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Abstract

Brazil nuts (*Bertholettia excelsa* H.B.K.) *shell, brown skin* and *edible part* from Eastern (E) and Central-Western (C-W) Amazon regions were evaluated for their selenium (Se) distribution, physical chemical characteristics regarding proximate composition, nut ratio and water availability (moisture content and water activity). Nuts samples were collected from two factories located in Manaus and Cameta cities (C-W and E, respectively) to where they are delivered from each State (Amazonas and Para) for processing. Se analysis was carried out by mass spectrometry with inductively coupled plasma, proximate composition by the AOAC official methods and nut ration by gravimetry. Se levels varied among nut parts from the *shell* to edible part as follows: *shell*<*brown skin*<*edible part* with 0.44/0.31<20.58/6.12<44.13/19.25 mg/kg for Manaus/Cameta. Regarding lipid and protein content, they were low for *shell* and *brown skin*, however high in dietary fiber (57.17%). The *shell/brown skin/edible part* ratio was 4.2/3.8 g (1.2). Brown skin weight was only 1.2±3.05 g of the whole nut. Se is a powerful antioxidant for human metabolism balance being the highest concentration detected in the *edible part*. From the discarded nuts parts, the *brown skin* had the highest percentage of that element, especially those from C-W region which could be used as a source of Se in different food applications. As expected the *shell* and *brown skin* showed low level of lipids and proteins, however quite high fiber content, especially the *brown skin* which is quite promising on the food fiber enrichment point of view.

Keywords: Amazon basin, Brazil nut, brown skin, nut shell, selenium, waste

Introduction

Selenium (Se) is an essential metabolism trace element that can be found under different forms (inorganic, metallic, oxyanions and organic). It can be present in soils at different levels being wide-spread in China, Russia, South and North America which is derived from seleniferous matters (Johnson et al. 2010; Fordyce, 2005; Broadley et al. 2006). The organic Se forms is produced by its link to sulfur amino acids (cysteine-Cys and methionine-Met) forming selenoCys (SeCis) and selenoMet (SeMet) (Suzuki, 2005). Once the Se compounds are recognized as Se species, they are transformed to selenide, an intermediate metabolite, and then used for the Se-proteins (enzymes) synthesis or excreted. The SeMet can be transformed to SeCys through trans-selenation, and be lysed by β-lyase or y-lyase enzymes to selenide. It is important to emphasize that; the SeMet can be used for protein synthesis in the body without distinction between SeMet and Met by the t-RNA (Suzuki, 2005; Schrauzer, 2000).
Se intake through food varies worldwide due to the different levels found in different food diets. The Se content in plants is affected by its concentration in the soil they are grown. Similarly, its concentration in animal products depends upon the Se content in the diet they eat (Barclay et al. 1995; McNaughton and Marks, 2002; Pacheco and Scussel, 2007; 2011). The same is true for humans. However, in this case, they may also meet Se needs by taking supplements and/or vitamin complexes that include minerals (Fairweather-Tait et al. 2011, Manfio et al. 2012).

Se is known for its antioxidant activity and in the therapeutic aspect, for its chemo preventive and antiviral properties. Appropriate amounts of this element are essential for the human body to have a balanced health. Several physiological roles are assigned to the presence of Se-proteins, such as DNA synthesis, that depends upon the presence of Se in the catalytic site of TrxR **(**Fairweather-Tait et al. 2011; Rayman, 2005).

Certain types of nuts, especially those of tropical origin, have relatively high levels of Se. In a study conducted with different tree nuts authors reported increasing levels of Se in different nuts: *hazelnuts* (0.008 to 0.036 mg/kg); *macadamia* (from 0.034 to 0.087 mg/kg) and *cashew* (from 0.17 to 0.39 mg/kg) nuts. They reported that Se levels of these nuts were far below the high levels found in the same study for *Brazil nuts* (*Bertholettia excelsa* H.B.K.) with 0.85 to 6.86 mg/kg (Barclay et al. 1995).

Whereas the *edible part* of the Brazil nut is known to contain high amounts of Se, which varies with the level present in the Amazon soil (Pacheco and Scussel, 2007), no information on the shelled nut factory wastes *shell* or *brown skin* are reported to date. Therefore the aim of this study was to determine the Se levels in the dry shelled Brazil nut wastes for the possibility of further Se utilization, thus adding value to the Brazil nut producing chain. This is the first study describing the Se distribution in Brazil nuts different parts.

