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Chemical Composition, Minerals, Physicochemical Properties and Antioxidant Activity in *Camu Camu* Seed Oil

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The aim of this work was to perform chemical and physicochemical analysis of oil of the *camu camu* (*Myrciaria dubia* (H.B.K) Mc Vaugh) seeds obtained at Caçari beach, on the banks of the Cauamé river, in Boa Vista city, Roraima state, Brazil. From the seeds, the dark green oil was 2.88% yield. The physical and chemical properties of the oil through the ^{1}H and ^{13}C NMR spectra: iodine content (55.39 g I_2 $100g^{-1}$), saponification index (178.14 mg KOH g^{-1}), acidity (1.46 mg KOH g^{-1}), olefinic/aliphatic hydrogen ratio (0.36), average molecular weight (934.16 g mol^{-1}). Analysis of the signal displacements in ^{1}H and ^{13}C NMR spectra confirmed the presence of the fatty acid functional groups and unsaturated fatty acid unsaturations, so the concentration of linolenic acid was 0.43%, acid 12.02% oleic, oleic was 62.49% and saturated fatty acids 25.10%. The quantitative analysis of minerals by ICP-OES showed concentrations of elements: K, Na, P, Si (> 100 ppm); Mg, S (10 to 100 ppm) and Ti (<100 ppm). The antioxidant activity, through the DPPH method, shows that *camu camu* oil has a high antioxidant percentage (IC_{50} = 114.72 µg mL^{-1}).

1. Introduction

The *camu-camuzeiro*, a shrub species native to the Amazonian *camu camu* (*Myrciaria dubia*) fruit, is found on the banks of rivers, lakes and *igapós*, especially in groups of black and acid waters (Villachica, 1996). In Roraima, Brazil, camu-camu fruits, known as caçari, have a high content of vitamin C (6112 mg 100 g^{-1} of pulp), with an index higher than that of other more popular citrus fruits (Yuyama, 2012).
The fruit populations are concentrated in the middle and upper Amazon River, in the eastern part of the Andes and in Amazonian countries such as Colombia, Venezuela, Guyana, Bolivia, Peru and Brazil (Andrade, 1995). The native forms in Roraima, its vitamin C content and its promising position in the market for pharmaceuticals, cosmetics and food preservatives corroborate with the chemical analysis of *camu camu*.
In the Brazilian Amazon, some *camu camu* production technologies are studied by the Brazilian Agricultural Research Corporation (EMBRAPA). Some improved materials with high productivity can reach 10 kg and 23 kg of fruit/plant/crop. In addition, some cloning techniques propose the vegetative multiplication of the species (Yuyama, 2011).

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Thus, the chemical analysis of vegetable oil, its minerals and the profile of major fatty acids is pertinent. This work therefore aims to chemically characterize the seeds of *camu camu*, since the knowledge of the chemical composition of seeds is an important factor of guidelines for the use of biomass as a food source for men and animals.

2. Materials and Method

2.1 Collection, preparation of sample and obtaining crude oil

The *camu camu* fruits were collected at Caçari Beach in Boa Vista city, Roraima, Brazil. The fruits were brought to the Environmental Chemistry Laboratory, Nucleus of Research and Post-Graduation in Science and Technology of the Federal University of Roraima (NPPGCT/UFRR). The seeds were removed, sanitized and dried in an air circulating oven at 50 ^{o}C for 72 hours, then ground and sieved between 20-40 Mesh (Santos et al., 2015). To obtain the *camu camu* seed oil, the Soxlhet extraction method was used, approximately 100 mL of hexane was used. The extraction was done for 3 hours in triplicate. The oil-hexane mixture was treated with anhydrous sodium sulfate and filtered. Thereafter, the mixture undergoes a separation process by evaporation. The solvent was recovered through the distillation process and then reused. The oil obtained was placed in a nitrogen environment and stored in a refrigerated environment.

2.2 Analysis of *camu camu* seed oil by ^{1}H NMR and ^{13}C NMR spectroscopy

For ^{1}H NMR analysis an amount of 10 mg of the sample was dissolved in 0.5 mL of $CDCl_3$ with spectra recorded on a 500 MHz spectrometer from the central analytical of University of São Paulo with the following conditions: 45° pulse, relaxation time 1 s, acquisition time 2.049 s, scanning width 7,997.6 Hz, line width 0.2 Hz, and number of repetitions 64, with total time of 3 minutes and 21 s. Chemical shifts are presented in ppm, using TMS as internal standard.
For ^{13}C NMR analysis an amount of 10 mg of the sample was dissolved in 0.7 mL of $CDCl_3$ with spectra recorded on a 500 MHz spectrometer from the central analytical of University of São Paulo with the following conditions: 45° pulse, relaxation time 1.132 s, acquisition time 0.845 s, scanning width 18,883 Hz, line width 1.0 Hz, and number of repetitions 160, with total time of 5 to 12 minutes. Chemical shifts are presented in ppm, using TMS as internal standard.

