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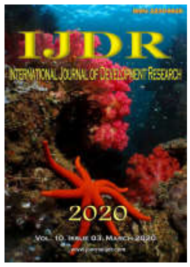

RESEARCH ARTICLE OPEN ACCESS

COMPARATIVE PARAMETERS OF PHYSICOCHEMICAL AND SPECTROSCOPIC PROPERTIES AND THE FATTY ACID PROFILE OF BRAZIL-NUT OIL (*Bertholletiaexcelsa HBK*) OF DIFFERENT HARVEST SEASONS

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ABSTRACT

The objective of this research was to compare the physical-chemical, spectroscopic and fatty acid profiles of Brazil nut oils from the 2018 and 2019 harvests. The methodological procedures followed the internationally accepted standards of the American Oil Chemist's Society. The analysis of the physical-chemical properties showed oils with high quality for human consumption, within the quality standards defined by Brazilian legislation. In relation to the different harvests, during the analyzed period, they did not present differences maintaining their high quality as a function of time. The spectral bands show chemical groups characteristic of long-chain fatty acids, without the presence of compounds related to oxidative processes. The fatty acid profiles showed high quality in constituents related to the fatty acids components of the omega family (ω-3, ω-6 and ω-9) showing the maintenance of the functionality of the products extracted from this oilseed, without changes in the chemical constituents in the analyzed crops.

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INTRODUCTION

The use of oilseeds' fat content is one of the most important activities in the Brazilian agribusiness. However, its composition can be altered by intrinsic and/or extrinsic factors such as climate, geography, agronomic variety extraction technology, and storage, among other things. Such factors may also affect its constitution, reduce its nutritional quality, and induce acceleration of oxidative processes. Accordingly, studying storage and comparing different stages of vegetable oils may be strategically important for food research. The extraction practices and utilization of these oils may have a wide range of interests and applications thus becoming a field of technological experiments similar to value adding (Santos*et al.*, 2019a; Pinto *et al.*, 2018; Serra *et al.*, 2019; Teixeira *et al.*, 2019).

The stability of these oils can be a major problem. The degradation of its lipid components is caused by oxidation, hydrolysis, polymerization, pyrolysis, and absorption of flavors and odors. The most important among these is oxidation - responsible for major damage by altering sensory characteristics, nutritional value, functionality, and toxicity (Costa *et al.*, 2016; Santos *et al.*, 2019). Some intrinsic and extrinsic variables may be related to the processes that lead to lipid deterioration, and fatty acid composition, presence of anti- and pro-oxidants, oxygen exposure, radiation, high temperatures, water content, and presence of metals and metalloproteins can directly affect the rate of oxidation reactions. These degradation processes can occur during the production, processing, transport, and storage stages. Other components such as proteins, vitamins, and pigments may also be negatively affected (Pinto *et al.*, 2018; Santos *et al.*, 2019a).

Ontheotherhand, changesmayoccurdueto natural orextrinsic factors such as mineral richnessorpovertyofthesoil, temperature and relative humidity, soilp Hand leaching, rainfall, degree of pollution of rivers that flow into the nuttrees, andalternatingrainyanddryseasons, among others. Therefore, thes eextrinsic factors can cause changes in fruits' composition and consequently lead tovariations that can modify their growth and structureby increasing ordecreasing the number of compounds of great importance for the fruits induced by the lack of precurs or sources of these compounds or by their premature harvest (Serra *et al.*, 2019; Teixeira*et al.*, 2019). All of these factors may also significantly modify the components of the Brazil nut. The harvest season of this fruit is very distinctive; it starts from November and December and lasts until mid-March, which may hinder crop standardization and prevent possible comparisons between studies. Therefore, especial attention should be given to storage and evaluation of possible changes in the composition during this period (Elzupir, 2019; Pereira *et al.*, 2019; Serra*et al.*, 2019). Oil is the most abundant component of Brazil nut, and its quality depends directly on the state of conservation of the kernels from which it was extracted, the efficiency of the extraction process, its exposure to the promoters of lipid oxidation, and the presence of hydrolytic enzymes. These aspects can affect several particular properties of the oil, such as fatty acid composition and initial state of oxidation, among others (Cardoso*et al.*, 2017; Elzupir, 2019). These oxidative processes directly influence the composition of the oils with the production of primary and secondary products, which are characteristic of the stages of degradation and oxidation of the matrices altering the spectral bands of its natural composition affecting sensory, nutritional, and commercial aspects of the products since they can change the by-products and formulations developed from them (Cardoso *et al.*, 2017; Pereira *et al.*, 2019; Santos *et al.*, 2019a). Given the above considerations, the objective of this study is to compare the physicochemical and spectroscopic properties and the fatty acid profiles of the Brazil nut oil obtained from two harvest seasons investigating possible variations in the quality of these oils during storage.