Material and Methods

Samples: Consisted of dry (processed) in-shell Brazil nuts from two Amazon regions, medium size Type (40-50 mm), individual weight average of 8.07 g (min 5.14; max 10.6 – De-Mello and Scussel, 2007). They were from the Eastern (E) and Central-Western (C-W)* regions of the Brazilian Amazon basin kindly provided by the factories: Renemero (Cameta city, Para State-PA) and CIEX (Manaus city, Amazonas State-MA), respectively. Initial moisture content (mc) and water activity (a_w) of 5.30/6.02% and 0.61/0.55 for E/C-W. No aflatoxin contamination (up to the method LOQ = 1.34 $ng.g^{-1}$ - Sobolev, 2007). **Figure 1. A** shows the location of the nut trees of the Brazilian Amazon region and **Figure 1. B** the points of harvesting of each State. A total of 45 kg each were utilized for the study. *Important to emphasize that Manaus city-MA, although is considered located in the Western region, it is very close to the Eastern (E) region border, thus called here C-W (**Figure 1.B**). We utilized and referred for this study to the official geographic location of the two main Amazon regions as reported by Pacheco and Scussel (2007).

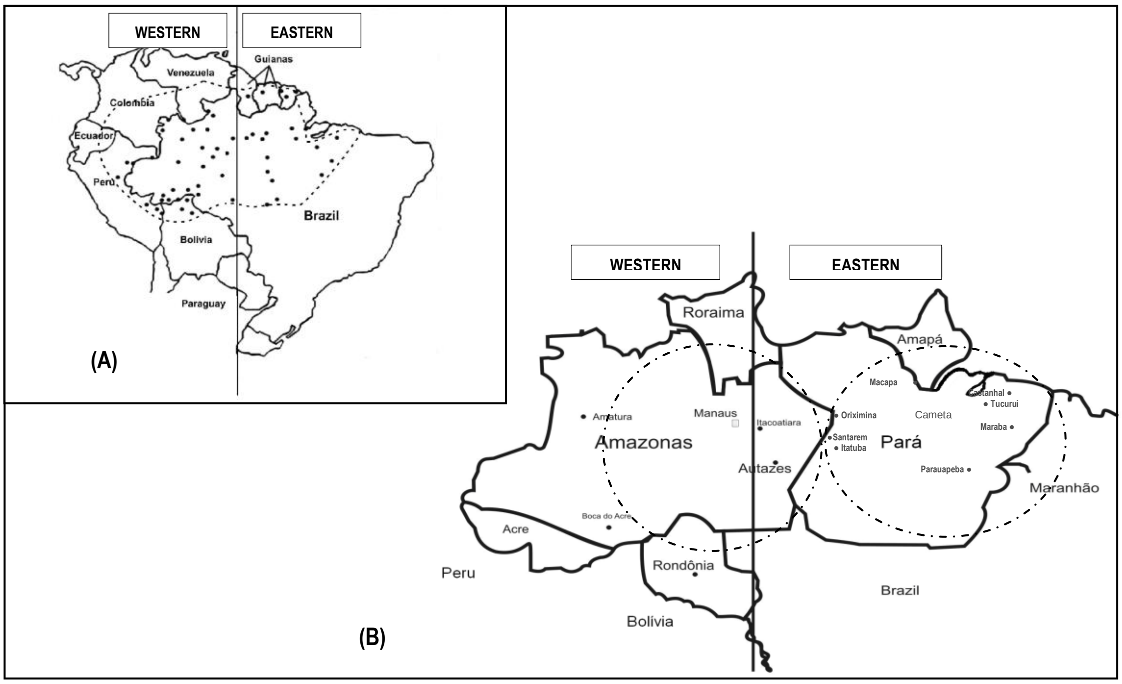

Figure 1: (**A**). Brazil nut tree distribution in the South American Amazon basin - Western (occidental) and Eastern (oriental) regions. [---] delimited area of the Brazil nut tree distribution. [•] some of the major extractive areas of Brazil nuts; (**B**). Northern Brazilian States comprising the Amazon basin, and the regions of sample collection (Central-Western region: Manaus city and Eastern region: Cameta city) where nuts collected from each region [-•-] converge to factories reception for selection and processing (dry in-shell or shelled Brazil nut) (Pacheco; Scussel, 2007, modified by Manfio & Scussel)

Standards, Reagents and Solvents: Se standard Nist. Hydrogen peroxide and nitric acid, supra pure grade were from Veteco. Citric acid, glacial acetic acid, hydrochloric acid, sulfuric acid, trichloroacetic acid, ammonium acetate, sodium sulphate sodium hydroxide, potassium sulfate, copper sulfate, titanium dioxide, silver nitrate, anhydrous methyl red indicator, all analytical reagent grade from Vetec. Ferric chloride from CRQ and tartaric acid, analar from HiMed. The solvents acetone, ether, ethanol, petroleum ether, were all analar and fromVetec.

