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Bioactive Compounds and Antioxidant Activity of Camu-Camu (Myrciaria dubia (Kunth) Mc Vaugh) Grown on a Non-Flooded Land Ecosystem

Adele Salomão-Oliveira[1,*], Helyde Albuquerque Marinho[2]

[1]Department in Food Science, Universidade Federal do Amazonas (UFAM), Manaus-AM, Brazil
[2]Department of Research, Society and Health, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus-AM, Brazil
*Corresponding author: adelesalomao@gmail.com

Abstract Camu-camu (*Myrciaria dubia* (Kunth) Mc Vaughn) stands out as a major Amazonian native fruit with broad market perspective, since it is an exceptional source of ascorbic acid and other antioxidants present in its composition. This study aimed to determine the freeze-dried camu-camu fruit's bioactive compound characteristics as well as antioxidant, physical and chemical activities. The samples were from the Manaquiri-AM district, non-flooded land ecosystem. Centesimal analyses have ranked camu-camu as a "very low density" food presenting: 91.24% moisture, 7.49% carbohydrates and low protein, lipids, ash, and fiber content values. The energy value showed to be 36.97 kcal. Camu-camu went throught freeze-drying process and parameters pH, Aw, total phenols, flavonoids and ascorbic acid content analyses. The values found in this study concerning the antioxidant activity by using DPPH IC_{50} sequestering method, showed to be 26.70 $\pm$ 0.76 µg / mL and the acid ascorbic estimated value is 3.04±0.06 $g.100^{-1}$. The results demonstrate non-flooded land camu-camu to be able to be used as a natural antioxidant promoting good health as well as contributing to the Amazon region's folk economy.

Keywords: *Amazonian fruit, antioxidant, ascorbic acid, bioactive compouds, non-contagious chronic diseases*

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1. Introduction

Camu-camu (*Myrciaria dubia* (Kunth) McVaugh) is a native Amazonian fruit tree species, known as water araçá, floodplain araçá, caçari and other names, as well. It occurs spontaneously on river banks and lake shores, its highest population and variety concentrations are to be found in Peruvian Amazon, extending to Venezuela, Colombia and Brazil [1].

Camu-camuzeiro is a shrub belonging to the family Myrtaceae. Its fruit is round-shaped, smooth and glossy-skinned, ranges from dark red to dark purple-colored, weighs from 4.5 to 15.5 g, and holds from one to four seeds, when ripe. This cherry-like fruit plays an important role on human nutrition, since it is a major bioactive substances source standing out for its, even higher than that found in most citrus fruits, ascorbic acid content [2].

There is currently an ever-growing concern on human health and encouraging the search for healthy foods through the consumption of both fresh and processed fruits, since many of them have shown to be rich in antioxidant substances, which helps to lower the chronic degenerative diseases incidence and, thus delay the early ageing process. Natural antioxidants have aroused great interest throughout the world, especially in the past few years, due to the health benefits brought about through their consumption as well as use by food, cosmetics and pharmacological industries [3].

Post-harvest camu-camu fruits shelf life has shown to be quite short, due to their high breathing rates. Depending on ambient temperature, in around three to five 5 days, this fruit starts exhibiting high physiological perishability, which confines its transport and storing time. Thus, the development and use of appropriate technology for better preserving the fruit and its quality characteristics becomes paramount, since it extends the time for the products conservation and commercialization, facilitating its transport and storage. Lyophilization or cold dehydration (freeze dry) stand out amongst the employed techniques, since it is a preservative or chemical product free process used for preserving and increasing the foods stability, enabling to reduce nutritional losses and, retain high nutrient and bioactive substance concentrations [4].

Under this context, this study aims to assess the fresh and lyophilization-submitted camu-camu's antioxidant capacity, *in vitro* bioactive compound content and, physicochemical characterization.

