

*Full Length Research Paper*

# Determination of total phenolic compounds, antioxidant activity and nutrients in Brazil nuts (*Bertholletia excelsa* H. B. K.)

Selvin Antonio Saravia Maldonado<sup>1\*</sup>, Ismael Montero Fernández<sup>2</sup>, Ricardo Santos Alemán<sup>3</sup>, Jhuni Abrahan Marcia Fuentes<sup>4</sup> and María da Conceição Campelo Ferreira<sup>5</sup>

<sup>1</sup>Faculty of Earth Sciences and Conservation, National University of Agriculture, Catacamas, Olancho, Honduras.

<sup>2</sup>Department of Organic and Inorganic Chemistry, University of Extremadura, Cáceres, Spain.

<sup>3</sup>Department of Food Science, Louisiana State University, United States.

<sup>4</sup>Faculty of Technological Sciences, National University of Agriculture, Catacamas, Olancho, Honduras.

<sup>5</sup>Post-graduate Program in Biodiversity and Biotechnology, Bionorte, Federal University of Roraima, Boa Vista-RR-Brazil.

Received 27 March, 2020; Accepted 5 June, 2020

*Bertholletia excelsa* is native to the Amazon, and it grows in the forests that border the great rivers of the Amazon. This tree brings interest in the commercialization of its fruit, which is called the urchin and almond. *Bertholletia excelsa* has antioxidant properties, and it is rich in phenolic compounds. In this research, the antioxidant capacity of the Brazilian nut from the city of São João da Baliza was evaluated by the DPPH method with a percentage of inhibition of  $70.04 \pm 0.07\%$ . In addition, the quantification of the total phenolic compounds was carried out by spectrophotometry according to the Folin-Ciocalteu method using gallic acid as standard. A value of total phenolic compounds expressed as gallic acid equivalents of  $1.62 \pm 0.11$  mg GAE.  $g^{-1}$  was obtained. On the other hand, the minerals were determined by nitric-perchloric digestion highlighting the high concentration of potassium  $581.21 \pm 14.12$  mg  $100 g^{-1}$  and calcium  $171.32 \pm 0.84$  mg  $100 g^{-1}$ , as a source of micronutrients, as well as iron  $2.15 \pm 0.07$  mg  $100 g^{-1}$  and manganese  $1.49 \pm 0.08$  mg  $100 g^{-1}$ .

**Key words:** Functional foods, minerals, bioactive foods, amazon.

## INTRODUCTION

The Brazil nut (*Bertholletia excelsa* HBK), is also known by other names such as castanha-do-brasil, castanha-do-para or castanha-da-amazônia (Brazil). This is a tree of great size, with large and emerging crown, forming large colonies or groups known as chestnut trees (Fernandes and Alencar, 1993). It belongs to the Lecythidaceae

family, including 200 widely distributed species (Mori, 1992). In the State of Roraima, they are found in the municipalities of São João da Baliza, Cujubim and in the region of the Itá settlement, among others.

Regarding production, Brazil is one of the world's largest producers of Brazil nut fruit with more than 35

\*Corresponding author. E-mail: saraviaselvin@yahoo.com.

tons per year (Tonini, 2007). This fruit have 544 mg of Selenium per ounce according with National Institute of Health (NIH, 2020). It is a non-wood forest product of importance in the local and international markets (Peres et al., 2003), its importance being emphasized and highlighted for populations living in rural areas, which it used goes for medicine applications as is the case of the bark, which is used in tea making as a natural remedy for anemia, intestinal problems and hepatitis (Cymerys et al., 2005). The consumption of nuts presents an important impact in the reduction of cardiovascular diseases (Kim et al., 2018) as well as helping to improve the serum lipid profile (Colpo et al., 2013). In terms of profitability, the edible part of the Brazil nut is the seed, being a source of economic income for local populations engaged in harvesting where, according to FAOSTAT (2013), the export of the seeds provides a source of income of approximately US \$ 166 million for Brazil together with Bolivia and Peru. Authors such as Duchelle et al. (2011) pointed out that collector who extract Brazil nut from almost all protected areas can yield a 43% of total income within households.

