

Full Length Research Paper

Qualitative evaluation and biocompounds present in different parts of camu-camu (*Myrciaria dubia*) fruit

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Camu-camu fruits have high functional importance and great potential as food. Knowing and determining the nutrients present in its fruits is crucial, in order to understand the relationship between consumption and human health. For this reason, the objective of this work was to evaluate qualitative attributes, such as organic acids content, antioxidant capacity and functional potential of different parts of camu-camu fruit. The fruit used in the present study were collected in the state of Amazonas, Brazil, and analyzed at the University of Florida, Gainesville, U.S.A. Seed, peel, pulp, pulp+peel, and whole fruit were evaluated regarding pH, soluble solids, titratable acidity, and levels of ascorbic acid, phenolic compounds, anthocyanins, and flavonoids. Antioxidant activity was evaluated by the Ferric Reducing Antioxidant Power (FRAP) and Diphenyl-1-picrylhydrazyl (DPPH) methods. Based on the results, camu-camu fruit proved to be a good source of acids, and the pulp is the part that is most concentrated in organic acids. Also, the pulp presents the highest amount of phenolic compounds and highest antioxidant activity. The peel had relatively higher amount of pigments, anthocyanins and flavonoids, and higher concentration of ascorbic acid, proving that it can also be used as a source of bioactive compounds.

Key words: Functional food, antioxidant activity, Ferric Reducing Antioxidant Power (FRAP), Diphenyl-1-picrylhydrazyl (DPPH), pigments, Caçari.

INTRODUCTION

Camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh), also known as 'caçari', 'araçád'água', or 'sarão', belongs to the family Myrtaceae, and is native to the Amazonian floodplains and lake (Maeda et al., 2007). For being a

fruit species with great nutraceutical and technological potential, the interest of the scientific community on this species has increased in recent years.

Fruits are important nutritional sources, since they are

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rich in vitamins, minerals and carbohydrates. Some species have higher content of a specific nutrient when compared with others. The increasing interest in camu-camu fruit is due to its remarkable ascorbic acid (vitamin C) content, and it is known as "King of Vitamin C" or "Super Fruit". In the state of Roraima, Brazil, the fruit is known as 'çaçari', presenting mean values of 3,571 to 7,355 mg ascorbic acid 100 g⁻¹ pulp (Aguiar and Souza, 2016; Chagas et al., 2015; Grigio et al., 2015; Grigio et al., 2016).

In addition to vitamin C, these fruits contain other antioxidant compounds, such as carotenoids, anthocyanins and other phenolic compounds which are valuable in human nutrition (Silva, 2012). These compounds help in the fight against and prevention of free radicals, increasing immune resistance and delaying the early or natural aging of cells (Santos et al., 2009).

However, the *in natura* consumption of camu-camu fruits is low due to the high content of acids (Azevedo et al., 2014), limiting its current consumption to the producing regions. The rest of the production is exported in the form of pulp, mainly to the United States, Europe and Japan (Akter et al., 2011), for the development of products that are considered natural sources of biocompounds or antioxidants.

Several studies have been carried out with the by-products obtained from fruit pulping, and they denote that the peel has great potential as promising sources of bioactive compounds, even though it is underutilized. These compounds can be used as functional food, not only in the Amazon region, but also in other parts of the world (Fracassetti et al., 2013; Azevedo et al., 2014; Azevedo et al., 2015). Moreover, other by-products that are considered waste could have great economic, nutritional, and functional potential. The seed, for instance, has also proved to be excellent source of minerals, with potential to be used in biotechnological development (Sousa et al., 2015).

Vitamin C has vital effect on the brain, since neurodegenerative diseases usually involve high levels of oxidative stress. Moreover, ascorbic acid has positive therapeutic implications against Alzheimer's disease, Parkinson's disease, Huntington's disease and stroke (Harrison and May, 2009). Thus, the potential of camu-camu to minimize the risks of these diseases is extremely significant.

