Physicochemical characterization, fatty acid composition, and thermal analysis of *Bertholletia excelsa* HBK oil

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ABSTRACT

The present study aimed at characterizing the oil extracted from Bertholletia excelsa H.B.K. almond, a native species from the Amazon region. Analytical methods used for oils and fats were employed through pharmacopoeia assays, AOCS (American Oil Chemists Society) standard methods as well as those recommended by ANVISA (National Health Surveillance Agency) such as acidity, peroxide value, saponification index, iodine value and refractive index, pH and relative density, and also thermoanalytical analyses (thermogravimetry, differential thermogravimetry and differential thermal analysis) as well as chromatographic analysis (gas chromatography). The characterization assessments of B. excelsa oil showed results indicating that the oil contains polyunsaturated fatty acids in large proportion. The termoanalytical tests indicated that B.excelsa oil showed thermal stability up to 220 °C, These results showed that the oil extracted from B. excelsa has acceptable characteristics and is of good quality.

Key words: Brazil nut oil, quality control, oxidative stability

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INTRODUCTION

In recent years, there has been a growing trend of using raw materials of natural origin in pharmaceuticals and cosmetics. This has caused the race to oils extracted from Amazon native plants, causing a rapid and significant expansion of the domestic and international market of these products. The oil industrialization is one of the most important activities of Brazilian agribusiness as its products are used in the food, cosmetics, and pharmaceuticals industries.[1]

The *Bertholletia excelsa* one of the riches of the Amazon forest is an important exporting component in the region. Its exploitation plays a key role in the socioeconomic organization of large areas of the Amazon forest extraction. Food greatly appreciated for its taste, the oil of *B. excelsa* also has nutritional qualities. The fruit of the chestnut tree, commonly called hedgehog, has a woody and quite hard shell. It can contain 15-24 seeds, whose size varies between 4 cm and 7 cm length. These seeds also have a very hard and rough peel and much appreciated almond.[2] The almond consists of 60-70% fat, 15-20% protein of good biologic quality, liposoluble Vitamins (A, E), and minerals (Ca, Fe, Zn, Na, K and Se).[3] The almond nut contains 13.8% palmitic acid, 8.7% stearic acid, 31.4% linoleic acid, and 45.2% oleic acid,[4] and it also contains a small quantity of palmitoleic and myristic acids.[5]

The oil extraction takes place on an industrial scale by hot or cold pressing. In lab scale, the solid-liquid extraction is commonly employed, with the aid of solvents.[6] The crude oil at room temperature is fluid with light yellow color, with a characteristic pleasant aroma and flavor.[7] The ways in which the oil extraction is processed can influence its characteristics. Factors such as exposure of the almonds to air, light, heat, exposure of the material to reagents, temperature variations, contribute to decrease the quality of the final product.

The stability of the oil may be altered by these factors which contribute to make it less stable to atmosphere changes and temperature rises.[8,9] The thermal stability

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of vegetable oils is a determining factor in their quality. Thermogravimetry (TG), derivative thermogravimetric curve (DTG), and differential thermal analysis (DTA) are used to evaluate the decomposition profile and the thermal stability of the oil as well as to register enthalpy variations.[10,11] These methods are widely used in quality control of vegetable oils because they quickly provide, data on the stability of the oil, regarding their thermal behavior.[12,13] These techniques have attracted great interest of researchers because of their use for the characterization of drugs both from natural and synthetic source, as well as in food products, polymers, cosmetics, and pharmaceuticals.[14-16]

Standard methods for the analysis of oils and fats American Oil Chemists Society (AOCS)[17] and pharmacopoeia assays,[18] such as indexes of acid, saponification, iodine, peroxides, determination of potential of hydrogen (pH), and density are important since they indicate the quality and authenticity of the oil.

The purpose of this study was to characterize the oil extracted from kernels of *B. excelsa* (Brazil-nut) by standard methods of analysis, chromatographic techniques and thermoanalytical analysis, to assess the quality of the oil to be used as raw material in the technology of products in pharmaceutical and cosmetic segments.

