BRAZIL NUT (*BERTHOLLETIA EXCELSA* HBK) SEED KERNEL OIL: CHARACTERIZATION AND THERMAL STABILITY.

Vicente Queiroga Neto[1], Olaf Andreas Bakke[1],Cintia Maria Pinto Ramos[2], Pushkar Singh Bora[2], Juan Carlos Letelier[2], Marta Maria da Conceição[3].[1]*

ABSTRACT

Brazil nut shows a high nutritive and commercial value, and represents an important export product from the north region of Brazil. The oil extracted from its kernel presents a high coefficient of digestibility, and the flour is a protein rich product. This is mixed with wheat flour in recipes of a highly nutritive value bread. Also, its flour composes pre-cooked food and animal feed. The objective of this work was to extract the oil of Brazil nut kernel and study its physic-chemical, chemical and thermal properties. The mean oil content in the Brazil nut kernel is 68.58%. Mean values for the physic-chemical characteristics of this oil are: specific density-0.94, refractive index-1.4451, peroxide index-2.92, acidity index in oleic acid-0.71, iodine index (Hülb)-97.8 and saponification index-187.5 indexes. Chromatographic analysis resulted in the following mean values of fatty acids: saturated (28.62%) - C7:0, C8:0, C12:0, C13:0, C17:0 and C19:0-traces (<0.06%), C16:0-15.45%, C18:0-12.83%, C20:0-0.07%, C22:0-0.09% and C24:0-0.19%, monounsaturated (32.03%) and polyunsaturated (39.24%) - C16:1 - traces, C18:1 – 31.95%, C24:1 – 0.08%, C18:2 – 39.14% and C18:3 – 0.10%. TG/DTG curves showed that the process of thermal decomposition of the oil occurs in three phases: decomposition of polyunsaturated, monounsaturated and saturated oils, respectively. DSC curve revealed one endothermic event due to thermal decomposition at Ti = 340°C and Tf = 395°C, and with ΔH = 30.33 Cal/g.

Uniterms: Brazil nut, oil, physical and chemical properties, chemistry, thermogravimetry.

ÓLEO DA AMÊNDOA DA SEMENTE DE CASTANHA-DO-PARÁ (*BERTHOLLETIA EXCELSA* HBK): CARACTERIZAÇÃO E ESTABILIDADE TÉRMICA.

RESUMO

A castanha-do-pará é dotada de elevado valor nutritivo e comercial, sendo um dos produtos de destaque na pauta de exportação da região norte do Brasil. O óleo da amêndoa apresenta bom coeficiente de digestibilidade e a farinha é um produto rico em proteína. Alem disso, é utilizada em misturas com farinha de trigo para fabricação de pão misto de valor nutritivo elevado. O objetivo deste trabalho foi extrair o óleo da amêndoa e estudar suas propriedades físico-químicas, químicas e térmicas. A média do teor de óleo na amêndoa foi de 68,58%. As propriedades físico-químicas apresentaram os seguintes valores médios: densidade específica –0.94, índice de peróxido – 2.92, acidez em ácido oléico - 0.71, índice de iodo (Hülb) – 97.8 e saponificação–187,5. A análise cromatográfica revelou a seguinte composição média em ácidos graxos: saturados - 28,62%: C7:0, C8:0, C12:0, C13:0, C17:0 and C19:0-traces (<0.06%), C16:0-15.45%, C18:0-12.83%, C20:0-0.07%, C22:0-0.09% and C24:0-0.19%, monoinsaturados (32,03%) e poliinsaturados (39,24%) - C16:1-traços, C18:1-31,95%, C24:1-

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[1] Programa de Pós-Graduação em Zootecnia/CSTR - UFCG. Av. Universitária, s/n, Bairro Santa Cecília, CEP: 58708-110., Patos - PB. E - mail: vqneto@cstr.ufcg.edu.br

[2] Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos/CT – UFPB João Pessoa –PB, Brasil. E-mail: ppgcta@ct.ufpb.br

[3] Universidade Federal de Campina Grande, Cuité – PB, Brasil. E-mail: martamaria8@yahoo.com

0,08%, C18:2-39,14% e C18:3-0,10%. Curvas TG/DTG mostraram que o processo de decomposição térmica do óleo ocorrem em três etapas:, decomposição dos ácidos graxos poliinsaturados, monoinsaturados e saturados, respectivamente. A curva DSC indicou 1 evento endotérmico, referente a decomposição térmica, com Ti=340ºC, Tp=395ºC e ΔH = 30.33 Cal/g.