Camu-camu has great functional and bioactive potential. Besides being rich in vitamin C, it has high levels of phenolic compounds, such as flavonoids. Some of these phenolic compounds also have antioxidant and anti-inflammatory properties, with potential to combat chronic diseases induced by the stress, when the fruits are consumed as part of the diet (Fujita et al., 2015). Antioxidants are chemical compounds that can prevent or reduce the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen. Antioxidants

have the ability to react with free radicals and thus restrict their effects on the organism (Couto and Canniatti-Brazaca, 2010).

Several studies have reported camu-camu as a food that helps spermatogenesis. The species also present antimutagenic potential, and helps treat diseases such as Alzheimer's and Parkinson's, diabetes, hypertension and obesity (Schwertz et al., 2012; Gonzales et al., 2013; Borges et al., 2014; Carvalho-Silva et al., 2014; Gonçalves et al., 2014; Azevedo et al., 2015; Fujita et al., 2015; Langrey et al., 2015). For this reason camu-camu has attracted great interest in the scientific environment, in order to exploit all its functional potential, nutraceutical and nutritional, which are still unknown and under-exploited.

Due to the high functional importance and to the great potential of camu-camu fruit, the knowledge and determination of its nutrients is imperative in order to understand the relationship between consumption and human health. And mainly phytochemical concentration differences between different parts of the camu-camu fruit are not known. For this reason, the objective of this work was to evaluate qualitative attributes, antioxidant capacity and functional potential, of different parts of camu-camu fruit.

MATERIAL AND METHODS

The camu-camu fruit used in the experiment were collected from a private property, in planted area, in Amazonas, located on Highway AM 010, km 98, Rio Preto da Eva-Brazil.

The fruit were collected at a mature stage, which was established in previous studies (Grigio et al., 2015). After harvest, fruit were taken to the Post-Harvest Laboratory of Embrapa Roraima, where they were cleaned and selected for the absence of damages, washed in running water, and sanitized with 0.02% sodium hypochlorite (NaClO) for 30 min, following Brazilian National Health Surveillance Agency (ANVISA) recommendations. After the hygienization, fruits were processed according to the treatments. Some fruits were properly pulped in an industrial pulper, with no water, by separating the pulp from the peel and the seeds; other fruit were kept intact; and some other fruit had only the seeds removed, keeping the pulp+peel for the treatments.

The treatments consisted of: peel+pulp, pulp, peel, whole fruit and seed. Totaling five treatments composed of four replicates each, where each replicate was from a sample of 30 fruits.

After the separation of the material according to the treatments, samples were lyophilized, stored in aluminized bags, and transported to the University of Florida, Gainesville, FL, U.S.A. for analysis.

The material was rehydrated according to the previously calculated moisture content equivalent to each treatment, until obtaining 10 g of fresh sample. The material was centrifuged in a refrigerated centrifuge at 4°C, 12,000 rpm, for 20 min, and the supernatant was removed for the following analyses:

pH

This was determined by reading 5 mL aliquot of the sample, using

an 814 USB sample processor.

Soluble solids (SS)

Soluble solids were estimated by the reading, using a portable refractometer (SOLOESTE, model RT-30ATC), with automatic temperature compensation (10 to 30°C). Results were expressed in °Brix (Institute Adolf Lutz, 2008).

Titrateable acidity (TA)

This was determined by titration, following the methodology described by the Institute Adolf Lutz (2008). Results were expressed in mg of citric acid 100 g⁻¹ sample.

Ratio (SS/TA)

This was calculated by the solid soluble:titrateable acidity ratio.

Ascorbic acid

The samples were extracted using 0.5% oxalic acid and titrated with 2,6-dichlorophenolindophenol (Ranganna, 1986). Results were expressed in mg of ascorbic acid 100 g⁻¹ sample.

Total flavonoid content

This was determined by the aluminum chloride colorimetric assay (Zhishen et al., 1999), using quercetin as standard. For the extraction, methanol solution and 5% aluminum chloride were added. After 30 min, spectrophotometer readings were performed at 441 nm. For each sample, a blank containing only sample and methanol was made. Results were expressed in µg of quercetin equivalent g⁻¹ sample.

