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Bioactive compounds in and antioxidant activity of camu-camu fruits harvested at different maturation stages during postharvest storage

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ABSTRACT. The present study was carried out to evaluate the antioxidant activity of and the content of bioactive compounds in camu-camu fruits harvested at different maturation stages and stored. The fruits were harvested in the municipality of Cantá, Roraima State, Brazil. The experimental design was completely randomized, with three replications, in a factorial arrangement consisting of three different maturation stages (immature, semi-mature and mature) and eight days of storage (3 x 8). The fruits were analysed every day regarding total vitamin C, carotenoids, anthocyanins, flavonoids, total phenolic compounds, and antioxidant activity (FRAP and DPPH). According to the results obtained, the interaction of maturation stages x eight days of storage had a significant effect according to the F test at 5% probability. The highest antioxidant activity (FRAP) was observed in the fruits harvested in the semi-mature stage, providing a longer shelf life. The carotenoid pigment, flavonoid, anthocyanin, and vitamin C contents were higher in the fruits harvested in the mature stage, and this stage was the most suitable for obtaining these functional biocompounds. Additionally, in mature fruits, the highest mean content of total phenolics and antioxidant activity (DPPH) were observed during storage. It was concluded that the mature stage is the most recommended for the extraction of pigments and antioxidant biocompounds from camu-camu fruits.

Keywords: anthocyanins; biocompounds; phenolic compounds; *Myrciaria dubia*; multivariate statistics; nutraceutical properties.

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Introduction

Camu-camu (*Myrciaria dubia* (Kunth) Mc Vaugh), also known as "caçari", "araçá-d'água", or "sarão", is a species of the Myrtaceae family native to Amazon floodplains and lakes (Maeda, Pantoja, Yuyama, & Chaar, 2006). Its geographical distribution is limited to the river courses and extends from the state of Pará - Brazil (Tocantins and Trombetas Rivers) to Peru, under the name of camu-camu. In the Central Amazon (regions of Manaus and Manacapuru, with the Javari, Madeira and Negro Rivers), and in the state of Roraima (in the banks of natural lakes and the Cauame River), it is known as "caçari" (Chagas et al., 2015; Smiderle & Sousa, 2010).

The consumption of native and exotic fruits and their derivatives has significantly increased in recent years. In addition to the global demand for new flavours, consumers have looked for fruits that are nutritious and healthy, that present important biomolecular compositions and that work as functional foods. The increasing interest in camu-camu fruits is related to their outstanding vitamin C content. It is currently considered the fruit with the highest vitamin C content (Chagas et al., 2015).

The high ascorbic acid content is a major qualitative attribute of camu-camu's commercial marketing. However, a factor that contributes to the restriction of its use is the pulp's very acidic taste and the peel's bitterness. Thus, research on its best use is necessary. Camu-camu nectar is an alternative to the use of camu-camu fruits since the nectar is a natural, nutritious drink that is ready for consumption and easy to process (Maeda et al., 2006).

Chirinos, Galarza, Betalleluz-Pallardel, Pedreschi, and Campos (2010), working with camu-camu in Peru, detected higher vitamin C contents in immature fruits than those in other maturation stages. However, Neves,

Silva, Pontis, Flach, and Roberto (2015) working with camu-camu in different maturation stages detected higher vitamin C content at 88 days after anthesis, i.e., at the beginning of ripening, with decreases in subsequent periods, when fruits were completely red. This period also corresponded to higher total phenolic content and antioxidant activity, meaning the vitamin C content shows the functional potential of camu-camu fruits.

However, there are reports in which ascorbic acid content was found in mature fruits. This variation in ascorbic acid concentration can be related to acidity, since the acids present in greater quantities in the immature fruit facilitate the degradation of ascorbic acid (Pinto, Jacomino, Silva, & Andrade, 2013; Villanueva-Tiburcio, Condezo-Hoyos, & Asquieri, 2010).

There is a large amount of information regarding the best harvesting stage of camu-camu fruits. This fact may be related to the high genetic variability of the fruit (Yuyama, 2011), which significantly influences the vitamin C content, suggesting the need for further studies. According to Maeda, Pantoja, Yuyama, and Chaar (2007), the concentration and stability of vitamin C in camu-camu vary according to the species, maturation stage, processing time and temperature, pH, and presence of oxygen and enzymes. Degradation of bioactive compounds may occur during processing and/or storage.