MATERIALS AND METHODS

Raw material

The *B. excelsa* oil was purchased from Amazon Oil Industry (Ananindeua, Pará, Brazil). The crude oil sample without the addition of preservatives, batch number OCA 001/10 whose extraction occurred on 10/01/2010 was performed by cold pressing (press expeller coupled with a kettle, 500 kg/h) from the milled almond nut. The analytical tests for the characterization of the oil were performed at the Laboratory for Research and Analysis of fuels, Chemistry Department and Drugs Quality Control Laboratory, Faculty of Pharmacy, Federal University of Pará, UFPA, from March to December 2010.

Determination of physico-chemical characteristics

Determination of relative density

Analyses were carried out by direct reading in glass hydrometer (pycnometry) at 25°C. The method used was recommended by the Brazilian Pharmacopoeia V edition.[18]

Refractive index-performed by a 2WAJ Abbe model refractometer. Method recommended by the Brazilian Pharmacopoeia.[17] It is specific for each oil, within certain limits. It is related to the degree of binding saturation, but it is affected by other factors such as free fatty acids content, oxidation, and thermal treatment.

Peroxide values-expressed in milliequivalents of active oxygen/kg of oil, calculated from the iodine released from potassium iodide, operating under the conditions indicated in the method proposed by AOCS Cd 8–53.[17] The value found is an evaluation criterion for incipient rancidity (pre-rancidity, characterized by the formation of unstable peroxides) and indicates the conservation state of fatty matter.

Iodine index

It is the measure of oils and fats unsaturation and is defined as the amount of iodine in grams calculated as the iodine absorbed by 100 g of the sample. The method AOCS Cd 1-25 was used for this determination.[17]

Saponification value

It is defined by the amount in milligrams of potassium hydroxide required to saponify 1g of oil or fat. The method recommended by the AOCS Cd 3c-91 was used.[17]

Acid value

This index is expressed as the number of milligrams of potassium hydroxide required to neutralize the free acids of 1 g of sample. Free fatty acids are determined in oil in methanol solution, by titration of sodium hydroxide solution using phenolphthalein as indicator. The content of free fatty acid was calculated based on the molecular weight of the predominant acid. The method recommended by AOCS Cd 3d-63 was used.[17]

Determination of pH

The determination of the pH was performed without previous dilution of the oil and performed by inserting the electrode directly into the samples at a temperature of 25°C ± 2°C. The potentiometer model pH 21/mv m (Hanna) was used. Pharmacopeial method recommended by the National Agency for Sanitary Surveillance.[19]

Fatty acid composition of the oil

Esterification was performed by the method Khan and Scheinmann, followed by the determination through gas chromatography, AOAC method Ce 1-62.[17] A SHIMADZU CG 14A gas chromatograph equipped with a flame ionization detector was used. A capillary column CP WA × 52 CB (25 m × 0.25 mm); DF 1 μm was used. Carrier gas flow (He) 1.0 mL/min. Injector temperature 200°C, detector temperature 250°C and the column temperature 190°C for 60 s, with increasing ratio of 2°C/min until reaching the maximum temperature of 250°C, standing there for 35 min.[20]

Thermoanalytical assays

The curves were obtained from approximately 4 mg sample using an alumina crucible and subjecting them to a temperature range between 25°C and 600°C under dynamic

atmosphere (flow of 50 ml/min), heating rate 10°C/min.[21] A thermal analyzer Shimadzu TGA-50 was used.

RESULTS AND DISCUSSION

Physicochemical characteristics

Table 1 shows the results of the physico-chemical analysis of *B. excelsa* oil. According to the results found in this study, we observed that the refractive index and density at 25°C for *B. excelsa* oil were 1.466 and 0.911 g/ml, respectively, which are in accordance with the values found by Ferreira e*t al.*[22] As these indexes vary inversely with the temperature, these results can be considered satisfactory. The iodine value was 95.0, allowing the analysis of the degree of the unsaturation of the oil and providing the amount of iodine absorbed per 100 g of the sample within the expected range. The pH value found was 3.80 which characterized as acid. The saponification number was 178.5 indicating that the oil contains polyunsaturated fatty acids in a large proportion. The value obtained in this study was lower than the one found by Ferreira *et al.*,[22] whose value was 198.5 mgKOH/g.