MATERIAL AND METHODS

Approximately 5 kg of export medium size Brazil nuts, from the 2018 and 2019 harvest seasons from the city of Belém do Pará were used. The oil was extracted immediately after harvest in both seasons; the nuts were vacum packed and stored at -18 °C. The lipid extraction was carried out by hydraulic press extraction (SMB -15 t). The nuts were pre-baked in an oven with forced air circulation (MARCONI) for 24 hours. The oils were filtered under vacuum through a filter paper (10 mm porosity) to remove residues from the extraction which could affect the results of the physicochemical, spectroscopic, and fatty acid composition analyses.
PHYSICOCHEMICAL CHARACTERIZATIONS OF OILS
The acidity of the oil obtained by hydraulic press was determined according to the AOCS official method Cd 3d-63 (AOCS, 2004). The saponification index was obtained according to the AOCS official method Cd 3-25 (AOCS, 2004). The peroxide value of the oil was determined according to the acetic acid-chloroform method, AOCS Cd 8-53 (AOCS, 2004).The density of the oils was determined using a digital densimeter (KEM KYOTO ELECTRONICS, Model OF-130) at 25 °C. The density value obtained was read directly on the device.

The Viscosity was determined using a Cannon-Fenske viscometer (SCHOTT Geräte, Type N. 23520) was used in the viscosity analysis of the oils in accordance with ISO 3105, ASTM 446 at a temperature of 40 °C. The refractive index was determined, according to the method of AOCS Cc7-25 (AOCS, 2004) using an Abbe type refractometer. The water content was determined according to the AOCS (AOCS, 2004).

Infrared Absorption Spectroscopy: Infrared absorption spectroscopy of the samples was obtained using a Perkin-Elrner (model X 1760 FT-IR) spectro photometer with KBr pellet (100 mg) and 5 µL of oil in the optical range 4000 400 cm^{-1} with a resolution of 4 cm^{-1}.

Characterization of Fatty Acid Profile: Methyl esters of fatty acids were prepared following the procedure described by the international organization for standardization ISO 5509 (1978). Samples of 1.0 g of oil were weighed in a test tube containing 10.0 mL of n-heptane, following by agitation. Next, 0.50 mL of NaOH and 2.0 mol/L in methanol were added, and the tube was agitated again for 20 seconds. After phase separation, thesupernatant was collected for later gas chromatograph analysis. The analyses were performed using gas chromatography (GC, Varian 430). The chromatographic conditions were as follows: a fused silica capillary column SP-2560 (Supelco USA), 100 m long and 0.25 m internal diameter, coated with 0.2 µm polyethylene. The operating conditions were: split ratio 50:1; column temperature of 140 °C for 5 minutes; programmed rate of temperature increase of 4 °C per minute up to 240 °C with helium gas as the carrier gas, isobaric pressure 37 psi, linear velocity of 20 cm/second; make-up gas – Helium – at 29 mL/minute; injector temperature of 250 °C, Varian CP8410, autosampler; and detector temperature of 250 °C. The qualitative composition was determined by the comparison of retention times of the peaks with those of the respective fatty acid standards. The quantitative composition was determined by area normalization, expressed as a mass percentage in accordance with AOCS Official Method Ce 1-62 (AOCS, 2005). The values correspond to averages of analyses results in triplicates.