Different types of flavonoids, such as flavonoids, flavonols, flavanones, and anthocyanins, are found in camu-camu fruits. The phenolic compounds present in camu-camu fruits depend on the maturation stage (Akter, Oh, Eun, & Ahmed, 2011; Chirinos et al., 2010). It is noteworthy that carotenoid pigments, flavonoids, anthocyanins, vitamin C and other antioxidant compounds are present in higher contents in the peel, giving the fruits greater antioxidant activity. The peel is the most suitable part of the fruit for the extraction of these functional biocompounds (Myoda et al., 2010; Neves et al., 2015).

Several studies have been carried out in different segments aimed at discovering new nutritional and functional sources. Epidemiological studies have demonstrated the protective effect or even therapeutic effect of diets rich in fruits and vegetables with high antioxidant potential, as well as in the treatment of chronic diseases, and camu-camu has caught attention for being rich in antioxidants (Borges, Conceição, & Silveira, 2014; Carvalho-Silva et al., 2014; Silva et al., 2012; Dastmalchi et al., 2012; Ellinger et al., 2012; Gonçalves, Lellis-Santos, Curi, Lajolo, & Genovese, 2014; Langley, Pergolizzi Jr., Taylor Jr., & Ridgway, 2015; Melo et al., 2006).

In this context, this study was carried out to determine the bioactive compounds and the antioxidant activity levels of camu-camu fruits harvested at different maturation stages and stored.

Material and methods

The camu-camu used in the experiment were harvested from native plants located on the banks of Lake Morena in the city of Cantá, state of Roraima, Brazil (02° 27' 45" S, 60° 50' 14" W). After harvest, the fruits were carefully packed in plastic boxes to prevent crushing of the fruit and transported to the Postharvest Laboratory of Embrapa Roraima, where the undamaged fruit were selected and cleaned with 0.02% sodium hypochlorite (NaClO) for 30 minutes.

Subsequently, the fruits were classified according to the maturation stage based on the peel colour: immature (completely green peel), semi-mature (50% green peel and 50% purple peel), and mature (100% purple peel). According to Neves et al. (2015), these colours correspond to 60, 81, and 102 days after anthesis, respectively. Then, the fruits were stored in the laboratory at an approximate temperature of 22 ± 2°C and relative humidity of 70 ± 3%.

The experimental design was completely randomized with three replications in a factorial arrangement (3 x 8), consisting of three different maturation stages (immature, semi-mature, and mature) and eight days of storage during two cycles. Each replication consisted of 30 fruits (approximately 300 g fruit).

The fruits were analysed daily. Every day, seeds were removed, and the remaining material (pulp + peel) was processed and homogenized for the following analyses:

Total Vitamin C: Determined by the methodology of Terada, Watanabe, Kunitomo, and Hayashi (1978). One gram of sample and 3 mL oxalic acid were homogenized and centrifuged at 4°C under rotation of 6,000 rpm for 20 minutes. One millilitre of the supernatant was added to 3 mL 0.5% oxalic acid, 3 drops 2,6-dichlorophenolindophenol sodium salt solution, 1 mL 2,4-dinitrophenyl hydrazine solution at 2%, and one drop thiourea solution. Afterwards, the samples were placed in a boiling water bath for 15 minutes and rapidly cooled in an ice bath. During the ice bath, 5 mL 85% sulfuric acid was added. The absorbance was measured

with a spectrophotometer at 520 nm after cooling. The results are expressed in g 100 g^{-1} of fresh sample.

Carotenoids: Determined according to the methodology of Linder (1974) and Witham, Blaydes, and Devlin (1971). Three millilitres of Tris-HCl buffered acetone was added to 200 mg of sample, and the mixture was homogenized and centrifuged for 5 minutes at 2,000 rpm. The supernatant was removed with the aid of a pipette, and the absorbance was measured in a spectrophotometer at 470 nm. The results are expressed in µg 100 g^{-1} of fresh sample.

Anthocyanins: Determined according to the methodology of Linder (1974) and Witham et al. (1971). The absorbance was measured in a spectrophotometer at 537 nm. The results are expressed in µg 100 g^{-1} of fresh sample.