In nutritional terms, it is food that provides considerable amounts of energy, rich in proteins and significant in antioxidant activity (Cozzolino, 2001). Furthermore, it is rich in nutrients such as iron, magnesium and manganese, which can be used at the same time as dietary supplements (Chunhieng et al., 2004). Therefore, the objective of this research was to evaluate the proximate composition, mineral content, total phenolic compounds and antioxidant activity in Brazil Chestnut grown in the city of São João da Baliza, Roraima State (Brazil).

## MATERIALS AND METHODS

### Collection and preparation of samples

The samples were collected in the Municipality of São João da Baliza in 2016, then the collected fruits were selected in 10 units according with NTON 17002-02 (2002), were taken to the Laboratory of the Agronomic Research Center, at the Center for Agricultural Sciences, campus Cauamé, Federal University of Roraima, where the fruits were opened and the seeds were separated, previously washed with distilled water and then with a 1% sodium hypochlorite solution and, finally, again with distilled water. The samples were frozen in an ultra-freezer at -80°C for subsequent lyophilization in a LIOTOP L101 lyophilizer for 48 h until the material was completely dried, then they were ground and sieved between 30 and 40 Mesh, then stored in closed bags and protected from light until the time to do the analysis.

### Proximate and mineralogical composition

The bromatological parameters evaluated in the Brazil Chestnut were carried out according to the methodology described by IAL (2008). To determine the moisture, the samples were placed for 6 h at 105°C until constant weight. Subsequently, to determine the percentage of ash, the samples were incinerated in a muffle at

600°C. The total percentage of proteins was carried out by digestion followed by determination Kjeldahl method. Determination lipid percentage was carried out by means of a Soxhlet extraction system and, finally, the carbohydrate determination was carried out by difference according to Equation 1.

$$\text{Carbohydrates} = 100 - (\% \text{Moisture} + \% \text{Ash} + \% \text{Lipids} + \% \text{Proteins}) \quad (1)$$

Finally, to determine the total energy value, Equation (2) described by Mendes-Filho et al. (2014) was used.

$$\text{Energy value (kcal/100 g)} = (P \times 4) + (L \times 9) + (C \times 4) \quad (2)$$

P = value of protein (%), L = lipid value (%), C = carbohydrate value (%), 4 = conversion factor in kcal determined in calorimetric pump for proteins and carbohydrates and 9 = conversion factor in kcal determined in a calorimetric pump for lipids.

The extraction of the minerals into the Brazil nuts was done according to the methodology described by Embrapa (2009), in which the perchloric nitric digestion (3:1) was used in TECNAL model TE 0079 digester block, washed with distilled water up to 25 mL for subsequent analysis. Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) were determined by Flame Atomic Absorption Spectrophotometry (FAAS) Shimadzu AA-7000, coupled with ASC-7000 auto sample. The potassium (K) composition was determined by means of flame photometry. Calibration was performed with standard solutions prepared from commercial standards of 1000 mg L<sup>-1</sup> Qhemis High Purity PACU 1000-0125. Phosphorus (P) was determined by with the ultraviolet molecular absorption spectrophotometry technique was used using a SHIMADZU UV-1800 model, according to the Embrapa (2009), by formation of the colorimetric reaction with ammonium molybdate ((NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub>) where the recordings were record at λ = 660 nm.

### Determination of total phenolic compounds and antioxidant activity

A determination of the total phenolic compounds was made according to the methodology proposed by Wolfe et al. (2003) and modifications, being expressed as a quantification of the total amount extract as mg AG 100 g<sup>-1</sup> sample. A determination of the antioxidant activity of the different extracts, supported by the extinction method of the absorption of radical 1,1-diphenyl-2-picril hydrazil (DDPH), by spectrophotometry of molecular absorption in visible ultraviolet measured at 515 nm (Miranda and Fraga, 2006).

