thus, Reddish-green/day fruits stored for up to nine days of refrigeration showed better post-harvest characteristics.

4 Conclusions

The optimal ripening stage for the harvesting of camu-camu was the “reddish-green” stage in order to preserve its physicochemical characteristics and antioxidant activity. Moreover, the bioactive properties of these fruits were preserved during the first nine days of storage under refrigeration at 15 °C.

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