Flavonoids: Determined according to the adapted spectrophotometric method (Awad, Jager, & van Westing, 2000; Santos & Blatt, 1998). Samples of fresh material were weighed and soaked in 20 mL solution containing 70% methanol and 10% acetic acid. After that, the samples were placed in an ultrasonic bath. After filtration, the samples were centrifuged at 10,000 rpm. The supernatant was transferred to sample tubes, and aluminium chloride and acetic acid were added at 10%. The mixture was stirred and then allowed to stand for 30 minutes. The absorbance was measured at 425 nm, and the results are expressed in mg rutin 100 g^{-1} of fresh sample.

Phenolic compounds: Determined according to the spectrophotometric method of Folin-Ciocateau, described by Singleton, Orthofer, and Lamuela-Raventós (1999). Samples were diluted, and a 0.5 mL aliquot of the diluted sample was transferred to a sample tube. Then, 2.5 mL Folin-Ciocalteu reagent was added, and the mixture was diluted with water (1:10). The mixture was allowed to stand for 5 minutes. Then, 2 mL 4% sodium carbonate was added, and the sample tubes were allowed to stand for 2 hours under light. The absorbance was measured in a spectrophotometer at 740 nm. The results were expressed in mg of gallic acid 100 g^{-1} of fresh sample.

Antioxidant activity (FRAP): Estimated using the reduced iron method (FRAP), following the procedure adapted by Rufino et al. (2006). Approximately 1 g of sample was added to 40 mL of 50% methanol, which was homogenized and allowed to stand for 60 minutes at room temperature. After this period, the samples were centrifuged (15,000 rpm) for 15 minutes, and the supernatant was transferred to a 100 mL volumetric flask. The supernatant was added to the residue of the first extraction and 40 mL acetone at 70%, and the mixture was homogenized and allowed to stand for 60 minutes at room temperature. After one hour, the samples were centrifuged again (15,000 rpm) for 15 minutes, the supernatant was transferred to a volumetric flask containing the first supernatant, and the volume was accounted for with distilled water. The extract and the FRAP reagent were placed in a water bath at 37°C. The absorbance was measured at 595 nm, and the results are expressed in µmol ferrous sulfate g^{-1} of fresh sample.

Antioxidant activity (DPPH): Determined in terms of oxidation inhibition potential, using the radical 2,2-diphenyl-1-picrilidrazil (DPPH) as reference (Brand-Williams, Cuvelier, & Berset, 1995). One gram of sample was weighed, and 10 mL ethyl alcohol was added. The sample was homogenized and centrifuged at 6,000 rpm for 50 minutes. After that, the supernatant was separated with the aid of a pipette, the solution was conditioned in a dark flask in an ice bath, and 3 mL ethanol was added to the solution. The absorbance was measured in a spectrophotometer at 517 nm from 500 µL of the sample extract with the addition of 300 µL DPPH solution. The results are expressed in µmol TEAC (Trolox equivalent antioxidant capacity) g^{-1} of fresh sample.

The data collected on each day of evaluation were submitted to analysis of variance by the F test at 5% and to polynomial regression analysis using R software (R Core Team, 2018). Multivariate data analysis by principal component analysis (PC) was carried out using the software INFOSTAT (Di Rienzo et al., 2016), where the observations were the different maturation stages and storage and the variables were the antioxidant activity and the content of bioactive compounds.

Results

According to the results obtained, the camu-camu fruits presented similar behaviour during the two evaluated cycles. For all the tested variables, the interaction (maturation stages x days of storage) presented a significant effect by the F test at 5% probability.

The total vitamin C content (ascorbic acid + dehydroascorbic acid) of camu-camu fruits during storage showed a higher mean value in immature fruits (Figure 1). However, this value decreased to 6,602 mg 100 mL^{-1} pulp on the fifth day and to 5,511 mg 100 mL^{-1} pulp on the eighth day of evaluation. It is possible that dehydroascorbic acid was still being synthesized and thus increased the vitamin C content in the initial days

of storage.