## RESULTS AND DISCUSSION

The amount of total phenolic compounds was carried out by the Folin-Ciocalteau method, which determined a quantity of total phenolic compounds in Brazil nut of 1.62 ± 0.11 mg GAE g<sup>-1</sup>, being used as gallic acid standard. According to Braga et al. (2010), who studied the concentration of phenolic compounds in different Amazon fruits, including Brazil nut, whose values range between 0.43 and 1.23 mg GAE g<sup>-1</sup>, being the values obtained in this work higher than those determined by this author.

Costa and Jorge (2011), presented values of quantity of phenolic compounds for Brazil nut of 1.69 mg GAE g<sup>-1</sup> close to those found in this work. The percentage of antioxidant activity was also determined using the DPPH

**Table 1.** Nutritional and mineralogical composition in the seeds of *Bertholletia excelsa* H.B.K.

Bromatological composition						
Carbohydrates (%)	Proteins (%)	Lipids (%)	Moisture (%)	Ashes (%)	Energetic value (kcal 100 g <sup>-1</sup> )	
10.20± 0.15	17.31± 0.13	66.11± 0.32	2.87± 0.17	3.51± 1.14	705.03	
Mineralogical composition (mg 100 g <sup>-1</sup> )						
P	K	Ca	Mg	Fe	Cu	Zn
9.5± 0.12	581.21± 14.12	171.32± 0.84	412.34± 1.17	2.15± 0.07	0.89± 0.07	3.61± 0.13

method, obtaining a percentage of antioxidant activity of  $70.04 \pm 0.07\%$ . This high antioxidant activity in Brazil nut, gives this seed a high added value since it causes a reduction in lipid oxidation, and on the other hand, when consumed, they reduce the risk of development of pathologies such as arteriosclerosis, brain dysfunction and some types of cancer (Pyrzynska and Biesaga, 2009).

Table 1 showed the results of the bromatological composition of the Brazil nut pulp, as well as its mineral composition. The values analyzed for the Brazil nut, mainly with the lipids that presented the greatest energy contribution with 66.11% followed by proteins with 17.31%. This high percentage of fatty acids confers a nutritional value added, which in its chemical composition of these fatty acids is unsaturated fatty acids such as oleic followed by linoleic acid, according to the determined values of fatty acid dices by Ferreira et al. (2019), the Brazil nut has a 37.7% oleic acid and between 27.8 and 32.6% linoleic acid. The results of the bromatological analysis presented in this work, are in agreement with those presented by Ferreira et al. (2006) who obtained an energy value of 680.20 kcal 100 g<sup>-1</sup> next to that obtained in this investigation.

The concentration of both macro and micronutrients minerals were determined and potassium with a concentration of  $581.21 \pm 14.21$  mg 100 g<sup>-1</sup>, followed by magnesium with a concentration of  $412.34 \pm 1.17$  mg 100 g<sup>-1</sup>. The value of magnesium obtained in this work for the Brazil nut was slightly higher than that of another edible Brazil nut called *Sapucaia* nut (*Lecythis pisonis* Cambess), whose magnesium concentration is 343.4 mg 100 g<sup>-1</sup> (Carvalho et al., 2012). The consumption of magnesium is important, since this element is involved in various metabolic reactions such as the glycolysis, synthesis of ATP, proteins and nucleic acids (Volpe, 2013). The recommendations of this element being in agreement with the Recommended Dietary Reference Intake (1999), which are of 420 mg per day for men and 320 mg per day for women. In the Brazil nut, minority nutrients were also detected, among them zinc with a concentration of  $3.61 \pm 0.13$  mg 100 g<sup>-1</sup>, followed by iron, with a concentration of  $215 \pm 0.07$  mg 100 g<sup>-1</sup>. Zinc is a necessary element for the proper functioning of all cells in the body, being essential for the proper development of

the immune system, diabetes control and improvement of stress levels among other benefits (Freitas et al., 2016).

This nutrient, on the other hand, establishes a certain synergism with other minor nutrients such as iron and copper, since zinc absorption can be affected by iron supplementation in terms of excess zinc intake, it can reduce absorption of copper (Pinheiro et al., 2005). According to Cominetti and Cozzolino (2009), zinc demands for the organism increases with age, reaching concentrations of this mineral of 9.4 mg per day for men and 11 mg per day, for women.

