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Physical-chemical Properties and Chemical Composition of Brazil Nut Oil, *Bertholletia excelsa*, from State of Roraima, Brazilian Amazon

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The Brazil nut, *Bertholletia excelsa*, is a globose fruit (urchin) that has chestnuts or almonds in its interior, these being rich in Selenium and other minerals essential for the proper functioning of the human body, as well as linolenic (ω3), linoleic (ω6) and oleic (ω9). Its oil presents nutraceutical, functional and therapeutic properties of biotechnological interest. The objective of this work was to evaluate the physico-chemical properties and chemical composition of two samples of the *Bertholletia excelsa* oil, from chemo-types of the State of Roraima, Brazilian Amazon, by Gas Chromatography with a Flame-Ionization Detection-GC-FID, Atomic Emission Spectroscopy by Microwave Plasma-MP-AES and Medium Infrared Region Absorption Spectroscopy-MIR. The physico-chemical characteristics of the two samples are within the limits and parameters allowed by the norms of Brazilian legislation and comply with international recommendations. The most commonly found fatty acid was the oleic, 37.8% for the two samples. The concentration of the minerals in order of decreasing quantity in mg / 100g were Ca (22.3), Mg (5.4), K (3.0), Se (0.5), Mn (0.2), Cu (0.1) and Co (0.05). Thus, the quality of the oil from these evaluated chemical races is evidenced, being considered suitable for human consumption and indicated for systematic investigations of bioactivity for different biotechnological purposes, mainly food, pharmacological and therapeutic.
Keywords: Oleic Acid, GC-FID, Essential Minerals, Gas Chromatography.

1. Introduction

Brazil nut, *Bertholletia excelsa* (Lecythidaceae), can also be known as Brazil nut, Brazil nut, Brazil nut, Brazil nut, among others. This product is an important component of Sustainable Forest Management and contributes significantly to the economy and conservation of the forest, and has increasingly been valued not only for its bioactive properties but also for the socio-environmental contributions it promotes for the maintenance of tropical forests and positive influence on the trade balance. In Brazil, its area of exploration is concentrated in the north. Its fruit, known as urchin, is a capsule with a very hard, woody, spherical or slightly flattened shape, where in its seeds about 8 almonds or chestnuts weighing between 200 and 1.5 kg are conserved (Muller et al., 1995).
The height of the plant varies between 30-50 m, with a straight trunk of 100-180 cm in diameter, covered by brownish-gray bark and longitudinal grooved. Alternate leaves, simple, elliptic and narrowly aboved, 25-35 cm long. Flowers with yellow coloration, large and fragrant (Lorenzi, 2008).

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In recent works, the chestnut tree was used for medical purposes and was accompanied by 50% of the members of a research, who indicated the chestnut as a beneficial food for health and for the prevention of diseases in general, being recommended the consumption of 2 or 3 fresh nuts per day (Ferreira and Carniello, 2018).

This species, especially the oil of its chestnuts or almonds, has nutraceutical, functional, therapeutic and pharmacological properties widely investigated and prospected for various uses and applications. It is a food appreciated for its sensorial characteristics and for its food, nutritional, pharmacological and medicinal importance. Its chemical composition, in general, presents between 60 and 70% of lipids, expressively of unsaturated fatty acids, and of 15 to 20% of protein, being also rich in minerals like Selenium, Calcium, Phosphorus, Magnesium and Vitamins (Moreda-Piñeiro et al., 2018).

Research has shown that its oil decreases oxidative stress and modulates the gene expression of antioxidant enzymes in a nutrigenomic way (Schott et al., 2018), among other functions in the human body. Among its nutrients, amino acid rich proteins, lipid content, especially functional fatty acids such as linolenic (ω3), linoleic (ω6) and oleic (ω9) and selenium, are major constituents and exhibit antioxidant properties (Kluczkovski et al., 2015).

The Amazonian regions, in their biodiversity, reserve a wide variety of bioactive products (Serra et al., 2019) that can meet the demands of industries that are seeking more and more formulations that meet the requirements of the market in terms of multifunctional products. However, the influence of edafoclimatic and ecophysiological conditions on the physicochemical and phytochemical properties of botanical chemical breeds is known. Studies with chemotipes of the state of Roraima (Tonini, 2007), Brazil, of *Bertholletia excelsa* are scarce in this line of research. In this sense, to know some of these properties in the conditions of the Amazon Rainforest of Roraima is a central premise for a careful recommendation of its biotechnological exploration.

Not forgetting the importance related to the knowledge of the composition of the food and its respective presentation to the consumer, which is of fundamental importance, since the person who makes use of the food needs information about the nutrients, mainly in the aspects of quality and quantity, to ensure give him a good nutritional status that contributes to and satisfies the daily needs of the human body (Balbi et al., 2014).

In this context and knowing that the biological activities of Brazil nuts are vastly researched in several areas of knowledge, the objective of this work was to determine the physicochemical properties and chemical composition of two samples of *Bertholletia excelsa* oil, from chemotypes from Roraima state, Brazilian Amazon, by GC-FID, MPAES and MIR.