Unitermos: castanha do pará, óleo, propriedades físicas e químicas, química, termogravimetria.

INTRODUCTION

The Brazilian flora is rich in a variety of native and exotic plants with a high potential for exploration either due to the genetic pattern of the species or diversification of its culture or due to the fruits (Carvalho, 1996). Braga (1976) emphasised the importance of some of these fruits such as cashew nut and Brazil nut, and suggested that, if explored rationally, they would constitute an excellent option as a food source. Among these species, the *Bertholletia excelsa* produces a fruit in which the seed kernel possesses nutritive potential due to its high lipid and protein contents (Glória & Regitano-D' Arce, 2000).

The Brazil nut is one important plant of the Amazon tropical forest. Its fruits are commercially important due to the nut kernels, one of the principal export commodities of the northern Brazil region. It is consumed raw and participates in a variety of recipes (Muller, 1981).

Thermoanalytical techniques are commonly used to evaluate the stability of vegetable oils (Kowalski, 1989; Wesolowski, 1993) and have the advantage of providing information that conventional methods are incapable (Santos et al., 2004). Besides, these techniques require smaller quantities of samples, shorter periods of analysis, and offer smaller chances of error. The Differential Thermogravimetry curves (DTG) provide detailed information such as initial and final temperatures of a particular process. The thermogravimetric profiles for sunflower oil showed a level stretch indicative of stability up to about 200 °C. Thermal decomposition of the oil occurred in three stages, related to the decomposition of polyunsaturated, monounsaturated and saturated fatty acids, respectively (Souza et al., 2004).

Very little information on the lipid fraction of Brazil nut seed kernel is available except that of Ferreira et al. (2008), Silva, Cortesi And Rovellini (1997) on characterization physicist-chemistry and composition fatty acid, Gutierrez et al. (1997), Ribeiro et al. (1997), Assunção, Bentes and Serrya (1984) on oxidative stability of crude oil. So far, there is no available report on the thermal stability of this oil. Therefore, the present work was undertaken to determine the physico-chemical properties, fatty acid composition and thermal stability of the Brazil nut seed kernel oil.

MATERIALS AND METHODS

The Brazil nut seed kernel

Brazil nut kernels were acquired from the local market in Belém City in the State of Pará, Brazil. The kernels were dried (50 °C for 24 hours) in a forced air circulation dryer and the pellicles were removed by simple rubbing. The dried clean kernels were triturated in a domestic multi-processor (Arno SA, Brazil) at top speed and passed through the 40 mesh screen. The flour was immediately utilized for proximate analysis. For extraction of oil, the flour was packed in polyethylene bags and stored in a refrigerator.

Proximate analysis of seed kernel

Moisture, protein, lipid and ash contents were determined following standard AOAC (1993) methods. Total carbohydrate content was calculated by difference. Three different samples were analyzed in duplicate.

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Extraction of oil

The oil was extracted from dried and triturated seeds with hexane in a Soxhlet extraction apparatus for about 12 hours. After completion of extraction, the solvent was recovered. The residual solvent from the oil was removed in a boiling water bath. Triplicate samples were prepared and packed in 250 mL amber colored bottles and stored in a refrigerator.

Physical and physicochemical properties

The specific gravity and refractive index were determined at a temperature of 25 °C using a specific density bottle and a refractometer, respectively. Standard AOAC (1993) methods were used for the determination of acid, peroxide, iodine and saponification indexes. Three different samples of oil were analyzed in duplicate.