The manganese concentration found was  $1.49 \pm 0.08$  mg 100 g<sup>-1</sup>, being in agreement with the concentration values of this element in fruits determined by the FAO/WHO (2002) in which the concentration was found to be between 0.20 and 10.38 mg kg<sup>-1</sup>. Lastly, copper is another of the microelements that we do not synthesize and we need to incorporate into the diet, since it has enzymatic activity, as well as being involved in the development of the immune system and gene expression (Amancio, 2017; Lugo, 2017). The copper values found were close to those shown by Da Silva et al. (2010), who considered a value of  $1.35 \pm 0.19$  mg 100 g<sup>-1</sup>.

## Conclusions

The Brazil nut is rich in energy, mainly due to its composition of lipids and proteins, and it can be used nutritionally in the elaboration of functional foods for energy-protein diets. In this sense, it is worth highlighting the concentrations of minerals, especially micronutrients such as manganese, iron, copper, and zinc, as well as macronutrients such as magnesium, potassium, and phosphorus with wide nutritional interest.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

Amancio OMS (2017). Funções plenamente reconhecidas de nutrientes cobre. São Paulo: Série de Publicações ILSI Brasil.

- Braga ACC, Silva AE, Pelais ACA, Bichara CMG, Pompeu DR (2010). Atividade Antioxidante e Quantificação de Compostos Bioativos dos Frutos de Abricó (*Mammea americana*). *Alimentos e Nutrição* 21(1):31-36.
- Carvalho IMM, Queirós LD, Brito LF, Santos FA, Bandeira AVF, Souza AL, Queiroz JM (2012). Chemical characterization of sapucaia nuts (*Lecythis pisonis* Cambess.) from zona da mata mineira region. *Bioscience Journal* 28(6):971-977.
- Chunhieng T, Petritis K, Elfakir C, Brochier J, Goli T, Montet D (2004). Study of selenium in the protein fractions of the Brazil nut, *Bertholletia excelsa*. *Journal of Agricultural Food Chemistry* 52:4318-4322.
- Colpo E, Vilanova CDA, Reetz LGB, Duarte MMF, Farias ILG, Muller EI, Muller ALH, Flores EMM, Wagner R, da Rocha JBT (2013). A Single Consumption of High Amounts of the Brazil Nuts Improves Lipid Profile of Healthy Volunteers. *Journal of Nutrition and Metabolism* 1:1-8.
- Cominetti C, Cozzolino SMF (2009). Funções plenamente reconhecidas de nutrientes Zinco. São Paulo: Série de Publicações ILSI Brasil, 2009.
- Costa T, Jorge N (2011). Beneficial Bioactive Compounds Present in Nut and Walnuts. UNOPAR Científica. *Ciencias Biologicas e da Saude* 13(3):195-203.
- Cozzolino SMF (2001). Application of used in dietary reference intake. DRIs. ILSI BRASIL. São Paulo.
- Cymerys M, Wadt LHO, Kainer K, Argolo V (2005). Castanha. In: Shanley, P.; Medina, G. (Eds) frutíferas e plantas úteis na vida Amazônica. Belém: CIFOR and Imazon pp. 61-73.
- da Silva RF, Ascheri JLR, de Souza JML (2010). Influence of Brazil nut processing on the quality of nuts. *Ciência e Agrotecnologia* 34(2):445-450.
- Dietary Reference Intake for calcium, phosphorus, magnesium, vitamin D and fluoride (1999). Calcium and related nutrients: Overview and methods. Washington: The National Academic Press.
- Duchelle AE, Cronkleton P, Kainer KA, Guanacoma G, Gezan S (2011). Resource theft in tropical forest communities: Implications for non-timber management, livelihoods, and conservation. *Ecology and Society* 16(1):4.
- EMBRAPA (2009). Manual de análises químicos de plantas, solos e fertilizantes. Brasília.
- FAO/WHO (2002). Expert Committee on Food Additives. Safety evaluation of certain food additives and contaminants. Aflatoxins. Geneva: World Health Organization.