2. Material and methods

2.1 Collection, Exchange and processing

The fruits were collected in a completely randomized manner in native chestnut trees established in two experimental areas managed by the Brazilian Agricultural Research Corporation - EMBRAPA, a unit of Roraima, where each plot comprises a perimeter of 9 hectares. These parcels are located in the municipalities of São João da Baliza (SJB), 00°56'02''N - 59°54'41''W, and Caracaraí (CC), 1º48'51"N - 61º08'04"W, Roraima, Brazil. The climates of these municipalities are characterized, respectively, as Tropical Forest (Af) and Tropical Monsoon (Aw), according to the Köppen climate classification types. Genetic materials were duly registered in the National Genetic Heritage and Associated Traditional Knowledge Management System - SisGen, linked to the Ministry of the Environment - MMA, according to specific Brazilian legislation, under the number A534AE6.

For processing, the urchins were sectioned and the nuts were selected, separated from inert materials, washed with distilled water, and then destined to the Laboratory of Environmental Chemistry of the Federal University of Roraima - UFRR, linked to the Chemical Department of the same institution, where they were submitted to the drying process in a circulation oven 40 °C until reaching a constant mass. Soon after, they were milled in a Philips brand (Walita 600 W), obtaining a homogeneous sample of granulometry between 20-40 Mesh, again weighed to quantify the yield, and finally duly stored until the moment of extraction of the oil for further analysis.

2.2 Extraction of fixed oil from chestnuts

The extraction of the oil of the chestnut was done in triplicate, with a duration of 3 h each, using the Soxhlet system and the solvent hexane, as described by (Melo Filho et al., 2018). These samples were stored in an amber bottle and nitrogen atmosphere until the analyzes were carried out under nitrogen atmosphere for further analysis. Extraction yields were also quantified.

2.3 Characterization and determination of physico-chemical properties

The following parameters were evaluated: i) density; ii) viscosity; iii) melting point. It is added that the data of the viscosity variable were submitted to the regression study to obtain the temperature factor response curves at different degrees in the ascending order (25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80°C).

2.4 Identification of the functional groups by absorption spectroscopy of the medium Infrared (MIR)

The samples were analyzed in the Infrared region using the IRPrestige-21 Fourier Transform spectrophotometer (SIMADZU) spectrophotometer, using 1: 100 thallium tablets coupled to a universal holder for fixing rectangular windows. For these analyzes the following conditions were used: a) Measuring mode: Absorbance; b) Number of scans: 16; c) Resolution: 4 cm^{-1}; and, d) Wavelength range: 4000 - 400 cm^{-1}.

2.5 Analysis of fatty acids by gas chromatography

The analysis for the determination of saturated fatty acids was performed on an HP7820A Gas Chromatograph (Agilent) equipped with a flame ionization detector (GC-FID), as described by Ribeiro et al. (2018) and Santos et al. (2018).

2.6 Quantification of element through atomic emission spectroscopy by microwave plasma (MP-AES)

The samples were digested by the nitric-perchloric method (EMBRAPA, 1999) and the triplicate readings were performed using Microwave Plasma Atomic Emission Spectrometer - MPAES, Model Agilent Technologies 4200. The following parameters were considered: i) Alternating current voltage: 211.0 V; ii) Frequency of the alternating current: 60Hz; iii) Magnetron voltage 4312.0 V; iv) Magnetron current: 400.0 Ma; and v) Magnetron temperature 42.5 °C. The following chemical elements were quantified in order of decreasing concentration (mg / 100g): Calcium (Ca), Magnesium (Mg), Potassium (K), Selenium (S), Manganese (Mn), Copper (Cu) and Cobalt).

3. Results and discussion

3.1 Characterization and determination of physico-chemical properties

The yield of the oils extracted from the São João da Baliza (FO-SJB) and Caracaraí (FO-CC) from *Bertholletia excelsa* were respectively 52,07% and 61,14%, demonstrating the rich lipid source. The properties of the almonds of this species have been studied extensively, mainly for presenting functional activities (Solfrizzi et al., 2018), nutraceuticals (Dahl, 2018), therapeutics (Mazokopakis and Liontiris, 2018) and biological activities of biotechnological interest (Buthelezi et al., 2019).
The samples from the present study also showed the same density value, 0.91 $g.cm^{-3}$. The kinematic viscosity (KC), at 25 and 80 °C, was 883.37 and 151.74 $mm^2\ s^{-1}$ for FO-SJB and 899.35 and 151.38 $mm^2\ s^{-1}$ for FO-CC. Among the KC variations evaluated every 5 °C in increasing order of temperature, we can see an inversely proportional response between the viscosity and temperature factors, typical behavior of a Newtonian liquid, in which the viscosity value decreases with increasing temperature.