Fatty acid composition of the oil

Fatty acids were converted to their methyl esters (FAME) following the method of Hartman and Lago (1973). Fatty acyl distribution in the oil was determined in a gas chromatograph (HP 5890 Series II, Hewlett Packard) equipped with a flame ionization detector. 1.5 µl of the FAME sample was injected and the separation was carried out on HP-INNO wax capillary column (Hewlett Packard; 30m length, 0.25mm id. and 0.25µm film thickness). The carrier gas (helium) head pressure was maintained at 11.5 psi and the column flow rate was 1 ml/min. The oven temperature was held initially at 120^{o}C for 1 min, increased from 120^{o}C at 8^{o}C/min to 210^{o}C and then held at 210^{o}C for 45 min. The temperature of the injection port and of the detector was 250^{o} C and 280^{o} C, respectively. FAME were positively identified by matching their retention time with those of standards obtained from SIGMA Co (USA), which were also run under identical analytical conditions.

Thermal Stability of oil

The evaluation of the thermal stability Brazil nut kernel oil was determined through the thermogravimetric curves in a Thermal Analyzer Shimadzu TGA-50 in the air atmosphere with flux of 50 mL.min^{-1} and heating rate of 10 °C.min^{-1}. Ten milligrams of oil sample were subjected to a temperature range of 30 to 600 °C. The thermal stability was determined by the analysis of TG/DTG curves that registered mass loss of oil during the heating period. For calorimetric curve (DSC) about 10 milligrams of oil sample were subjected to heating at a rate of 10 °C.min^{-1} in a Shimadzu calorimeter DSC-50 in nitrogen atmosphere with flux of 50 mL.min^{-1}. The sample was heated up to 500 °C.

RESULTS AND DISCUSSION

A proximate analysis is of utmost importance to provide information on the basic constituents of a particular raw material. The result of the proximate analysis of dried Brazil nut flour was, in % (mean±SD), as follows: 4.91 ± 0.09 moisture, 68.58 ± 1.07 lipids, 16.50 ± 1.09 protein, 4.32 ± 0.80 ash, and 5.69 ± 1.02 carbohydrates (by difference). Similar values for lipid (66.8 and 67.0) and protein (13.6 and 16.08) contents were also reported in earlier works by Andrade et al. (1999) and Santos (1978), respectively. These results show that dried kernel powder is a good source of lipid and proteins (17.9 %) similar to other oleaginous seed kernels that are rich in these constituents.

Table 1: Proximate composition of dried *Brazil nut* seed kernel, obtained from three lots of dried and powdered seed kernels.

Constituents	% (mean ± SD)
Moisture	4.91 ± 0.09
Lipids	68.58 ± 1.07
Ash	4.32 ± 0.80
Protein	16.5 ± 1.09
Carbohydrates (by diff.)	5.69 ± 1.02

The results show the average value and standard deviation of the analysis of three oil samples.

The knowledge of the physico-chemical characteristics of the oil and fat compounds is important as it individualizes each lipid by means of specific indexes. Also, this knowledge allows the identification of the type of fatty acid present in the lipid (saponification index) and its unsaturation level (iodine index) (Branco, 1976). Table 2 presents the physico-chemical properties of Brazil nut seed oil. The saponification index was about 187.5 which is indicative that the oil contains fatty acids of long alifatic chain on average. The iodine index was 97.8, a value suggestive of the presence of significant amounts of insaturated fatty acids. The acid and peroxide indexes represent the quality of the oil. The acid index of the oil was 0.71% as oleic acid, within the 0.3 to 2.0% range established by the Brazilian Ministry of Health (Brasil, 1998) and lightly higher than the acid index recommended by Codex Alimentarius (1993) for edible oils. Although acidity is caused by the hydrolytic rancidity of the seed, it could be easily minimized during the process of oil refinement. However, in spite of the fact that crude oil was analyzed in the present study, the peroxide index was 2.92 meq/kg, well below the limit of 10.0 meq\kg oil established by Codex Alimentarius (1993).

Table 2: Physico-chemical properties of *Brazil nut* seed kernel oil, obtained from three oil samples.

Physicochemical Property	Index (mean ± SD)
Specific density at 25^0 C	0.9400 ± 0,0012
Refractive index at 25^0 C	1.4451 ± 0,003
Acid index (oleic acid %)	0.71 ± 0,01
Saponification index (mg KOH/g oil)	187.5 ± 1,01
Iodine index (g of I_2/100g oil)	97.8 ± 0,22
Peroxide index (mEq.g/kg oil)	2,92 ± 0,17

The results show the average value and standard deviation of the analysis of three oil samples.