2. Material & Methods

2.1. Collecting Site and Fruit Transport

Camu-camu batches in the ripe physiological stage were harvested from non-flooded land ecosystem cultivars

at Experimental Station, on Manaquiri /AM County roadway km 8 (03º 25' 41" S and 60º 27' 34" W), 150 km from the city of Manaus, in three different months (June, August and September 2014) so they would constitute three independent samples. Fruits were packed in 5 kg batches stored into 10 L black polyethylene bags, placed in icepack-filled cold boxes and then transported to the nutrition biochemistry laboratory / INPA, on the same day (Figure 1).

Figure 1. Mature-colored camu-camu fruits

2.2. Selection, Cleaning and Sanitization

Characteristic mature-colored fruits were selected by excluding green and injured ones. Then, fruits were randomly mixed by taking the following steps: pre-washing under running water to remove macroscopic dirt, sanitized through being soaked in 50 ppm sodium hypochlorite solution for 10 minutes and then washed in running, drinking water [5].

2.3. Sample Preparation

Seeds were removed by hand with the aid of a previously sanitized stainless steel butter knife, so as perform fruit (flesh and skin) yield assessment. Remaining pulps were then immediately placed in sealed polyethylene bags and stored a freezer at (-18°C).

2.4. Physical Analysis Fresh Camu-Camu

Thirty (30) fresh camu-camu units were randomly weighed on a leveled and tared Shimadzer, 320 g capacity precision scale and diameter were mesured with a 0 to 8.0 mm range caliper.

2.5. Camu-Camu Pericarp (Rind and Pulp) Proximate Analysis

Fresh camu-camu pericarp's proximate composition was performed so samples would be characterized. Moisture, proteins (factor 6.25), hash and lipids analyses were carried out according to methodologies described by [6,7]. Total carbohydrates were determined through proximate composition and calorific value difference by Atwater conversion factors.

2.6. Crude Fiber Determination

Samples were desiccated and defatted, and digestion processes carried out in acid medium (1.25% H2SO4), followed by digestion in an alkaline medium (NaOH 1.25%) in the fiber determiner TE-146/8 50 TECNAL [7].

2.7. Pericarp Chemical Analyses and Lyophilization

Pericarp (rind and pulp) freeze drying was processed through the benchtop freeze dryer: Brand: Virtis, SP Scientific, Wizard 2.0, model advance plus ES-53, 208/230 volts, amps 10, Hz 60 with an initial pressure at 100 mTorr.

2.8. pH and Aw

Hydrogen potential was determined through mod: Q400AS 90-240 V, 10W, Quimis benchtop pH meter [7]. The achievement of determining the water activity at 25 °C was performed using the apparatus AquaLab - Dew Point Water Activity Meter 4 TE, mod. S4TE, 110 w Brazilian BrasEq- Equipment Ltda.

2.9. Determination of Total Phenols, Flavonoids and Free Radicals Sequester (DPPH e ABPS).

The determination of the content of total phenols present in the samples was done by the Folin-Ciocalteu method, with modifications [8] and gallic acid standard slope (EAG). To perform the test in triplicate, the extracts were first diluted in ethanol at a concentration of 1 mg/ml. Then, 10 µL and / or standard gallic acid (diluted in ethanol at a concentration of 1 mg / mL) were added to each extract in each microplate well. Right after that we added 50 µL of Folin-Ciocalteu reagent 1/10 diluted in distilled water. The plate was incubated at room temperature for 8 min. Finally, we added 240 µL more of sodium carbonate. For the white 50mL of water, 10µL extract and / or standard and 240 µL of sodium carbonate, were utilized. The plate was then incubated at room temperature for 3 min and, then, the absorbance reading determination was done at 620 nm wave length on ELISA reader. Results were expressed in total phenols mg in galic acid equivalent (GAE). Findings were attained using the following calculation:

$$\text{Total phenols} = \frac{\text{Extract absorbance} - \text{White absorbance}}{\text{Standard absorbance}} \text{x} 100.$$