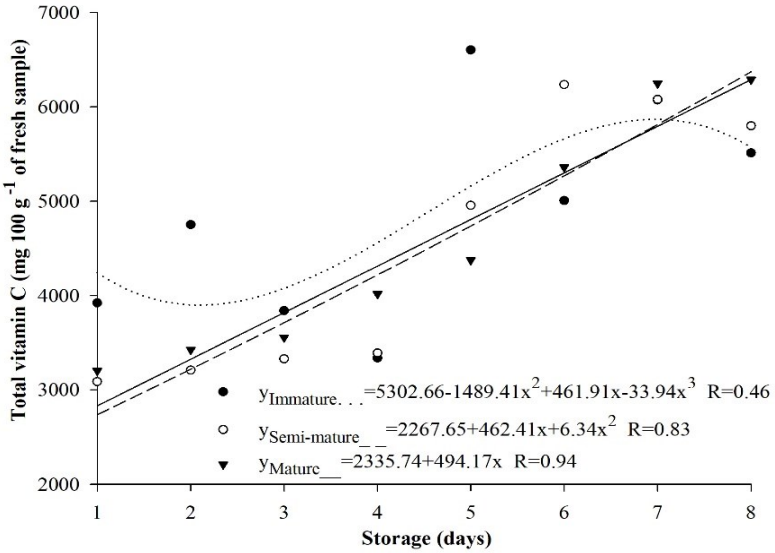

Figure 1. Total Vitamin C content in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

Carotenoids not only are important precursors of vitamin A but also present a considerable degree of antioxidant activity. The mature fruits showed the highest values of this pigment during the whole experiment (Figure 2), visually indicated by the reddish-coloured peel; together with anthocyanins and chlorophyll, these components give colour to camu-camu fruits.

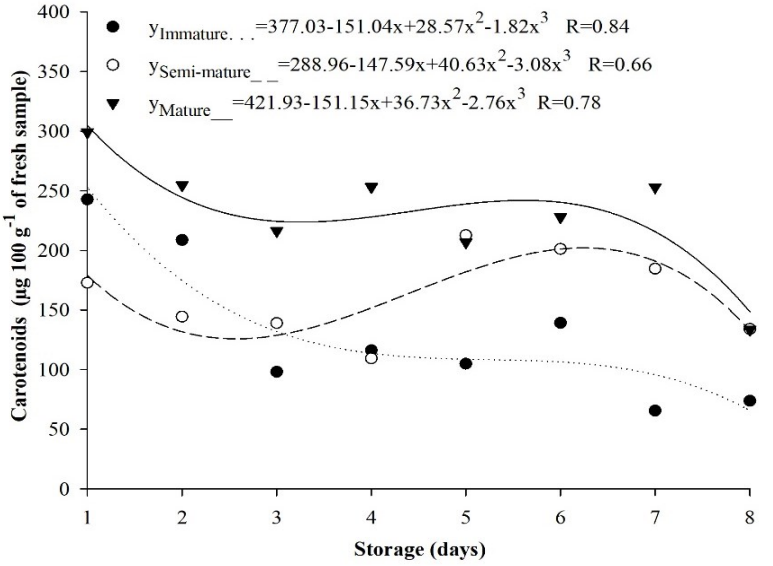

Figure 2. Carotenoids content in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

The mature fruits also had the highest anthocyanin values, with a peak of 2,513.28 µg 100 g^{-1} pulp on the sixth day, showing that even after harvest, the anthocyanin biosynthesis remains active, with an abrupt decrease of 77% from the sixth to the eighth day of storage (Figure 3).

Regarding the flavonoid content, the mature fruits showed the highest levels in the experiment, followed by the semi-mature and immature fruits. On the last day of evaluation, the mature fruits showed an increase in these values (Figure 4).

The phenolic compounds and mature fruits presented the highest means, followed by the semi-mature and immature fruits (Figure 5).

The analysis of the antioxidant activity by the FRAP method showed that the fruits at the semi-mature stage showed a higher mean value during storage. However, the highest values were observed in the mature

fruits on the sixth and seventh days of storage, with values of 2,108 and 2,167 µmol ferrous sulfate g^{-1} pulp, respectively (Figure 6).

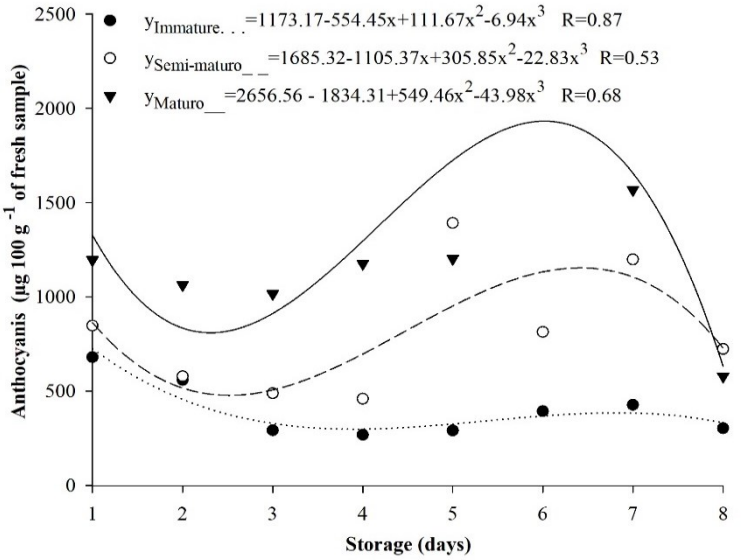

Figure 3. Anthocyanins content in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