Total anthocyanins content

This was determined according to the method described by Lee and Francis (1972), using cyanidin as standard. An acidified solution of methanol (methanol:HCl, 85:15, v:v) was added to the samples. Once being homogenized, samples were stored in the dark. After 24 h, samples were read in a spectrophotometer at 520 nm. Results were expressed in µg of cyanidin equivalent g⁻¹ sample.

Phenolic compounds

This was determined according to the Folin-Ciocalteu spectrophotometric method described by Singleton et al. (1999). A 20 µl aliquot of the sample was diluted in 1.58 mL water. Afterwards, 100 µL of the Folin-Ciocalteu reagent was added and wellmixed. After 30 s to 8 min, 300 µl of the sodium carbonate solution was added and stirred. Solutions were allowed to stand at 20°C for 2 h, and the absorbance of each solution was determined at 765 nm. Results were expressed in mg of gallic acid g⁻¹ sample.

Antioxidant activity (DPPH)

Total antioxidant activity was determined by the oxidation inhibition

potential, using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) as reference (Brand-Williams et al., 1995). One gram of the sample was weighed, 10 mL of ethyl alcohol was added and homogenized, and the mixture was taken to centrifuge at 6000 rpm for 50 min. Subsequently, the supernatant was separated using a pipette, and the solution was stored in a dark bottle in an ice-bath, added to 3 mL ethanol. The standard curve was made with ascorbic acid. Absorbance was read using a spectrophotometer at 517 nm, in 500 µL of the sample extract, added to 300 µL of the DPPH stock solution. Results were expressed in mg of ascorbic acid equivalent g⁻¹ sample.

Antioxidant activity (FRAP)

Total antioxidant activity was also estimated by the iron reduction method (FRAP), following the procedure adapted by Rufino et al. (2006). One gram of sample was added to 40 mL 50% methanol, which was homogenized and allowed to stand for 60 min at room temperature. Afterwards, samples were centrifuged (15,000 rpm) for 15 min, and the supernatant was transferred to 100 mL volumetric flask. Forty millimeter 70% acetone was added to the residue of the first extraction, which were then homogenized and allowed to stand for 60 min at room temperature.

One hour later, samples were centrifuged again (15,000 rpm) for 15 min, and the supernatant was transferred to the volumetric flask containing the first supernatant, and the volume was completed with distilled water. The extract and the FRAP reagent (Acetate buffer, 0.3 mol/L, 2,4,6-Triz(2-pyridil)-s-triazine (TPTZ) solution, 8.0 mmol/L and ferric Chloride solution, 20 mmol/L) were brought to a water bath at 37°C. The standard curve was made with ascorbic acid. Absorbance reading was performed at 595 nm. Results were expressed in mg of ferrous sulfate g⁻¹ fruit.

Data were subjected to analysis of variance, and the means were tested by the Tukey test at 5% probability using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Results show statistically significant difference for all the treatments tested. When comparing the pH, only the seed was different from the others, with mean value higher than that of the other treatments (Table 1). Thus, the seed presented lower acidity when compared with the other parts of the fruit. Values lower than those observed in the present study have been reported in the literature (Akter et al., 2011; Barreto et al., 2013; Rufino et al., 2009).

The seed had the highest content of soluble solids, followed by whole fruit, peel, and peel+pulp, which did not show statistically significant difference between them. Finally, the pulp had the lowest soluble solids, with mean value of 5.53 °Brix, similar to the reports found in the literature regarding the pulp (Barreto et al., 2013; Fujita et al., 2013; Grigio et al., 2016) and the fruit (Rufino et al., 2009). Thus, it can be inferred that camu-camu seeds likely have the highest sugar content. Similar behavior was observed by Daiuto et al. (2014), who detected higher sugar contents when evaluating avocado seed. This greater amount of sugar is possibly the necessary source for seed germination.