Total flavonoids determination was performed in triplicate [9]. Extracts were diluted in ethanol in the concentration of 1 mg/mL. Thirty (30) µL of each extract and/or standard quercetine (diluted in 80% ethanol in the concentration of 0.1 mg/mL) and 90 µL of 95% ethanol were added into each microplate well. Then 6 µL of 10% aluminum chloride and 6 µL of 1M potassium acetate, both diluted in distilled water, were added. For the white, 30 µL of extract and/or standard quercetine and 270 µL of

ethanol, were utilized. The plate was incubated at room temperature for 30 min and, then, the absorbance reading was determined on ELISA reader at a 405 nm wavelength.

Findings were first attained by utilizing the samples and standard absorbance difference between the respective whites, and then, the flavonoids concentration determination was calculated:

$$\text{Flavonoids} = \frac{\text{Mean, sample absorbance}}{\text{standard absorbance difference}} \text{x} 100.$$

Antioxidant activity determination was carried out in triplicate, according to the free radical reduction method DPPH with modifications [10]. Extracts were diluted in 1 mg/mL-concentrated ethanol. Then, 30 µL of each, equally-concentrated extract and/or standard quercetine were added into each microplate well, in their respective-seriated dilution. Next, 270 µL of DPPH radical were added into every microplate well. And 30 µL of ethanol and 270 µL of l DPPH radical were used as controls. Then, the plate was incubated in the dark at room temperature for 15min and, the absorbance reading in 492 nm wavelength, was done next. The free radical sequestering capacity was attained in two ways: through inhibitory concentration value (IC_{50}), which represents the amount of substance needed to reduce the initial DPPH concentration by 50%, and also through radical oxidation inhibition percentile as calculated by equation:

$$\text{Antioxidant Potential} = 100 - \left(\text{Abs}^{\text{sample}} / \text{Abs}^{\text{control}}\right) \text{x} 100.$$

Antioxidant activity against ABTS free radical was determined according to the method described [11]. The ABTS radical action was prepared from potassium persulfate stock solution ($K_2S_2O_8$, MM 270.3g mol; 140 mmol L^{-1}), resting in the darkness a room temperature, for 12 horas before being used. The standard Trolox curve was used. Absorbance was measured, from the standard curve, at 734 nm and the findings expressed as µmol of Trolox g^{-1}.

2.10. Ascorbic Acid (AA)

The fresh and lyophilized samples ascorbic acid content's determination was performed using the analysis quantification method through high-performance liquid chromatography (HPLC), which consists of extracting the AA from the lyophilized product (100 mg) using 10 mL of extracting solution comprising water, 8% acetic acid (v/v), 1 mL of EDTA, 0.3N sulfuric acid and M-phosphoric acid (MPA) 3% (v / v) in triplicate, in ice bath, following the procedures described at 5°C [12]. The equipment used was the Thermo® chromatograph with automatic injector and quaternary pump, diodes array detector (HPLC-DAD) and, XCalibur® software-equipped computer for obtaining spectrometric data.

2.11. Carotenoids Determination

The method proposed was utilized for quantifying and identifying carotenoids. All method steps were performed light-sheltered and, pigment-containing extracts were secured with aluminum foil. Each carotenoid's identification was quantified from its own visible, Beer law-based maximum absorbance spectrum. The following formula was used to quantify carotenoides content [13]:

$$g \text{ of Carotenoids} = \frac{\text{MaximumAbsorbance x Sample Volume (mL)} \text{x} 10^6}{100\% \ E^{1\%} \text{x Sample Weight (g)}}.$$

2.12. Chromium Determination

Analysis was performed by absorption spectrometry with electrothermal atomization (Graphite furnace), brand: Perkin-Elmer, model: Simaa-6000, according to the Health Canada Applications Chemist SCP Science methodology [14].