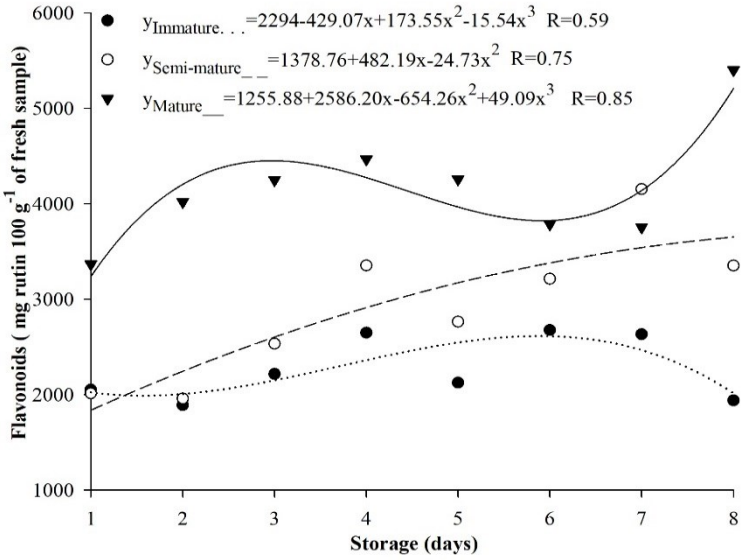

Figure 4. Flavonoids content in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

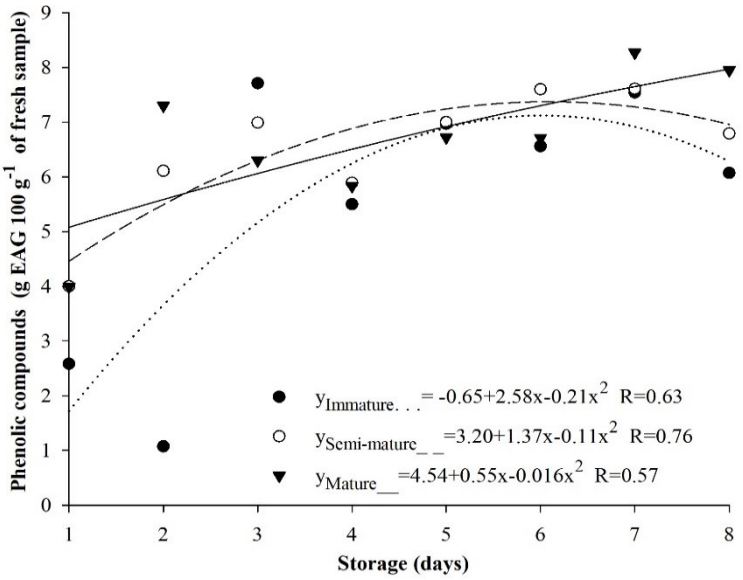

Figure 5. Phenolic compounds in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

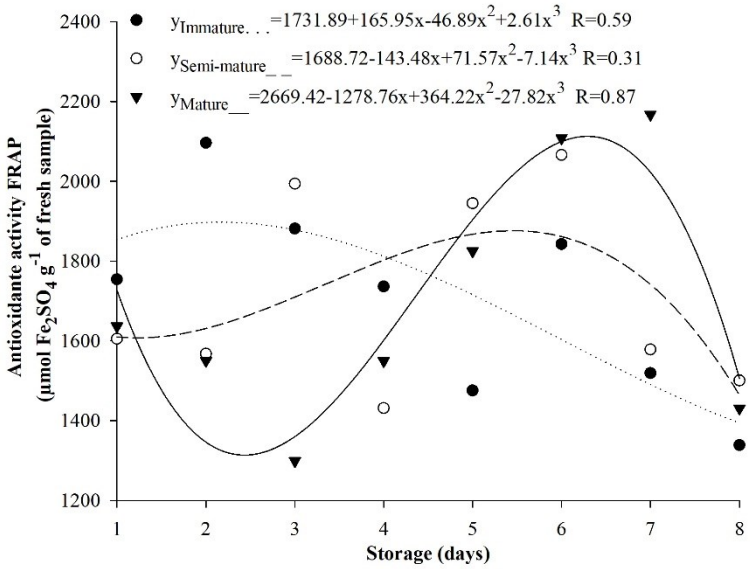

Figure 6. Antioxidant activity (FRAP) in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