Table 1. pH, soluble solids (SS), titratable acidity (TA), ratio (SS/TA) in different parts of camu-camu fruit.

Parameter	pH	Soluble solids (°Brix)	Titratable acidity (mg citric acid 100 g ⁻¹ sample)	Ratio (SS/TA)
Peel+Pulp	3.12±0 ^B	6.93±0.2 ^B	4.05±0 ^B	10.79±0.2 ^{BC}
Pulp	3.09±0.1 ^B	5.53±0 ^C	4.86±0 ^A	10.39±0 ^{BC}
Peel	3.16±0 ^B	7.00±0.2 ^B	2.96±0.1 ^C	9.93±0 ^C
Whole fruit	3.09±0 ^B	7.26±0 ^B	4.69±0.1 ^A	11.79±0.1 ^B
Seed	3.93±0 ^A	13.86±1.3 ^A	4.33±0.1 ^B	19.19±1.3 ^A

*Means±standard deviation followed by the same letter in the column do not differ by the Tukey test at 5% probability.

Table 2. Anthocyanins, flavonoids, phenolic compounds, antioxidant activity (FRAP and DPPH) and ascorbic acid in different parts of camu-camu fruits.

Sample	Anthocyanins (µg g ⁻¹ sample)	Flavonoids(µg g ⁻¹ sample)	Phenolic compounds(mg g ⁻¹ sample)	Antioxidant activity (FRAP) (mg g ⁻¹ sample)	Antioxidant activity (DPPH) (mg g ⁻¹ sample)	Ascorbic acid (mg 100 g ⁻¹ sample)
Peel + Pulp	50.7±1.5 ^B	440.9±4.9 ^C	130.6±2.9 ^B	11.7±0.2 ^A	8.53±0 ^{AB}	4,426±90 ^A
Pulp	13.6±1.3 ^D	91.5±6.5 ^D	141.2±3.9 ^A	11.7±0.3 ^A	8.46±0 ^B	2,919±55 ^C
Peel	107.2±2.6 ^A	550.8±12.3 ^A	98.1±0.6 ^D	11.7±0 ^A	8.53±0 ^{AB}	3,068±135 ^{BC}
Whole fruit	29.9±0.6 ^C	517.6±21.0 ^B	117.7±5.9 ^C	11.5±0.4 ^A	8.59±0 ^A	3,204±61 ^B
Seed	n. ^d	49.6±2.8 ^E	n. ^d	8.4±0.2 ^B	2.45±0 ^C	551±31 ^D

*Means±standard deviation followed by the same letter in the column do not differ by the Tukey test at 5% probability. ** n.d = not detected.

The highest titratable acidity index was observed in the pulp, according to the method of the Instituto Adolf Lutz, and it did not statistically differ from the whole fruit, followed by the seed and the peel + pulp, which also did not have statistically significant difference between them. The peel had the lowest acidity index. These results are consistent with those found in the literature (Rufino et al., 2009; Akter et al., 2011; Barreto et al., 2013; Fujita et al., 2013).

The pulp and the whole fruit had higher values of acid. Results indicated that a large number of acids are present in the pulp of camu-camu fruits, corroborating the literature that reports camu-camu fruit to be an extremely acidic one (Rufino et al., 2009; Grigio et al., 2015; Maeda et al., 2006). On the other hand, the lowest values were observed in the peel. This fact is consistent with the fact that camu-camu pulp is the part of the fruit that contains the highest contents of acids, since in the treatments with pulp these values were always higher than in the treatments with peel or seed.

Regarding the acceptability index, or ratio, higher values were observed for the seed, since it contained a greater quantity of Brix, a proxy for soluble sugars, consequently denoting more expressive values to this ratio, followed by the whole fruit, peel+pulp, and pulp, which did not differ statistically between treatments. The lowest soluble solids/titratable acidity ratio was observed peel+pulp and pulp.