2.13. Experimental Design and Statistical Analysis

Analyses were performed according to entirely random design (ERD), with three replications. The results were analyzed by analysis of variance (ANOVA) and the F-test mean comparison Tukey test, both at the probability level of 5% using the Minitab statistical software, version 14.

3. Results and Discussion

Table 1 shows the arithmetic averages of the results of the physical characteristics of camu–camu batches collected in different months. The total fruit weight ranged from 3.16 g to 18.65 g and averaged 9.63±3.00 g. As to the mean height and diameter, they showed to be 2.50±0.27cm and 2.70 cm, respectively. There was a relationship between weight, height and diameter ($p<0.05$). The average yield of the pericarp of the fruits formed by the ensemble of bark and pulp was 7.39±1.76g, corresponding to 76.8% (Table 1). The weight of the seeds varied between 19 and 28% of the total weight of camu-camu, thus, the yield would range from 71 to 80 %, which corroborates the findings (71 and 83 %) found in literature [15,16].

Table 1. Camu-Camu Fruit's Physical Analysis.

Fruit Variables								
	Weight (g)				Yield (g)			
Batch*	$\bar{X}$	SD	Mín	Max	$\bar{X}$	SD	Mín	Max
1	11.60	±0.58	5.06	18.65	6.35	±0.74	3.38	11.46
2	9.74	±0,31	6.16	13.32	7.46	±0.20	5.30	9.81
3	7.53	±0.44	3.16	16.70	5.25	±0.26	3.16	10.75

* (Harvest month) batch 1- June, batch 2- August and batch 3- September.

Table 2 shows camu-camu fruit (*Myrciaria dubia* (Kunth) McVaugh) proximate analysis. It is fundamentally important to know the raw material's composition when applying different technological processes, which along with interfering in the overall quality aspect, influence the final product's sensory attributes and stability. The

nutritional composition of the pericarp (pulp + peel) of camu-camu highlights the high moisture content (91.24%) and negligible protein and lipid values. A food's water content is related to energy density (ED) defined as the energy available per weight unit (kcal / g or kj / g), thus, camu-camu is classified as food of "very low density", that is of ED, between (0 and 0.7 kcal / g) according to the CDC energetic density [17]. The carbohydrate content indicates camu-camu not to be a good source of this nutrient, according to the Brazilian population's Food Guide, which refers to the food's ED as a strategy for promoting a healthy diet since consuming low ED food contributes to diminish the caloric total and, consequently, prevents weight gain along with chronic non-transmissible diseases [18]. Camu-camu's energetic value (36.97 kcal) as well as ashes and fibers contents (2.46%) corroborate the findings of literature [19]. According to what Agência Nacional de Vigilância Sanitária [20] establishes, a solid food can only be said to be a source of fiber when the latter represents at least 3% of its composition.

Table 2. Centesimal Composition of the Pericarp of Camu-Camu (*Myrciaria dubia* (Kunth) McVaugh) Grown on Non-Flooded Land, as Expressed in g.100^{-1}

Composition (%)	Values Mean and SD
Moisture at 105º C	91.24±0.02
Proteins	0.46±0.01
Lipids	0.57±0.00
Total Carbohydrates *	7.49±0.04
Ashes	0.23±0.03
Fibers (g)	2.46±0.29
Total Energetic Value (kcal)	36.97

Pericarp values expressed in g.100^{-1}, with their standard deviations with determinations in triplicate.
*Calculation by difference.

Food characteristics such as pH and water activity (WA) should be assessed in order to attain products bearing longer shelf life. Deep-frozen camu-camu was ranked as very acid (2.75 ± 0.01), (Table 3) according to the following classification (Little-acid (pH>4.5), (Acid (pH between 4 and 4.5) and (Very acid (pH<4.0) [21]. The latter characreristic "very acid" may be related to the high concentrations of organic acids such as citric, ascorbic and malic acids, in addition to being a major intrinsic factor for narrowing the microorganisms' growth, maintaining the enzymatic activities, the product's flavor and overall conservation, in adition to favoring the ascorbic acid stability [22]. Several countries have been adopting the food's water activity (Wa) determination as compulsory "Standard". The WA lowermost limit for the growth of microorganisms in food has shown to be about 0.60. Deep-frozen camu-camu Ph and WA findings have demonstrated high potential when based on these two stability parameters [23].