- FAOSTAT—Food and Agriculture Organization of the United Nations statistics (2013). Produção e valor da produção da Castanha-do-brasil sem casca: Brasil, Bolívia e Peru. Available in: accessed July, 30th 2017).
- Fernandes NP, Alencar JC (1993). Desenvolvimento de árvores nativas em ensaios de espécies. 4. Castanha-do-brasil (*Bertholletia excelsa* H.B.K.), dez anos após do plantio. *Acta Amazonica* 23(2):191-198.
- Ferreira ES, Silveira CS, Lucien VG, Amaral AS (2006). Caracterização FÍSICO-química da amêndoa, torta e composição dos ácidos graxos majoritários do óleo bruto da castanha-dobrasil (*Bertholletia excelsa* H.B.K.). *Alimentos e Nutrição* 17(2):203-208.
- Ferreira MCC, Neto MF, de Melo ACGR, Fernández IM, Chagas EA, Ferraz VP, Ribeiro PRE, Melo Filho AA (2019). Physical-chemical Properties and Chemical Composition of Brazil Nut Oil, *Bertholletia excelsa*, from State of Roraima, Brazilian Amazon. *Chemical Engineering Transactions* 75(1):391-396.
- Freitas EC, Silva ACM, da Silva MV (2016). Análises de minerais zinco e manganês presentes na farinha do morango. *Revista Brasileira de obesidade, nutrição e emagrecimento* 10(60):303-307.
- Instituto Adolfo Lutz (IAL) (2008). *Physicochemical methods for food analysis* (IV ed.) São Paulo.
- Kim Y, Keogh JB, Clifton PM (2018). Nuts and Cardio-Metabolic Disease: A Review of Meta-Analyses. *Nutrients* 10(1):1935.
- Lugo NT (2017). El zinc y el cobre: Micronutrientes esenciales para la salud humana. *Acta Médica del Centro* 11(2):79-89.
- Mendes-Filho NE, Carvalho MP, de Souza JMP (2014). Determination of macronutrients and minerals nutriente of the mango pulp (*Mangifera indica* L.). *Perspectivas da Ciência e Tecnologia* 6(1):22-36.
- Miranda ALP, Fraga CAM (2006). Atividade següestradora de radical livre, determinação do potencial antioxidante de substâncias bioativas, *Practical Studies for Medicinal Chemistry*, Ginebra: IUPAC.
- Mori SA (1992). The Brazil nut industry - Past, present and future. In: PLOTKIN, M.; famolare, L. (Eds). *Sustainable Harvest and Marketing of Rain Forest Products*. Washington: Island Press. pp. 241-251.
- National Institute of Health (2020). Selenium: fact sheet for health professionals. Office of Dietary Supplements. <https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/>
- Norma de Procedimientos para muestreo de productos vegetales. NTON 17002-02 (2002). Comision Nacional de Normalización Técnica y Calidad del Ministerio de Fomento, industria y comercio. Norma técnica Nicaraguense (NTN).
- Peres CA, Baider C, Zuidema A, Pieter LHO, Wadt KA, Kainer DAPG, Silva R P, Salomão LL, Simões ERN, Franciosi FC, Valverde R, Gribel GH, Shepard JR, M Kanashiro P, Coventry DW, Yu AR, Watkinson FRP (2003). Demographic threats to the sustainability of Brazil nut exploitation. *Science* 302:2112-2114.
- Pinheiro DM, Porto KRA, Menezes MES (2005). A Química dos Alimentos: Carboidratos, lipídeos, proteínas, vitaminas e minerais. Marceió/Alagoas: UFAL.
- Pyrzynska K, Biesaga M (2009). Analysis of phenolic acids and flavonoids in honey. *Trends Analytical Chemistry* 28(7):893-902.
- Tonini H (2007). Castanha-do-Brasil: uma espécie chave na promoção do desenvolvimento com conservação. Boa Vista: EMBRAPA Roraima.
- Volpe SL (2013). Magnesium in disease prevention and overall health. *Advances in Nutrition* 4(3):378-383.
- Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. *Journal of Agricultural Food Chemistry* 51(1):609-614.