The fatty acids composition of the seed kernel oil is shown in Table 3. Fatty acids from 7 to 24 carbon atoms containing up to three double bonds were detected. The saturated fatty acids represented 28.62% of the total fatty acids, predominantly $C_{16:0}$ (15.45 %), $C_{18:0}$ (12.83 %), $C_{20:0}$ (0.07 %), $C_{22:0}$ (0.09 %) and $C_{24:0}$ (0.19 %). Other saturated fatty acids, such as $C_{7:0}$, $C_{8:0}$, $C_{12:0}$, $C_{13:0}$, $C_{17:0}$ and $C_{19:0}$, were also identified in trace quantities. Unsaturated fatty acids represented 71.3 %, of the total fatty acids. C18:1 (31.95 %) and linoleic acids (39.14 %) together contributed about 99.7% of the total unsaturated fatty acids. The monounsaturated fatty acid concentration (32.03 %) was smaller than that reported for canola (61.5%), olive (61.9%) and was larger than soy (22.9 %) and sunflower (23.2) (BRUZZETTI, 1999). However, its polyunsaturated fatty acid concentration (39.24%) was inferior than all mentioned species (Bruzzetti, 1999). Also, linolenic acid (C18:3) was present in low (0.10%) concentration. Fatty acids C18:2 and C18:3, representatives of the ω-6 and ω-3 families, are essential for humans because they are the precursors of arachidonic acid which metabolically transform to long chain polyunsaturated fatty acid such as eicosapentaenoic (20:5) and docosahexaenoic (22:6) fatty acids. Among other functions, these fatty acids are important in the formation of eicosanoids that includes prostaglandin (PG), tromboxan (TXA), prostacyclin (PGI) and leucotrien (LTB). These substances play an important role in the mediation of immunologic, allergic, inflammatory reactions, and in the control of hemostasy (Calder, 1993; Voss, 1994). Behenic acid was also found in small concentration (0.09%). This fatty acid has been reported as an anti-nutritional factor (Balogun & Fetuga, 1985), because it inhibits the action of digestive enzymes in the digestive tracts of human and animals.

Table 3: Fatty acid composition of *Brazil nut* kernel oil, obtained from three oil samples

Fatty acid	% (mean ± SD)
Saturated fatty acids	28.62
C7:0	TR
C8:0	TR.
C12:0	TR.
C13:0	TR
C16:0	15.45 ± 0.07
C17:0	TR
C18:0	12.83 ± 1.04
C19:0	TR
C20:0	0.07 ± 0.02
C22:0	0.09 ± 0.07
C24:0	0.19 ± 0.12
Monounsaturated fatty acids	32.03
C16:1	TR
C18:1	31.95 ± 1.49
Polyunsaturated fatty acids	39.24
C18:2	39.14 ± 0.12
C18:3	0.10 ± 0.04

The results show the average value and standard deviation of the analysis of three oil samples.

Thermal Analysis

The oxidative process in vegetable oils is characterized initially by the oxidation forming secondary products – peroxides. The following phase corresponds to the decomposition of monounsaturated fatty acids, mainly oleic acid and the polymerization of the substances remaining from the previous phase.