Several studies have highlighted flooded areas grown camu-camu fruits to bear various compounds with antioxidant activity, which include polyphenols, ascorbic acid and carotenoids. Literature related to this nonflooded ecosystem-grown fruit's antioxidant potential as well as the obtained findings standardization is still very poor, dificulting comparirons, even though reported data have shown to be unanimous when reporting its remarkable antioxidant capacity. Table 3 shows the findings pertaining to the bioassays on antioxidant activities as determined through DPPH and ABTS methods, in addition to the constitution of compounds bearing antioxidant activity such as ascorbic acid and carotenoids from non-flooded land ecosystem grown camu-camu freeze-dried pericarp. Findinds in this study have shown freeze-dried material-reconstituted camu-camu pericarp to present 1,492.8 ± 0.76 mg GAE 100 g^{-1} mean value (Table 3), grading it as a remarkable phenolic compounds source. This value corroborates with the published literature reporting that high concentration ranging from 1,370 to 2,110 mg GAE 100 g^{-1}, accounts for the fruit's sensory bitterness and astringency characteristic, which narrows its acceptability [22]. It is known that the phenolic compouds content may be influenced by several factors, such as, maturity, crop species, geographical origin, growth level, harvesting and storing process conditions. According to the studies, the camu-camu's epicarp (peel) presents the highest ascorbic acid and phenolic compounds concentration [2].

To assess a plant's antioxidant capacity, one must get the highest extraction from its bioactive compounds that present differentiated polarity, thus, a determined solvent's solubility may be the peculiar phytochemical characteristic that accounts for there being no universal procedure to do it. Therefore, the utilization of different methodologies is indicated to determine the antioxidant effect. Thus, antioxidant activity values as determined by (DPPH and ABTS) methods and ascorbic acid content are found in Table 3.

The estimated fruit's antioxidant capacity showed to be 26.70±0.76 (IC_{50} µg. mL^{-1}), demonstrating it to be efficient. Several studies have indicated phenolic compounds to be the ones most responsible for the antioxidant capacity in fruits, yet, other studies have suggested it to be vitamin C. Studies found low correlation (r^2<0.4) between antioxidant activity through the DPPH method, and ascorbic acid suggesting the antioxidant capacity of camu-camu to be derived from phenolic compounds. These phytochemicals' amount and profile vary according to fruit type, variety and maturation degree as well as crop edaphic and climatic conditions [1,24]. While other studies achieved 114.20 µg. mL^{-1} IC_{50} in the dry peel of ripe camu-camu peel, through the DPPH sequestration method [25].

The ascorbic acid contents detected on camu-camu pericarp showed significant antioxidant capacity contributing to the fruit's bioactive potential, corroborating the literature [24]. Ascorbic acid content values variation, per batch, may be expected, since the seed sowing was carried out as a domestication experimental process with grear genetic variability, in order to achieve the fruit's physicochemical stability. The deep-frozen camu-camu's ascorbic acid content values showed to be higher than those that have been published in the past few years (between 1.72 and 2.58 g/100g) [25]. By adopting the fruits's ascorbic acid content quantitative grading, camu-camu stands out (3.04±0.06 g^{-100}) as a fruit bearing a high content of this micronutrient, namely, (>50 mg/$^{-1}$). Hence, the determined ascorbic acid (Table 3) points out its remarkably high content value, since it shows to be higher than what has

been recommended for the nutrition of both male and female adults [26,27]. Several studies have shown the ripe camu-camu's peel to bear the highest ascorbic acid concentration along with anthocyanin pigments. In this maturating process, the fruit reaches full growth and maximum edible quality while still attached to the plant [1].