When analyzing the total anthocyanins content, statistically significant difference was observed among all the treatments. As expected, the content was higher in the peel of camu-camu fruits, due to the greater purplish pigment, with mean value of 107.19 µg g⁻¹ sample (Table 2), followed by peel + pulp, and whole fruit, with mean values of 50.70 and 29.90 µg g⁻¹ sample, respectively. Lower incidence of this pigment was observed in the pulp, and no traces of anthocyanins were detected in the seed. Similar behavior was observed by Solis et al. (2009), when evaluating the pulp, peel and seed of camu-camu fruits. The more mature the fruits, the higher was the anthocyanin content (Pinto et al., 2013).

In relation to the total flavonoids content, statistically significant difference was observed among all treatments. The highest values were: Peel (550.80), whole fruit (517.65), peel+pulp (440.92), pulp (91.54), and seed (49.56 µg g⁻¹ sample). The peel presented the highest concentration of these pigments, such as anthocyanins and flavonoids, which have antioxidant potential. Anthocyanins were responsible for the color of the peel, corroborating with other studies that evaluated the pulp, the peel and seed of camu-camu fruits (Solis et al., 2009).

Currently, camu-camu has been considered one of the richest foods, mainly due to its great amount of phenolic compounds, and consequently to its great antioxidant potential. The highest amount of phenolic compounds

was observed in the pulp, followed by pulp+peel, whole fruit, and peel, with mean values of 141.18, 130.57, 117.66 and 98.15 mg of gallic acid g⁻¹ sample. Phenolic compounds were not observed in the seed. Similar results have reported the pulp with the greatest amount of phenolic compounds (Solis et al., 2009). In particular this fruit is known for its content of ellagic acid derivatives (Francassetti et al., 2013), which we assume are preserved in our dried powdered samples.

Several studies have been carried out in order to evaluate the amount of phenolic compounds present in the fruits, and a great difference on the mean values had been observed between different studies. This difference may be due to genetic diversity or even to conservation methods and maturation stage of the fruit, resulting in the divergent data found in the literature (Bataglion et al., 2015; Fracassetti et al., 2013; Fujita et al., 2013; Neves et al., 2015; Solis et al., 2009; Rufino et al., 2010; Villanueva et al., 2010).

The FRAP method detected greater antioxidant activity than the DPPH method, and thus, the former is considered one of the most indicated to evaluate camu-camu fruits (Rufino et al., 2010). Higher antioxidant activity was observed in both methods when both pulp and peel were evaluated. However, antioxidant activity is very low in the seed. Much information is found regarding the antioxidant activity of camu-camu fruits, and several methods are being tested. However, all of them lead to the same result, proving that camu-camu is one of the fruits with the highest antioxidant activity, and with great potential for application and development as a functional food (Baldeon et al., 2015; Barreto et al., 2013; Bataglion et al., 2015; Fracassetti et al., 2013; Fujita et al., 2013; Neves et al., 2015; Rufino et al., 2010; Solis et al., 2009; Villanueva et al., 2010).

The highest concentration of ascorbic acid was observed for the peel+pulp, with mean value of 4.426 mg acid 100 g⁻¹ sample, followed by the whole fruit and the peel, which did not present statistical difference between them. On the other hand, the lowest values were also observed for the seed. The highest values were always observed in the treatments that contained the peel, which is in agreement with other results found in the literature (Zamudio, 2007; Imán-Correa et al., 2011). According to Fujita et al. (2013), the lyophilization process can generate losses of ascorbic acid and of other polyphenols. However, our study shows that the products can still retain considerable amounts of vitamin C, phenols, anthocyanins and antioxidants, even after being dried.

Conclusion

Results show that camu-camu fruit has good qualitative attributes. The pulp had the greatest amount of phenolic

compounds and antioxidant activity. Higher amounts of pigments (such as anthocyanins and flavonoids), and higher concentration of ascorbic acid were found in the peel, making it useful as a source of bioactive compounds as a food colorant with antioxidant qualities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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