RESULTS AND DISCUSSION

The results of physicochemical analyses of the Brazil nut oils are shown in Table I. The physicochemical analyses of crude Brazil nut oil, when compared to standards set by Brazilian legislation, can be classified into two maximum tolerance parameters defined by the Ministry of Health (MOH) through the National Health Surveillance Agency (ANVISA) - which controls the production of edible oils according to a technical regulation adopted by the RDC Nº. 270 of September 22nd, 2005. This regulation defines vegetable oils and fats as "products consisting mainly of glycerides of vegetable fatty acid (s) setting the following parameters: acidity and peroxide values with the maximum tolerance of 4.0 (mgKOH / g) and 15 (mEq / kg), respectively (BRAZIL, 2005). The results obtained for the acidity (0.47 ± 0.03 and 0.32 ± 0.05 mg KOH/g) of the oils of the two different harvest seasons studied show values similar to those reported by Santos *et al.* (2012). It can be seen that the values obtained are way below the maximum allowed by law (Brazil, 2005).The free fatty acid contents expressed as percentage of oleic acid show values of 0.24% and 0.16%, respectively. These results are similar to those found by Neto*et al.* (2009), 0.71%.

Table 1. Physicochemical characterization of Brazil nut oils – 2018-2019

Analysis	Brazil nut oil (2018)	Brazil nut oil (2019)
Acidity Index (mgKOH/g)	0.47 ± 0.03	0.32 ± 0.05
% GLA Oleic acid	0.24 ± 0.01	0.16 ± 0.01
Peroxide Value (mEq/kg)	4.06 ± 0.08	3.46 ± 0.06
Saponification (mgKOH/g)	187.77 ± 3.47	181.53 ± 1.40
Refractive Index	1.47 ± 0.00	1.46 ± 0.00
Density (g/cm^3)	0.91 ± 0.00	0.91 ± 0.00
Water Content (%)	0.05 ± 0.01	0.03 ± 0.01
Viscosity (cSt)	41.35 ± 0.03	41.06 ± 0.00

Data represent the mean ± standard deviation of triplicates (n = 3)

However, both values are below 2.5%, value found in the in the literature on vegetable oils. The saponification values obtained show little difference between the 2018 and 2019 harvest seasons, with rates of 187.77 and 181.53 (mgKOH/g), and Neto*et al.* (2009), 198.58 and 187.5 (mgKOH/g); this value is not defined by Brazilian legislation. The refractive index, relative density, and viscosity are related to specific characteristics of each vegetable oil such as predicting the liquid-like behavior of the oil, chain length, and unsaturation. The values of found for the refractive index and relative density showed no changes over the storage period remaining virtually unchangeable in the 2018 and 2019 harvest seasons following the same patterns when compared to those found These parameters are also not defined by the legislation (BRAZIL, 2005). The results obtained for water content show little difference between the harvest seasons (0.05 and 0.03%), a value that was not determined by Neto*et al.* (2009). Similar results were obtained in the viscosity analysis of the oils in the two harvest seasons (41.35 and 41.06 CST, respectively); parameters not found in the literature and not defined by Brazilian legislation (Brazil, 2005). The peroxide values obtained in the two harvest seasons, 4.06 and 3.46, like the acidity value results, indicate that the parameters related to oil quality preservation were kept constant, even during prolonged storage, keeping below the limits established by Brazilian legislation (Brazil, 2005). When compared with other oils extracted from oilseeds such as soybean, corn, safflower, and olive oil, the peroxide value results obtained for Brazil nut oils proved to be smaller (10.10 mEq/kg for soybean oil, 7.40 mEq/kg for corn oil, 7.75 mEq/kg for safflower oil, and 9.02 mEq/kg for oil olive oil (Champman*et al.*, 2009). This index is considered one of the parameters to assess the oxidative stability of oils and resistance to deteriorative processes. The comparisons confirm the quality of the Brazil nut oil, even during extended periods of storage, settling within the quality parameters established by the consumer market and the legislation (Brazil, 2005).

Infrared spectroscopic analysis of the oils: The absorption spectra in the infrared region were obtained from the measurement of the vibrational spectral bands present in the sample. This analysis is based on incident infrared radiation on the lipid matrix composition causingdetectable changes in the characteristics of each compound, in which the respective wavelengths are absorbed and recorded. The amount of energy absorbed is a function of the number of molecules present; the infrared equipment provides qualitative and quantitative information. The spectrum graph is the result of the relationship of transmittance of a sample as a function of the wavelength. This spectrum is a fundamental property of the molecule that may be used to characterize the sample and to determine its concentration. The absorption spectroscopy analysis in the infrared region for the oils studied (Figures 1 and 2) showed the same behavior and spectral peaks indicating the same characteristics for both vegetable oils from the two different harvest seasons studied. Figures 2 and 3 show the infrared spectra of Brazil nut oil. The presence of very similar broad bands in the range from 3447 cm^{-1} to 2854 cm^{-1} can be seen in the spectral peaks of 2018 and 2019 harvest seasons. These peaks are characteristic of the methyl (-CH3); methylenic (-CH2), and methyl (-CH) groups and are similar to those found in the spectral bands of Sapucaia, bacaba, tucuma oils(Santos *et al,*.(2019); Pinto *et al.*, 2018; costa *et al.*, 2016).