2.3 Analysis of the NMR spectra by Protóleos software

The program called PROTÓLEOS RMN ^{1}H aims to create an environment for the calculation of physicochemical and major fatty acid properties. This program was developed in a free environment to be used as a tool to support the analysis of vegetable oils, calculating physicochemical properties, such as: iodine index (II), acidity index (AI), saponification index (SI), average molecular weight (MW), ester content (EI), percentage of ester (%EI), further calculating the percentages of acids: oleic (ω-9), linoleic acid (ω-6), linolenic acid (ω-3) and saturated (SAT), in addition to calculating the area of proton (AP), olefinic protons (V), total protons (T), ratio of olefinic / aliphatic hydrogens. The program was written in PYQT (free software), where the data input fields are the signals: a, b, c, d, e, f, g, h, i, j, k corresponding to the integrals obtained from the spectra ^{1}H NMR of the oil. All calculations are done directly and automatically grant information by data entry (Farias, 2013).

2.4 Determination of minerals by ICP-OES

The Inductively Coupled Plasma/Atomic Emission Spectrometry (ICP-OES) is a well-established technique widely used in research laboratories. ICP-OES is a multielement analysis technique that operates over a wide linear range of concentrations (4 to 5 orders of magnitude), with fast analysis and low operating cost. Besides these advantages, another important factor is that the technique allows the direct analysis of liquid samples, in case of pneumatic nebulization. Subsequently, the samples were digested using concentrated nitric acid and 30% hydrogen peroxide under microwave oven heating. The equipment used was ICP-OES of the brand Spectro, model Arcos, the procedure was carried out at the Analytical Center of the Institute of Chemistry of the University of São Paulo. Power applied: 1,400 W; Radiofrequency of the RF generator: 27.12 MHz; Plasma gas flow rate: 12 L min^{-1}; Aux gas gas flow: 1 L min^{-1}; Nebulization gas flow rate: 0.85 L min^{-1}; Sample introduction rate: 0.85 L min^{-1}.

2.5 Antioxidant activity

The methodology consists in measuring the extinction of the absorption of the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical at 515 nm. The determination of the antioxidant activity was performed in triplicate by UV-visible molecular absorption spectrophotometry method. The technique consisted of incubating for 10 minutes

500 µL of a 0.1 mM DPPH methanolic solution with 500 µL of solutions containing increasing sample concentrations (500; 1,000; 2,500; 5,000; 10,000; 15,000; 20,000; 25,000; 30,000; 35,000; 40,000; 45,000; 50,000 µg mL^{-1}) in methanol. The same procedure was followed for the preparation of the so-called control solution, but replacing 500 µL of the sample in 500 µL of methanol solvent. To the solution called "white", the solvent used was ethanol. The capture moiety DPPH percentage was calculated in terms of percentage of the antioxidant activity (% AA).

The samples are analyzed in a UV-Visible 515 nm wavelength molecular absorption spectrophotometer in order to evaluate the absorbance of the different sample concentrations and record the results. After the evaluation can be calculated the concentration of oil required to capture 50% of free radical DPPH (EC_{50}) by linear regression analysis (Carbonari, 2005).

3. Results and discussion

3.1 Yield of *camu camu* seed oil

Oil extracted from camu-camu seed had a dark green color. The yield obtained was 2.98%. This is considered to be very low compared to other vegetables, for example: *castanha do Brazil* (50-60%), sunflower (30-50%) and *pinhão manso* (40-50%) (Antoniassi and Freitas, 2017). This low yield is probably due to the fact that its seed is classified as amylaceous, that is, rich in starch, presenting low levels of oleosomes in its composition (Soares et al., 2012).

3.2 Analysis of *camu camu* seed oil by ^{1}H NMR

The ^{1}H NMR spectrum of the *camu camu* seed oil is shown below (Figure 1).