The antioxidant activity measured by the DPPH method in the immature fruits harvested at the beginning of the experiment was inferior to those of the other maturation stages (Figure 7). However, there was an increase in this activity, and from the fourth day, the immature fruit stood out compared to the other stages until the seventh day of storage.

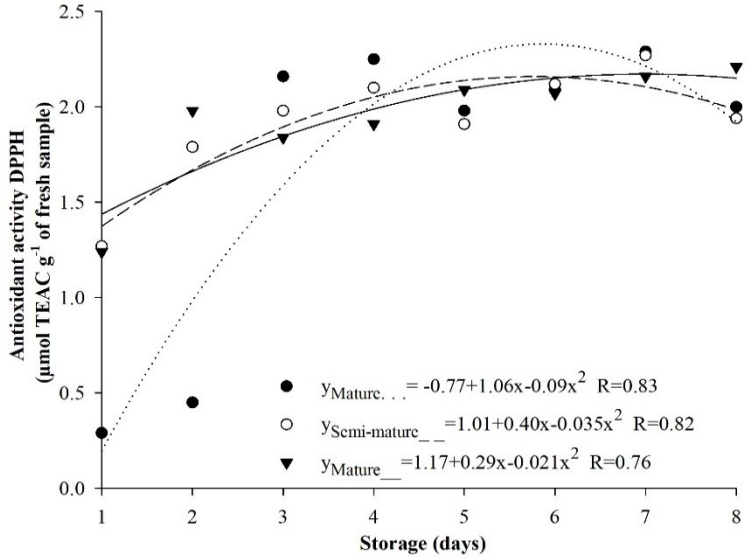

Figure 7. Antioxidant activity (DPPH) in camu-camu fruits harvested at different maturation stages and stored at 22°C and 70% RH.

The fruits harvested at the semi-mature and mature stages showed stable performance throughout the storage period, with mean values of 1.92 and 1.93 µmol TEAC g^{-1} pulp, respectively.

Multivariate analysis

According to the multivariate analysis, it was possible to observe the scores at different maturation stages during storage for the bioactive compounds and antioxidant activity of camu-camu fruits (Figure 8). The figure shows the variation in bioactive compounds and antioxidant activity of camu-camu as a function of maturation stage and days in storage, where it was observed that the principal components (PC) explained 69.9% of the data variability. PC 1 and PC 2 explained 36.59% and 31.29% of the data variability, respectively.

In PC1, the variables of vitamin C, phenolic compounds and antioxidant activity (DPPH) presented the highest correlations. In PC2, the flavonoids, antioxidant activity (FRAP), anthocyanins and carotenoids were the factors with the highest correlation in the analysis. Significant and positive correlations were observed between the antioxidant activity (DPPH) and these bioactive compounds according to the data matrix (data not shown): antioxidant activity (DPPH) and phenolic compounds ($r = 0.91$, $p < 0.05$), flavonoids ($r = 0.42$, $p < 0.05$), and anthocyanins ($r = 0.49$, $p < 0.05$), and a negative correlation was observed between antioxidant activity (DPPH) and carotenoids ($r = -0.41$, $p < 0.05$). Significant and positive correlations were also observed between the bioactive compounds: carotenoids and anthocyanins ($r = 0.78$, $p < 0.5$), vitamin C and phenolic compounds ($r = 0.44$, $p < 0.05$), phenolic and flavonoid compounds ($r = 0.45$, $p < 0.05$) and anthocyanins and flavonoids ($r = 0.49$, $p < 0.5$).