3.2 Identification of functional groups by Medium Infrared Region Absorption Spectroscopy (MIR)

The spectra obtained with respect to FO-SJB, demonstrated that the absorption in the range of 2918 to 2856 cm^{-1}, is characteristic of an axial strain of the CH (sp^3-s) binding to the methyl group (CH_3) with a strong band and intense in about 2918. The mean intensity absorption at 2856 cm^{-1} can be attributed to the methylene groups (-CH2). The absorption at 3003 cm^{-1} can be attributed to an axial strain absorption band H-C = (sp^2). The strong and characteristic band with absorption at approximately 1742 cm^{-1} refers to the carbonyl group (C = O).
A band with absorption at 1470 cm^{-1} was also attributed to a symmetrical angular deformation of the methylene group plane. The band between 1200 and 1050 cm^{-1} of medium intensity with the peak in the 1168 cm^{-1} range is characteristic of the saturated esters (COC) functional group, in the range of 1290 to 1040 cm^{-1}, alcohols, esters, ethers, carboxylic acids and fatty acids. And finally, the absorption at approximately 723 cm^{-1} can be attributed to an angular deformation outside the plane of the (C-H) groups.
The spectra obtained for Brazil nut oil for the FO-CC sample showed similar absorptions relative to the regions observed for the oil spectrum of the FO-SJB sample, so the observations made to the said FO-SJB are valid for the FO-CC. It is reiterated that the functional groups and absorptions found are within the limits of the ranges stipulated in the literature.

3.3 Analysis of fatty acids by gas chromatography (GC-FID)

It was observed in the two samples the presence of 8 fatty acids, Table 1, being these equivalents to the composition between fatty acids of unsaturated and saturated chains. Research on Brazil nut also observed a profile of fatty acids similar to those found in the present investigation. In the studies of Cicero et al. (2018) the presence of monounsaturated fatty acids (70.54%) was verified, where oleic acid (47.78%) was the most representative. Alan et al. (2018) complement that in addition to the oil, Brazil nut flour presents significant levels of linoleic acid, being the second fatty acid identified in this study. This demonstrates the biotechnological potential of both the products and the by-products of this almond, and can meet the demands of multifunctional products demanded by the industry.

Table 1: Fatty acids content of extracted oils (%) of dehydrated nuts of Bertholletia excelsa of São João da Baliza (FO-SJB) and Caracaraí (FO-CC), Roraima, Brazil, determined by CG-FID.

Fatty acids	Retention time (min)	FO-SJB (%)	FO-CC (%)
Myristic Acid	4.10	0.7	0.2
Palmitic acid	5.65	18.7	17.5
Palmitoleic acid	5.87	0.7	0.7
Stearic acid	7.26	11.5	8.3
Oleic acid (ω9)	7.46	37.7	37.7
Linoleic acid (ω6)	7.84	27.8	32.6
Linolenic acid (ω3)	8.19	0.6	0.2
Arachidic acid	8.82	0.3	0.3
Others		2.0	2.5
Total		100	100

3.5 Quantification of minerals through microwave plasma atomic emission spectroscopy (MPAES)

In general, the investigations with brazil nuts have been directed, mainly, to evaluate the Selenium (Se), due to its vastly reported antioxidant action. However, other minerals such as Manganese (Mn), Iron (Fe) and Copper (Cu) are also considered essential for the proper functioning of the human body and research shows that they help synergistically in antioxidant activity and other biological effects in the human body (Cicero et al, 2018b). In this sense, the minerals of the oils FO-SJB and Caracaraí (FO-CC), Table 2, were quantified in descending order of concentration.

Table 2: Concentrations (mg / 100g) of the minerals found in the extracted oils (%) of dehydrated nuts of Bertholletia excelsa of São João da Baliza (FO-SJB) and Caracaraí (FO-CC), Roraima, Brazil, by MPAES.

Minerals	*FO-SJB (mg/100g)*	*FO-CC (mg/100g)*
Calcium (Ca)	22.50	38.40
Magnesium (Mg)	5.24	7.49
Potassium (K)	3.00	10.80
Selenium (Se)	0.50	0.60
Manganese (Mn)	0.20	0.40
Copper (Cu)	0.10	0.20
Cobalt (Co)	0.05	-

Studies of the concentration of minerals present in Brazil nut oil by MPAES are scarce, however, in other investigations with the same objective, were observed by Pacheco, Scussel (2007) and Souza; Menezes (2008), that the minerals present in the composition in the evaluated samples have: phosphorus, potassium, magnesium, calcium and selenium with some differences of concentrations in relation to the present research. Chunhieng et al. (2004) corroborate that the presence of Magnesium, second element of higher concentration in the samples of the chemotypes evaluated in this study, and Manganese should be perceived as important elements from the nutritional and nutritional point of view.

4. Conclusion

The physico-chemical characteristics and properties of the oils analyzed in the experimental conditions of this study are in compliance and within the limits of the norms and recommendations of the Brazilian legislation of oils and fats of the National Agency of Sanitary Vigilance. - ANVISA, Brazil, and meet the parameters established by the Food and Agriculture Organization of the United Nations (FAO), demonstrating its quality for consumption and possible bioprospections.
The presence of most of the unsaturated fatty acids and essential minerals observed in the samples indicates the nutraceutical and functional potential of the oils, indicates their bioactive potential and guides the need for systematic studies of activity to prove the effectiveness of their bioactivity in different areas with an emphasis on biotechnology.

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