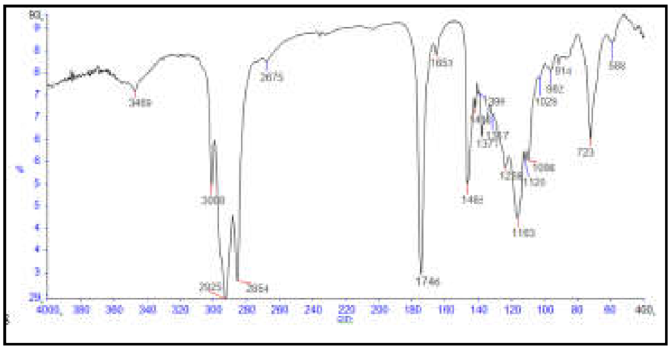

Figure 1. Brazil nut oil infrared absorption spectra, 2018 harvest

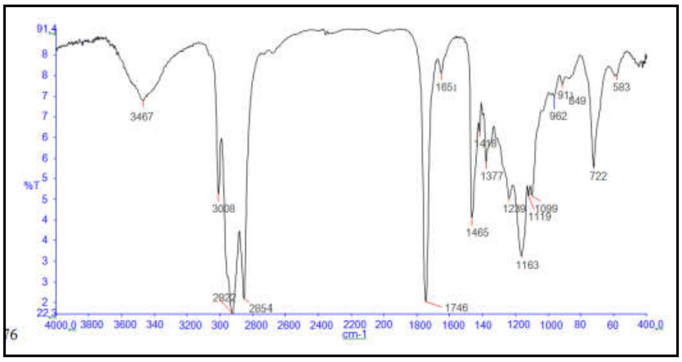

Figure 2. Brazil nut oil infrared absorption spectra, 2019 harvest

The bands lower than 1746 cm^{-1} are characteristic of carbonyl groups, (C = O), methyl esters, and triglycerides, common compounds in long chain fatty acids and evidence of vibrations frequency of carbonyl group of amides present in the protein portion. These bands are also present in the Brazil nut proteins, Sapucaia, bacaba (Santos *et al.*, 2019; Pinto*et al.*, 2018). The analysis performed by Shadangi, Mohanty (2014), with Sunflower seeds and Santos *et al.* (2019), with sapucaiaalmonds have a high content of protein, similar the brazil nuts, and hence it also contributes towards N-H stretching of the amide group. The carbonyl component that appeared due to the presence of lipids or ester are seen at 1743 cm^{-1}, similar to that obtained in this research. It can also be seen in both samples the presence of bands in the range of 1163 cm^{-1}, which is a characteristic of unsaturated ester functional group (C-O-C), and bands near 1290 to 1040 cm^{-1}; the last bands observed around 722 cm^{-1} can be related to the sequence of aliphatic fatty acid chains of and triacylglycerols (Pinto*et al.*, 2018; Santos *et al.*, 2019). When compared to the analysis performed by Santos *et al.* (2019) with sapucaia oil, oilseed from the same botanical family as the chestnut *Lecythidaceae* in the respective spectral bands, the spectroscopic analysis of the Brazil nut oil do not show significant differences since the patterns of group identification are classified within a range of values, in which small oscillations are allowed while keeping the elements defined in the same chemical group. A few spectral bands such as those of the group (CH_2) are present in the Brazil nut oil in several ranges from 722-2854 cm^{-1} with the presence of other intermediate groups within these ranges also. The same pattern occurred for the Sapucaia, Bacaba and tucumã oil withvalues ranging from 722 to 2923 cm^{-1} and little variation between these oils and the same chemical groups in these ranges (Santos *et al.*, 2019; Pinto *et al.*, 2018; Costa *et al.*, 2016). This comparative result between the same oil from different harvestseasons and other oils from different species shows that the compounds present in these raw materials maintain the same pattern of chemical composition as that of the Amazonianoil seeds found in the literature (Teixeira; Sousa, 2019). These results compared to those of other oils confirm the great diversity, quality, and amount of lipids in thesenuts, which shows, by comparing the spectraof the oils studied in different harvest seasons, that the aging process and proper storage during this period does not change the spectroscopic profile. Analyses of the fatty acid profile of Brazil nut oils confirmed the quality of the theseoils, as shown in Table II.