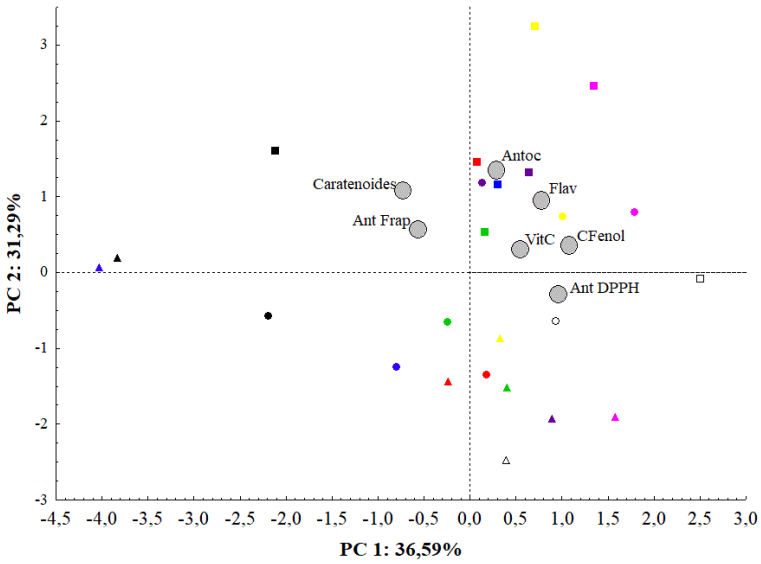

Figure 8. Principal component analysis (PC 1 and 2) performed with maturation stages (immature, semi-mature and mature) and storage days (1 to 8). Biplot (load variables and sample scores). Triangle corresponds to fruits in immature stage, circle corresponds to fruits in semi-mature stage and square corresponds to fruits in mature stage. Black symbols correspond to one storage day, blue to 2 days, green to 3 days, red to 4 days, purple to 5 days, yellow to 6 days, pink to 7 days, and white symbols to 8 days of storage.

The fruits in the immature ripening stage showed the lowest correlations with the bioactive compounds and antioxidant activity compared to the other stages on all storage days, showing only a small correlation with DPPH antioxidants to the 6th storage day.

The semi-mature stage of maturation on days 5, 6, 7, and 8 showed the highest correlations between the bioactive compounds and antioxidants. The semi-mature and mature stages of maturation on the 8th day had a higher correlation with the DPPH antioxidants. To couple the maturation stage, the fruits on days 2, 3, 4, and 5 strongly correlated with anthocyanins, flavonoids, vitamin C and phenolic compounds. The maturation stage on day 1 showed the highest correlation with carotenoids and antioxidant activity (FRAP).

The fruits in the semi-mature stage on the 5th day and those in mature stage on the 2nd, 4th, and 5th days had relatively high correlations with anthocyanins, and those in the mature stage on the 3rd day had a relatively high correlation with vitamin C, possibly due to the process of maturation and changes in peel colour.

Discussion

At the end of the experiment, the fruits harvested in the mature and semi-mature stages showed relatively higher vitamin C values (6,289 and 5,799 mg 100 g^{-1} pulp, respectively). The increased ascorbic acid content in the fruits during maturation is associated with increased synthesis of metabolic intermediates, which are precursors of ascorbic acid (Pinto et al., 2013).

Chirinos et al. (2010), working with camu-camu in Peru, detected higher total vitamin C content in immature fruits than in other maturation stages, confirming the results observed in this study. However, Imán Correa, Bravo Zamudio, Sotero Solís, and Oliva Cruz (2011) found that the more mature the fruits were, the greater were the vitamin C values when evaluating camu-camu peel + pulp.

The mature fruits presented a decrease in the amount of carotenoids during the experiment, varying from 300 to 133 µg 100 g^{-1} pulp, due to the increased physiological changes and to the advanced maturation stage. On the eighth evaluation day, the immature fruits presented amounts of carotenoids equal to those of the semi-mature fruits. Superior values (400 µg 100 g^{-1} pulp) were observed by Rufino et al. (2010) and Zanatta and Mercadante (2007) when evaluating camu-camu fruits. However, Neves et al. (2015) found lower values of total carotenoids in reddish-peeled fruits.

In immature fruits, higher carotenoid values were found at the beginning of the experiment than in the semi-mature fruits. From the second day of evaluation, these values decreased, reaching 70 µg 100 g^{-1} pulp. Neves et al. (2015) also found that the carotenoids present in camu-camu peel showed a decreasing trend with the advance of maturation, even when the peel colour was 100% green.

The mature fruits also had the highest anthocyanin values; these results are equivalent to those reported by Villanueva-Tiburcio et al. (2010), who found the highest amount of anthocyanins in mature and fresh camu-camu fruits when analysing three maturation stages. The immature fruits presented lower anthocyanin content than the other maturation stages during the whole experiment. The values found in this study were much lower than those observed by Maeda et al. (2006) and Rufino et al. (2010), who also determined the anthocyanin content of the epicarp of camu-camu fruits. This phenomenon is attributed to the storage time, which causes pigment loss (Maeda et al., 2007).