The thermogravimetric (TG) profile of Brazil nut seed oil (Figure 1) shows its thermal behavior in dynamic conditions. TG curve shows thermal stability of the oil untill a temperature of 209 ºC (T_i). The decomposition and carbonization processes follow the same three stages curve, ending at a temperature of 602 ºC (T_f). At this temperature mass loss reached 97%. In DTG curve (Figure 1) it could be noted more explicitly that the process of thermal decomposition of the oil occurred in three stages. In the first stage the temperature ranged from 209 to 408 ºC, mass loss was 53.7%, similar to the reported for olive (53.25%) and rice (53.19%), lower than the ones for canola (58.66%), and higher than those for sunflower (49.65%) and soy (51.31%) oils (Santos, 2002). Buzás et al. (1988) and Weast (1973) explain that the temperature range corresponding to the first stage could be attributed to the thermal decomposition of the polyunsaturated fatty acids. This stage is considered the most important one and represents the initial phase of the degradation of triglycerides, mainly composed of the polyunsaturated fatty acids. In this phase the oxidation of the polyunsaturated fatty acids take place. The second stage of thermal decomposition occurred at a temperature range of 408 to 492.0 ºC. The mass loss corresponded to 34.9% of the initial mass, higher than the loss of mass observed for all other oils reported by Santos (2002), ranging from 9.73% for olive to 14.65% for sunflower oil. The third stage of thermal decomposition occurred at a temperature range of 492 to 602 ºC. The mass loss at this stage corresponded to 97% of the initial mass. The second and third phases represented the decomposition of monounsaturated and saturated fatty acids, respectively, and other substances formed during the polymerization of the degradation products of the fatty acids in the earlier stage. High temperatures catalyze the reactions of hydrolysis and oxidation of oils (Dobarganes & Perez-Camino, 1991). The products of these reactions react among themselves and produce cyclic monomers, dimers, and polymers.

According to Fennema (1993) and Paul & Mital (1997), the thermal stability of the oil used in frying process depends upon its acid and peroxide indexes. Gennaro et al. (1998) reported that the thermal stability of the oil depends on the proportion of saturated and unsaturated fatty acids in triacilglycerides. Free fatty acids and other substances, such as carotenoids, sterols, phospholipids, phenolic substances and tocopherol, have been pointed out as responsible for thermal stability by the same authors. They conclude that the stability of virgin olive oil (*i.e.*: low level of auto-oxidation) is mostly due to the presence of natural phenolic components that makes their way to the oil during the extraction process.

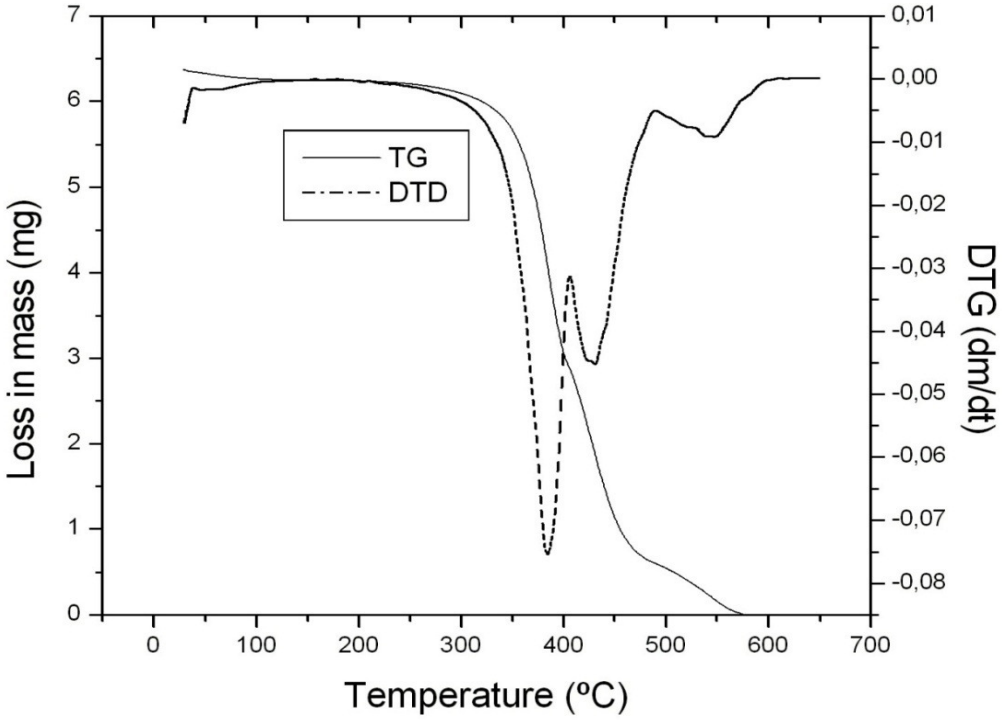

Figure 1 - TG/DTG curves of *Brazil nut* seed kernel oil.