Table 2. Comparison of fatty acid profiles of Brazil nut oil in the harvest seasons

FATTY ACIDS	Area [%] (2018 Harvest)	Area [%] (2019 Harvest)
Palmitic Acid (C16:0)	14.85 ± 0,10	14.24 ± 0.02
Stearic Acid (C18:0)	10.83 ± 0.05	11.20 ± 0.02
Oleic Acid C18:1n9)	38.65 ± 0.04	36.27 ± 0.03
Linoleic Acid (C18:2n6)	34.92 ± 0.00	37.53 ± 0.04
Alfa-linoenic Acid (C18:3n3)	0.07 ± 0.00	0.08 ± 0.00
Erucic Acid (C22:1n9)	0.27 ± 0.02	0.29 ± 0.00
cis-Docosahexaenoic Acid (C22:6n3)	0.01 ± 0.00	0.06 ± 0.00
Others	0.33	0.34
TOTAL	100	100

Data represent mean ± standard deviation of triplicates

The fatty acid composition obtained in the chromatographic analysis showed a high percentage of unstauration of the oils with approximately 75%, especially for C18:1 (38.64% and 36.267%) and C18:2 (34.91% and 37.533%), respectively in the oils studied, showing little change in their profiles. This can be explained by minor variations in the composition of oils, which may possibly occur due to the difference between the harvest seasons, places of collection, and storage, among others. These data are similar to those obtained by previous studies on nut oils, such as that of Brazil Santos *et al.* (2012), with an average of 75.6% of unsaturated acids and the same predominance patterns of C18:1 (39.3%) and C18: 2 (36.1%), for the composition of unsaturated fatty acids in the nuts with predominance of oleic and linoleic acids. The strong presence of unsaturated fatty acids does not attenuate the considerable proportions of acids such as stearic, palmitic, and erucic acids, besides the traces of other fatty acids. When compared with the other oils profile, the fatty acid profile of Brazil nut oil is quantitatively smaller in terms of the total of unsaturated fatty acids of other oils maintaining the predominance of oleic and linoleic acids. The functionality of these oils is due to the presence of ω-6, ω-9, and traces of ω-3 fatty acids, which have been directly associated with many health benefits, with great emphasis on the effects of reducing the LDL cholesterol, antioxidant properties, anti-free radical activity, and cardioprotective effects. These functions show the importance of these oils, including the Brazil nut oil with the presence of appreciable components, comparable to most widespread commercial oils (Cardoso*et al.*, 2017; Pinto*et al.*, 2019; Santos*et al.*, 2019a). With regard to organic systems, high levels of unsaturated fatty acids are important in brain development and function since they are involved in the formation of cell membranes in the central nervous system. Another essential function assigned to these acids is being responsible for building the immune system and its efficient operation and for the pro-and anti-inflammatory responses. The production of eicosanoids such as leukotrienes (LTB4), thromboxane (TXA 2), prostaglandins (PGE2), and prostacyclin from the conversion of linoleic acid into arachidonic and linolenic acid into eicosapentaenoic (EPA) and DHA acids (docosahexaenoic) can be considered as responsible for these functions (Cardoso *et al.*, 2017; Pinto *et al.*, 2019; Santos *et al.*, 2019a). The importance of these results for various industrial sectors may be associated with high aggregate potential; health benefits through regular consumption of foods containing omega series fatty acids; studies on use for food fortification or enrichment; or even use as dietary supplements and on drugs and cosmetics, among other industrial applications.