High concentrations of ascorbic acid can generate intense degradation of anthocyanins, since this instability is caused by direct condensation of ascorbic acid in 4 carbon atoms of anthocyanins (De Rosso & Mercadante, 2007; Nóbrega, Oliveira, Genovese, & Correia, 2015). In addition, the climatic conditions of the area (low rainfall and high temperatures) may have negatively influenced the anthocyanin content, since, according to Zanatta, Cuevas, Bobbio, Winterhalter, and Mercadante (2005), lower rainfall and higher temperatures may be responsible for higher anthocyanin content in camu-camu.

The behaviour of the flavonoid contents indicates a relationship between flavonoid content and maturation; that is, the more mature the fruits of camu-camu, the higher the flavonoid content. These figures are equivalent to those found in research carried out by Chirinos et al. (2010) and Neves et al. (2015), who found that the more advanced the maturation stage, the higher the flavonoid content.

Neves et al. (2015), working with camu-camu fruits in different maturation stages, observed that when the fruits are more reddish, the flavonoid contents (flavones and flavonols) in the peel reached 60,000 µg 100 g^{-1}, while the pulp presented no more than 2,000 µg 100 g^{-1} pulp.

This is due to high amounts of vitamin C, anthocyanins, carotenoids and flavonoids, which are phenolic compounds abundant in mature fruits. Values superior to those recorded in this study were observed Neves et al. (2015), who studied pulp + camu-camu freeze-dried peel and recorded values of up to 13,873 g equivalents of gallic acid 100 g^{-1} sample. On the other hand, Fujita, Borges, Correia, Franco, and Genovese (2013) found values similar to those of this study.

Villanueva-Tiburcio et al. (2010), working with fresh and dried camu-camu fruits in three maturation stages, verified that a large amount of phenolic compounds in the peel of camu-camu fruits was observed in the dry peel of semi-mature fruits. The same was observed for ascorbic acid content, since it is a phenol, and significantly influences the results of total phenolic compounds. Similar results were also observed Chirinos et al. (2010): the fruit studied showed high amounts of phenolic compounds. This fact can be explained by both the genetic variability and climate conditions of each producing area. These antioxidant activity values are lower than those found in the literature regarding experiments using the FRAP methodology to observe the antioxidant capacity of camu-camu fruits (Fujita et al., 2013; Rufino et al., 2010).

The antioxidant activity (DPPH) of camu-camu fruits is closely linked to the reducing power of ascorbic acid and other phenolic compounds present in different concentrations and in different maturation stages. Analyses have also indicated that losses were due to processing and storage, even cooled, which sharply reduced the antioxidant activity of these compounds compared to those found by other scientific investigations (Fracassetti, Costa, Moulay, & Tomás-Barberán, 2013; Rufino et al., 2010).

Chirinos et al. (2010) observed mean values of 153, 185, and 167 µmol TEAC g^{-1} pulp in immature, mature, and semi-mature camu-camu fruits, respectively. The absorbance used for reading can also influence and underestimate the remaining DPPH and thus influence the antioxidant activity of the sample (Prior, Wu, & Schaich, 2005). Further dilutions carried out during the analysis, as well as the soil and climatic conditions of the production area, may have significantly influenced the results. Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Hawkins Byrne (2006), when measuring the antioxidant activity of guava fruits by the methods of DPPH and FRAP, among others, found that the FRAP method showed the highest correlation with the contents of ascorbic acid and phenolic groups.

Conclusion

Postharvest storage in the semi-mature and mature ripening stages showed correlations with the bioactive compound contents and antioxidant activity of camu-camu fruits.

The highest antioxidant activity (FRAP) is observed in fruits harvested at the semi-mature stage, which allows the fruits to have a longer shelf life.

The carotenoid pigment, flavonoid, anthocyanin, and vitamin C contents are higher in mature fruits, and this stage is the most suitable for obtaining these functional compounds.

Immature fruits present higher mean total phenolic content and antioxidant activity (DPPH) during storage, showing that vitamin C indicates the functional potential of camu-camu and thus classifying it as a good source of antioxidant compounds;

The mature stage is the most recommended for the extraction of pigments from camu-camu fruits.

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