The acid and peroxide indexes are parameters that demonstrate the quality of the oil. The acid value obtained was 2.14 mgKOH/g, which is within the range of 4.0 mgKOH established by the National Agency of Sanitary Surveillance (ANVISA).[23] The acidity is due to the hydrolytic rancidity process during the storage of the oil of *B. excelsa* almond. The peroxide value was 12.0 mEq/kg, a value close to 15.0 meq/kg established by the ANVISA[23] and the above the limit of 10.0 mEq/kg established by the Codex Alimentarius.[24]

Peroxide value showed a slightly high value, possibly due to variations in the conditions of the oil conservation, such as changes in storage time, time for performing the assessments, as well as particularities during the extraction process, which resulted in an increased oxidation of the oil.[6]

According to Table 2, 11 different fatty acids were identified, and the predominant was the unsaturated ones such as oleic acid 38.5% (C18:1) and linoleic acid 31.26% (C18:2). Saturated fatty acids represented 25.55% of total fatty acids, mainly C16: 0-14.28%, C18: 0-10.61%; other saturated fatty acids such as C12:0 and C20:0 were also identified in trace amounts. Unsaturated fatty acids represented 70.19% of total fatty acids. C18:1 (oleic acid) - 38.50%; C18:2 (linoleic acid) - 31.26% were about 69.76% of total unsaturated fatty acids. Chromatogram of the *B. excelsa* oil is shown in Figure 1.

The percentage of oleic acid found in Brazil-nut oil is two times higher than that found in passion fruit oil, however its value is lower when compared with Buriti and Pracaxi oils.[25] Oleic acid is a long chain fatty acid, consisting 18 carbon atoms containing a double bond between carbons and it is considered an essential fatty acid (Omega 9) which is involved in human metabolism and the synthesis of hormones. Other acids present in Brazil-nut oil, such as linoleic acid (Omega 6) and linolenic acid (Omega 3) are also required in the human diet since they have beneficial effects in the prevention of cardiovascular diseases (arrhythmia and blood clotting) and hyperinsulinemia.[26]

Table 1: Physic-chemical characteristic of *B. excelsa* oil

Analysis	Results	Maxima values (ANVISA)
Refractive index (25°C)	1.466±0.00	NS
Relative density (g/mL)	0.911±0.00	NS
Acid value (mg KOH/g)	2.14±0.05	4.0 mg KOH/g*
Peroxi index (meq/g)	12.0	15 meq/g*
Saponification value (mg KOH/g)	178.5±0.05	NS
pH determination	3.80	NS

*Reference values for cold-pressed oils and unrefined.

Table 2: Fatty acids profile of *B. excelsa* oil

Fatty acids	Brazil nut oil	Pracaxi oil	Buriti oil	Passion fruit oil	Peanut oil
Undelic acid C11:0	0.0943	NS	NS	NS	NS
Lauric acid C12:0	0.2423	0.187	NS	NS	NS
Myristc acid C14:0	0.215	0.308	0.1	0.1	NS
Palmitic acid C16:0	14.2674	1.854	23	11	6-16
Palmitoleic acid C16:1	0.2899	0.047	NS	NS	NS
Margaric acid C17:0	0.0766	NS	NS	NS	NS
Stearic acid C18:0	10.6132	1.8626	NS	NS	NS
Oleic acid C18:1	38.5021	53.550	61	17	35-72
Linoleic acid C18:2	31.2651	13.050	6	68	13-45
Linolenic acid C18:3	0.1550	0.134	0.5	3.5	NS
Arachidic acid C20:0	0.3627	1.053	0.7	NS	1-3

NS: Not specified; *B. excelsa: Bertholletia excelsa*

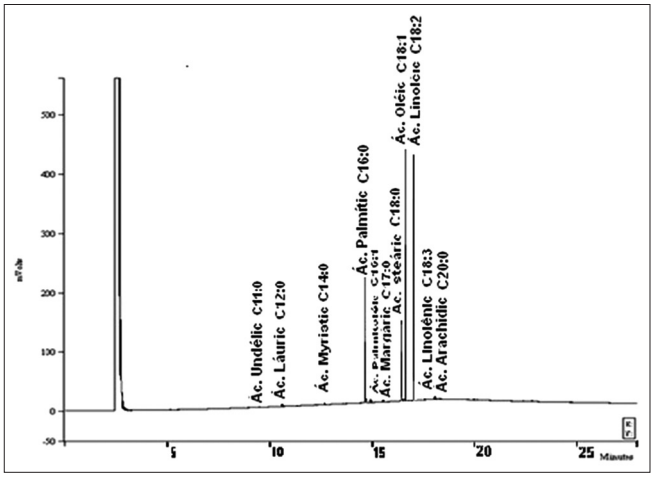

Figure 1: Bertholletia excelsa oil by gas chromatography.