Conclusion

The results obtained for the Brazil nut oil show that no differences were found in the physicochemical data comparing the two harvest seasons studied; the analysis of acidity and peroxide index confirm that when stored properly, the quality of the oil was maintained meeting the Brazilian legislation requirements.The spectroscopic analysis in the infrared region showed no differences in their bands in relation to the harvest seasons studied and the consequent storage process showing the stability of these oils, owing to the non-detection of spectra related to compounds resulting from oxidative processes in different stages of lipid degradation.The fatty acid composition is similar for the oils obtained from the two harvest seasons. It is worth higlighting the presence of omega functional fatty acids in both oils maintaining their quality even during storage.

REFERENCES

American Oil Chemists' Society (AOCS). Official methods and recommended practices of the american oil chemists' society, Champaign, IL., 2004.

American OilChemists' Society(AOCS). Official methods and recommended practices of the American Oil Chemists' Society, [AOCS]. 2005: Fatty Acid Composition by Gas Chromatography Method Ce 1-62, AOCS American Oil Chemists Society.

BRASIL. Resolução RDC/ANVISA/MS nº 270, de 22 setembro de 2005. Regulamentotécnicoparaóleosvegetais, gordurasvegetais e creme vegetal.DiárioOficial da RepúblicaFederativa do Brasil. Brasília, DF, 23 de setembro de 2005. Seção 1.

Cardoso B.R., Duarte, G.B.S.; Reis, B.Z.; Cozzolino,S.M.F. 2017. Brazilnuts: Nutritionalcomposition, health benefits and safety aspects. Food Research international.100, 9-18.

Champman T.M, Kim H.J, Min D.B. 2009. ProoxidantActivy of Oxidized α- Tocopherol in vegetable Oils. *Journal of Food Science,* 74, 536-542.

Costa, B.E.T.; Santos, O.V; Correa, N.C.F; França, L.F. 2016. Comparative study on the quality of oil extracted from two tucumã varieties using supercritical carbono dioxide. Food Science Techonology. v. 36, 322-328.

Elzupir, A.O. 2019. Seasonal variation and health implications due to aflatoxins in nuts sold in Riyadh region. *Revue françaised'allergologie.* 59,15–21.

International OrganizationFor Standardization 1978. ISO 5509.Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids.1-6.

Neto V.Q, Bakke O.A, Ramos C. M. P, Bora P.S, Letelier J. C, Conceição M. M. 2009. Brazil nut (*Bertholletiaexcelsa H.B.K*) seed kernel oil: characterization and thermal stability.Revista de Biologia e Farmácia. 03, 33-41.

Pereira E,Ferreira M.C,Sampaio K.A,Grimaldi R,Meireles J.A, Maximo G.J. 2019. Physical properties of Amazonian fats and oils and their blends.FoodChemistry, 278, 208-215.

Pinto R.H.H, Sena C, Santos OV, Costa WA, Rodrigues AMC, Carvalho Junior RN. 2018. Extractionof bacaba (*Oenocarpus bacaba*) oilwithsupercritical CO_2: Global yieldisotherms, fattyacidcomposition, functionalquality, oxidativestability, spectroscopic profile and antioxidant activity. Grasas aceites, 69, 1-8.

Santos O.V, Carvalho R.N, Costa C.E.F, LannesS.C.S. 2019. Chemical, chromatographic-functional, the rmogravimetric-differential ands pectroscopic parameters of the sapucaia oilobtained by different extraction methods. Industrial Crops and Products, 132:487-496.

Santos, O.V, Corrêa, N.C.F.; Soares, F.A.S.M.; Gioielli,L.A.; Costa, C.E.F.; Lanne, S.C.S. 2012. Chemical evaluation and thermalbehaviorofBrazilnutoilobtainedby diferente extraction processes. FoodResearchInternational. 47, 253-258.

Serra J.L, Rodrigues A.M.C, Freitas R.A,Meirelles A.J.A, Darnet S.H, Silva L.H.M. 2019. Alternative sources of oils and fats from Amazonian plants: fatty acids, methyl tocols, total carotenoids and chemical composition. Food research international**.**.116, 12-19.

Shadangi K.P, Mohanty, K. 2014. Kinetic study and thermal analysis of thepyrolysis of non-edible oilseed powders by thermogravimetric and differentialscanning calorimetric analysis. Renewable Energy, 63, 337–344.

Teixeira A.M, Sousa C. 2019. A review on the application of vibrational spectroscopy to the chemistry of nuts.Foodchemistry. 277, 713-724.0

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