Differential Scanning Calorimetry (DSC)

The DSC curve for the Brazil nut seed oil is shown in Figure 2. The energy transition begun at 340 °C and ended at 395.5 °C (Tf), with a variation in enthalpy of 34.33 cal/g. An endothermic peak was evidenced at 394 ºC (Tp). The endothermic transition in the oil corresponds to the thermal decomposition of the saturated and unsaturated fatty acids (NASSU, 1994) in nitrogen atmosphere while, exothermic transition is attributed to the process of auto-oxidation. The ΔH for this transition is sufficiently high (34.33 cal/g), suggesting that the oil offered a good resistance to oxidation in nitrogen atmosphere. The values of the enthalpy are negative because the oil is receiving heat for its decomposition. In any case, the bigger the size of the hydrocarbon chain, the higher the enthalpy of activation. The presence of antioxidants in the oil also influences its activation energy through the induction of enthalpy (SIMON et al., 2000). These authors observed that the enthalpy of oils containing antioxidants requires enthalpy of decomposition superior to oils that do not contain antioxidants. Kasprzycka-Guttman and Coziniak (1995) reported that the thermal decomposition of saturated fatty acids requires more energy than the unsaturated fatty acids.

The DSC profile was similar to that of canola and olive oil as reported by Santos (2002), and the enthalpy of the transition (ΔH) was superior to that of the soy (19.0 cal/g), corn (11.6 cal/g), sunflower (21.61 cal/g) and inferior to canola (35.31 cal/g), rice (51.13 cal/g), and olive (46.37 cal/g) oils.

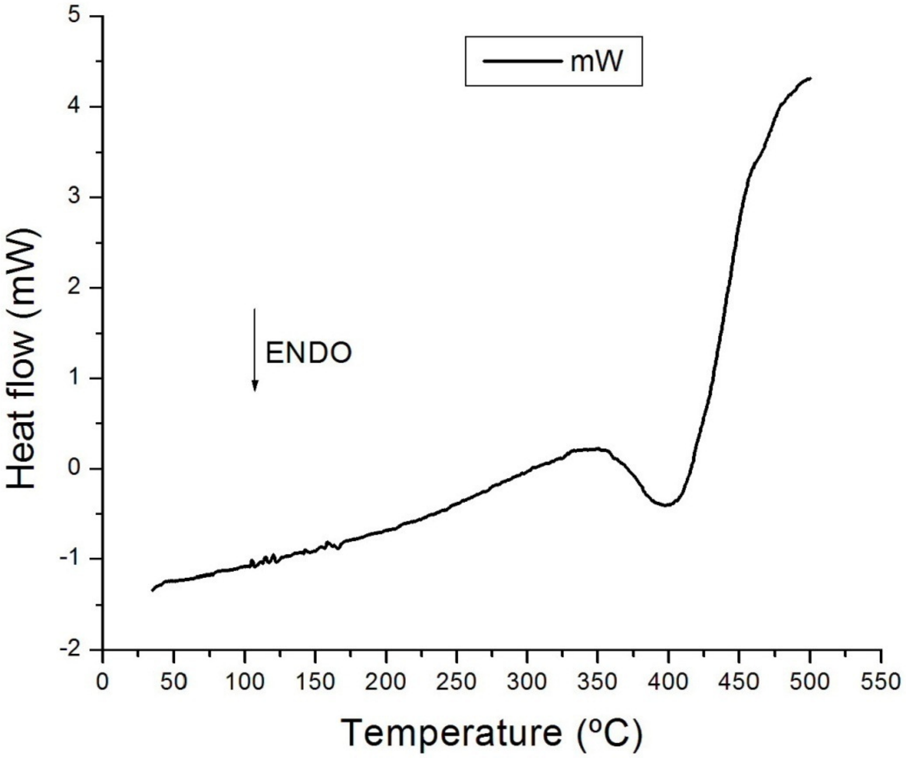

Figure 2 - DSC curve of *Brazil nut* seed kernel oil.

CONCLUSIONS

The studied oil showed physico-chemical properties within the limits preconized by the Brazilian legislation and are related with its fatty acid chemical composition, suggesting a high resistance to oxidative degradation. However, its nutritional quality, compared to other commercial oils, was negatively affected by its relatively low content of essential fatty acid.

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