The high content of unsaturated fatty acids present in the oil, mainly oleic, and linoleic acid favor the occurrence of oxidative degradation reactions. The results are similar to the results obtained by Gonçalves *et al.*[27] for polyunsaturated fatty acids, such as oleic acid in the proportion of 37.42%, as well as 24.83% of saturated acids such as palmitic and stearic, with 13.15% and 10.36%, respectively. The proportion of polyunsaturated linoleic acid found in the assessment resembles the results found by Tateo[4] which was 31.4%.

According to the fatty acid composition, the sample is within the limits established for vegetable oils by the RDC 270[22] and Codex Alimentarius.[24]

Thermal analysis

Figure 2 demonstrates the TG profile of *B. excelsa* oil showing its thermal behavior under dynamic conditions. The TG curve shows the thermal stability of the oil to a temperature of 220°C, similar to other experiments.[6,28] The decomposition and carbonization processes occurred in three phases of the curve ending at a temperature of 580°C. At this temperature, the mass loss reached 97%. In DTG curve [Figure 2], it can be seen more clearly that the thermal decomposition of the oil occurred in three steps, with loss of initial mass at the range of approximately 220°C and then a greater loss of mass in the range of 270–580°C. The values of mass loss are shown in Table 3.

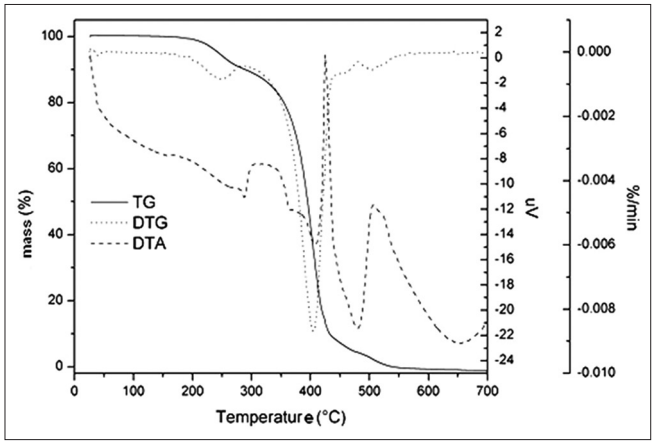

Figure 2: TG/DTG and DTA curves of the thermal behavior of Bertholletia excelsa oil

Table 3: Mass loss of *B. excelsa* oil by thermogravimetric analysis

Interval/°C	Result/%	Event
200-220	0.10	Evaporation of water
270-580	97.0	Decomposition of fatty acids
Total	97.10	Total mass

B. excelsa: Bertholletia excels

The first step is considered as the most important one and represents an early stage of the degradation of triglycerides, mainly composed of polyunsaturated fatty acids. At this stage, the oxidation of polyunsaturated fatty acids occurs. The loss of mass is complete at 580°C, leaving a residue of 3%, corresponding to the content of inorganic material or mineral salts. The mass loss at this stage corresponded to 97% of the original mass. High temperatures catalyze hydrolysis and oxidation reactions in oils.[29]

The graph corresponding to the DTA curve [Figure 2] shows one exothermic peak at a temperature range 400-450°C. At oxygen flow, the curves keep the standard of mass loss of <1% at the temperature range 200-220°C; in the second stage the temperature range stood at around 270-580°C with mass loss of 97% [Table 3].

The results obtained by TG/DTG and DTA in a dynamic atmosphere clearly showed the thermal behavior of the analyzed oil. By using these methods, it was possible to determine the thermal stability of this material, which is the determining factor in quality control of oils and fats during processing, storage, and industrial use.[28]

CONCLUSION

The results obtained by the physicochemical analyses carried out in *B. excelsa* oil are within the parameters established by the Brazilian legislation; the acidic characteristics, the high degree of unsaturation, indicated that the oil contains polyunsaturated fatty acids to a large extent. The techniques, by which assays were performed, are useful for quality control of the oil in the study and allowed obtaining satisfactory results which ratified its use as raw material for the pharmaceutical and cosmetic industries.

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