Studies have identified in camu-camu positive findings on physiological benefits in the prevention of diabetes and association with adiposity also seems to exist, since central adiposity and low vitamin C intake was associated [28].

Antioxidant activity can not solely be ascribed to ascorbic acid and polyphenols contents in camu-camu, since other antioxidant compunds, such as carotenoids can do it as well. The present study indicates total carotenoids contents to be 545.92±28.86 mg Eq (Table 3). This finding corroborates the 601.9±75.61 g/100g, content found by researchers [29], in camu-camu fruits from Mirandópolis/São Paulo. Epidemilogical and clinical studies have shown that low carotenoids intake or concentration in the blood are associated with macular degeneration. Amongst the benefits to human health, one may verify the cardiovascular disease risk reduction and skin protection from the damages caused by UV radiation [30]. Furthemore, it is able to reduce mutagenisis and, in physiological concentrations, it may inhibit human cancer cells growth, especially in prostate cancer with no evidence of toxic effects or cellular apoptosis [30].

As to the mineral as determined it was 7.60±1.72 μg^{-1} (Table 3), there being no difference regarding the sample batches ($p>0.05$). The adequate intake (AI) is 30 µg/day and 20 µg/day for adult men and women, respectively [27]. However, the recommendations for the micronutrient may be achieved by consuming the fruit when deep-frozen, since this process shows there to be an increase on the concentration of nutrients per weight unit as compared to that found in the fruit while still fresh [32]. Studies carried out showed chromium values to be 19.9±1.7 µg%, and their importance for the organism to be related to glycemy and lipids control [33,34].

Table 3. Chemical analysis and bioactive compounds contents of camu-camu fruit (*Myrciaria dubia* (Kunth) McVaugh) freeze-dried (pulp and peel).

Determinations	Sample Camu-camu
pH	2.75±0.03
WA	0.30±0.01
Total Phenols (mg GAE.100 g^{-1})	1,492.88±0.6
Total Flavonoids (mg EQ.100 g^{-1})	23.66±0.11
DPPH (IC50 µg/ml)	26,70±0,76
ABTS (µmol/g)	1,127.99±4.2
Ascorbic Acid (g.100^{-1})	3.04±0.06
Total Carotenoids (mg Eq. ß-caroteno.100 g^{-1})	545.92±28.06
β- carotene (mg.100 g-1)	71.81±3.25
Chromium (μg^{-1})	7.60±1.72

GAE: galic acid equivalent
QE: quercetin equivalent
Eq: equivalent.

According to what has been presented, the camu-camu deep-freezing process has shown to be a viable technology to be employed on the preservation of the fruit's chemical, nutritional and functional charecteristics as well as cultivation on nonflooded land ecosystem. Therefore, it becomes important to put forth feasible technological alternatives, which will warrant significant nutritional contents when the fruit is to be consumed, since the fruit *in natura* is little commercialized and sporadic due to its seasonality. Another aspect to be considered is the socioeconomical issue for the population, and on account of it being a fruit that grows in the natural environment on the banks of rivers, creeks, lakes and swamps, we suggest favoring the Amazonian riverside dwelling popupalions with an incentive for its commercialization on account of its bioactive characteristics reinforced by ascorbic acid and phenolic substances, which are important for promoting good health.

4. Conclusions

Based on the findings acquired under conditions of analysis, the nonflooded land ecosystem grown camu-camu showed, when frozen-dried, high antioxidant capacity, which is a remarkable source for vitamin C, high agroeconomic potential, being an alternative to add value and encourage the fruit's consumption in addition to contribute for prevention of chronic non-transmissible diseases (CND).

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Conflict of Interest

The authors declare no conflict of interest.

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