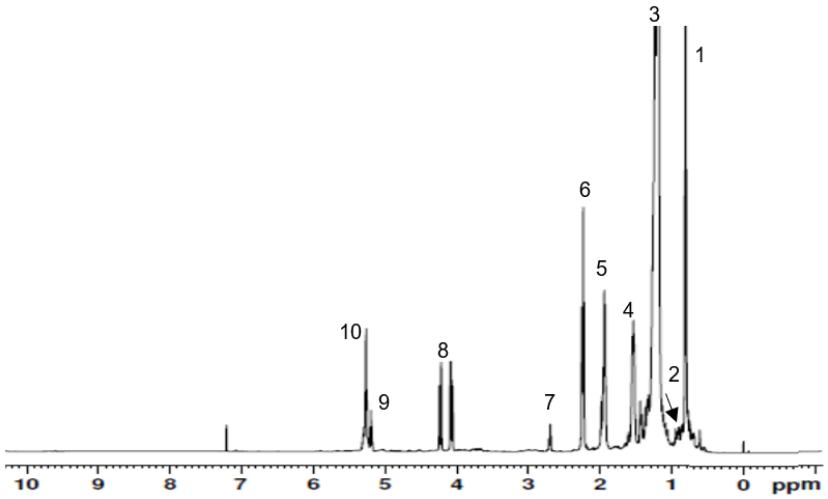

Figure 1: ^{1}H-NMR spectrum of camu camu oil.

The signals of the vegetable oils with protons associated with them are visualized in the spectrum (Figure 1) shown based on the unrefined oil, i.e. a crude oil. The signs are in agreement with the literature (Knothe and Kenar, 2004, Reda and Carneiro, 2006).

These signals from the *camu camu* oil spectrum (Figure 1) are assigned, where signal 1 is corresponding to the methyl protons; signal 2 indicates methylene protons which are in the β-position relative to the double bond, or are present at the position relative to the carbonyl group; signal 3 is corresponding to the methylene protons in position β in relation to the carbonyl group; signal 4 is due to α-methylene protons in relation to double bonds, they are also called allylic protons; signal 5 justifies the methylene protons in position α in relation to the carbonyl group; the signal 6 corresponds to the protons in position α in relation to the two double bonds, also called bis-allylic protons. Signal 7 refers to the protons attached to carbons 1 and 3 in the glycerol group; the signal 8 corresponds to the bonded atoms to the carbon atom 2 in the same glycerol group, this signal is superimposed on signal 9 (methylenic protons of of the glyceryl) and signal 10, which corresponds to the olefinic protons (Guillén and Ruiz, 2003). The proportions of fatty acids were determined from the ^{1}H NMR spectrum (Guillén and Ruiz, 2003).

The amount of linolenic acid (ω-3), 0.43%, and linoleic acid (ω-6), 12.02%, in the *camu camu* oil are very inferior to that of the Sicilian lemon and Rangpur lime. The amount of oleic acid (ω-9), 62.49%, in *camu camu*

oil is higher than that of Sicilian lemon and Rangpur lime oils (Carneiro et al., 2005), but near sunflower oil (Corsini and Jorge, 2008). The saturated fatty acid content (SFA) for *camu camu* oil (25.1%) is compatible with the others compared to the literature, which indicates its tendency to the pasty form. The *camu camu* seed oil also has a saturated fatty acid content close to that of the 23.6% rice seed oil.
Table 1 presents Physicochemical and chemical properties of *camu camu* seed oil determined in this research and compared with others oils of literature.

Table 1: Physicochemical and chemical properties of the camu camu seed oil compared to the literature.

Properties	*M. duabia* **This research**	Melancia (Avila, 2012)	Soja (Reda, 2010)	Limão rosa (Reda, 2010)	Canola (Reda, 2010)	Milho (Reda, 2010)	Girassol (Reda, 2010)
II (g I_2 100 g^{-1})	50.39	114.9	124.20	101.40	103.09	110.60	119.50
SI (mg KOH g^{-1})	178.15	174.00	192.00	183.10	179.00	195.00	186.80
AI (mg KOH g^{-1})	1.46	0.26	0.30	0.28	0.08	0.12	0.31
MW (g mol^{-1})	934.16	951.50	875.30	912.90	929.30	862.40	798.90

Legend: II = iodine index; SI = saponification index; AI = acid index; MW = molecular weight.

In relation to the acid number, the value found for the oil no refined was 1.46 mg KOH g^{-1}. According to the Resolution RDC Nº 270 of ANVISA (2005), the maximum acid value allowed for oils and fatty refined is 0.6 mg KOH g^{-1}. Therefore, this presented value of the *camu camu* seed oil is an acid value superior to the stipulated for oils and fatty refined according to ANVISA.
According to description of calculations above, the saponification index is 178.15 mg KOH g^{-1}. This index is closely linked to the molecular mass of triglycerides. Thus, the lower this index is, the higher will be its molecular mass. For the *camu camu* seed oil, the value of saponification index is between the 174-195 interval of literature, Table 1.
Regarding the iodine index that measures the degree of unsaturation of fatty acids in vegetable oils, it is evident that the lower the unsaturation of the fatty acids present in the molecules of triglycerides, the smaller the capacity of iodine absorption and consequently the lower the value of the index of iodine.

3.3 Analysis of *camu camu* seed oil by ^{13}C NMR

^{13}C NMR spectrum analysis shows that δ 172.9-173 ppm corresponds to triacylglycerol carboxylates; δ 128.4-130.2 ppm corresponds to the vinyl carbons. Glycerol methylenes appear at δ 62.4 ppm. Alpha-carboxylic carbons appear in δ 34.17-34.36 ppm. The ω-3 carbons show absorption at 32.1-31.6 ppm. Methyls appear in δ 14-14.5 ppm as shown below (Figure 2).

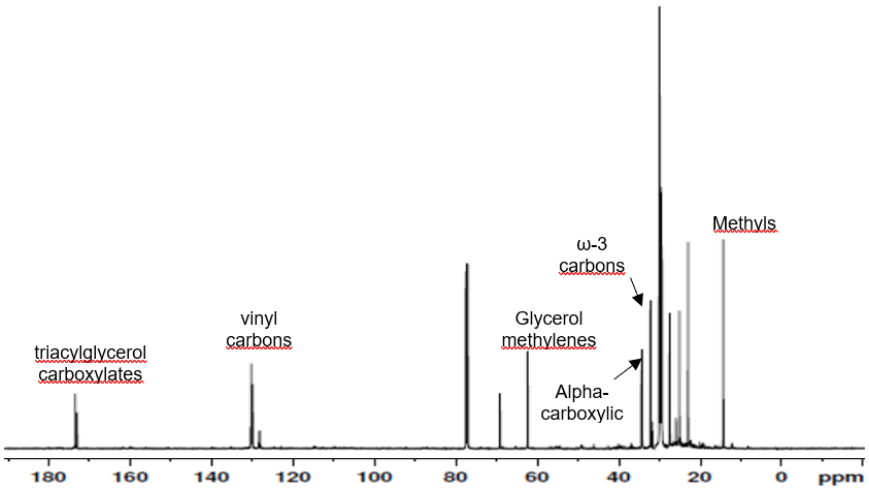

Figure 2: ^{13}C-NMR spectrum of camu camu oil.

3.4 Mineral analysis by ICP-OES

ICP-OES analysis of *camu camu* seed oil identified the presence of the following elements in *camu camu* oil: K, Na, P, Si (> 100 ppm); Mg, S (10 a 100 ppm) and Ti (< 100 ppm). The levels of potassium concentration

(K), sodium (Na), phosphor (P) and silicon (Si) presented the highest values of minerals in the chemical composition of the *camu camu* seed oil: K: 456.95 ppm; Mg: 60.36 ppm; Na: 190.35 ppm; P: 448.40 ppm; S: 60.36 ppm; Si: 413.15 ppm; Ti: < 0.04 ppm. Minerals have great importance in human health, their insufficiency or deficiency in food and / or supplementation can lead to problems in physical development as well as chronic diseases in children, young adults and the elderly (Panziera et al., 2011; Melo et al., 2005; Velásquez-Meléndez et al., 1997).

3.5 Antioxidant activity

The determination of the antioxidant activity of the fixed oil of *camu camu* in different concentrations. The result shows that the antioxidant percentage increases proportionally with the concentration. The correlation between the antioxidant activity (%) and the oil concentration used (Y=0.3969 x +4.4687) with R^2 = 0.9941, providing a very good correlation coefficient, presenting good linearity, providing an EC_{50} = 114.72 μg mL^{-1}, presenting a very high value in comparison to the positive control employed (quercetin = 0.94 μg mL^{-1}).
According to Finley et al. (2011), it is of great importance to find foods that act as antioxidants, that is, substances that prevent or delay the oxidative processes caused by reactive oxygen species (ROS). These ROS cause from deterioration of food (Finley et al., 2011) to DNA damage (Diplock et al., 1998), where chronic diseases and aging are associated (Langley et al., 2015). Thus, in this way, Langley et al. (2015) points out that in the United States the consumption of *camu camu* goes beyond nutritional, it is used as functional food, and the idea is to obtain more information of its biological potential that will add value and consequently greater visibility in the United State market.

4. Conclusion

The physicochemical analyzes of the oil of the *camu camu* seeds indicated that the conditions of storage and extraction were stable, which can be corroborated by the results of the tests according to the literature data. The ^{1}H NMR technique was important for determination of the acidity, iodine number, saponification index and average molecular weight of the *camu camu* seed oil due to its speed and precision. At the same time, ^{1}H NMR allowed quantification of fatty acid composition in *camu camu* oils. Analysis of fatty acid composition showed that *camu camu* seed oil has a saturated fatty acid content compatible with other oils given in the literature. Among the unsaturated fatty acids present in the composition, oleic acid is present in greater amounts in the oil. The mineral analysis of the oil revealed the presence of potassium (K), phosphorus (P), sodium (Na) and silicon (Si). The antioxidant activity of the oil, as measured by the DPPH method, indicated the potential of the oil as an antioxidant.

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