Equipament and Apparatus: Microwave digestion system, model Ethos Plus, from Milestone, inductive coupled plasma spectrometer ICP, D Nexion 300, from Perkin Elmer. Dissecator with ∅ 200 mm from Diprolab, autoclave from Fanen, microbiological oven from Tecnal, analytical balance, AY220 from Shimadzu and metal crucibles of ∅ 70 mm. Peristaltic pump, model MS-Reglo from Ismatec, commercial Brazil nuts cracker from CIEX, blenders, 1 l of Metvisa, pressure cooker, 7 l, Panelux, microscope stereoscopic from CarlZeiss Jena. The scan electronic microscope - SEM, JSM, from Jeol, Gold coater, SCD500 from Leica, digestive Kjeldahl system, model Q327-26B, SPLabor, Kjeldahl flasks, 800 ml from SPLabor, analytical balance, Metla Toledo, Adventurer Pro, water activity meter, Aqualab, Soxhlet extractor apparatus, model MA-491, SPLabor, microbiological oven, 315SE, from Fanem, spectrophotometer, model 8425A from Hewlett-Packard and vertical autoclave modelAV50, Phoenix.

Brazil Nut Samples Preparation. (a) heating: Nuts from each region were submitted to autoclavation/ (to facilitate the *brown skin*/*shell* detaching off the *edible part* - as carried out in the factories) and left cooling to proceed for next step: **(b) shelling.** That was carried out utilizing a commercial nut cracker and **(c) nut parts separation.** Each part was separated into different containing as *brown skin*, *shell* and *edible part* (**Figure 2**), followed by weighing, grinding in a blender and portions separate for Se, proximate composition, mc and a_w analysis (*n=5*).

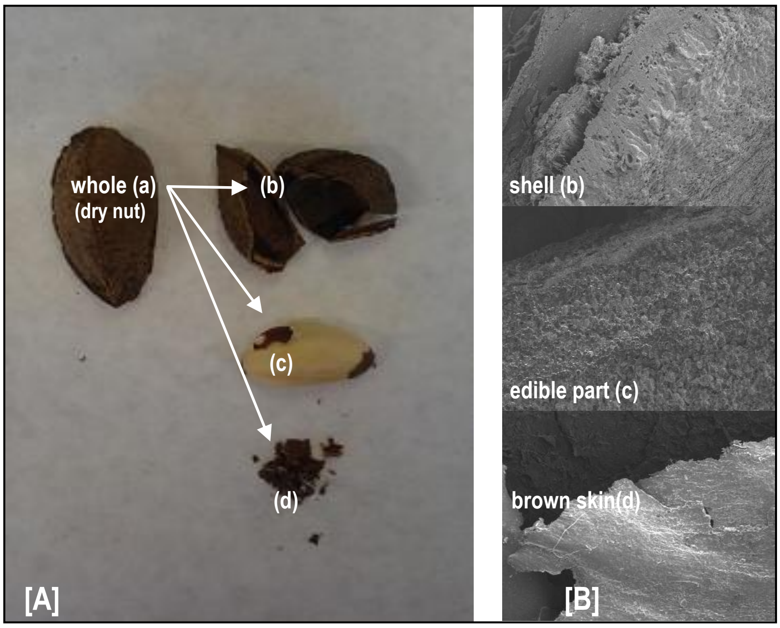

Figure 2: Brazil nut (*Bertholettia excelsa* H.B.K.) samples utilized for the Se distribution study: [a] whole, (b) shell, [c] edible nut and [c] brown skin [A] microscope sterioscopic, x1; [B] scan electronic microscope (x50 10Kv, 500 µm).

Selenium Analysis: They were performed by ICP (US EPA, 1996). Briefly, samples were digested (0.4g) with HNO_3 and hydrogen peroxide in microwave digestion system followed by ICP analysis. The limit of quantification (LOQ) was 3.50 mg/kg, which was defined as the lowest point of the calibration curve with high repeatability. The recovery was 92 % (*n=5*).

Proximate Composition of Brazil-Nut Parts: The nuts *shell*, *brown skin* and *edible part* content of lipids, proteins, dietary fiber, carbohydrates and ash were analyzed according to the AOAC official methods (AOAC, 2005).

Temperature (60 and 121°C) and Pressure Effect on Se Levels: Nut parts were submitted to 60°C (dehydrating temperature – similar to in-shell dry nut process) in a microbiological oven and to 121°C (sterilizing temperature – similar to shelled dry nut process) in an autoclave, both for 120 min. Se levels and mc were determined prior and after heating application.

Mc and A_w: were determined by gravimetry (AOAC, 2005) and by applied the Aqualab apparatus at 25°C, respectively.

Statistical Analysis: They were performed by variance analysis (ANOVA) and included the Tukey´s test to evaluate significant differences among the means ($p< 0.05$). The results were expressed as the mean values and standard errors.

Results and Discussion

From the data obtained it was possible to observe that the Se levels increased among the (a) nut parts and the (b) Amazon nut site of collection: from nut *shell* to *edible part* and from Cameta (E) to Manaus (C-W) cities. **Tables 1** and **2** show the data on Se levels, each part proximate composition, mc, a_w and weight ratio of each Brazil nut part evaluated.

Distribution of Se in the Brazil nut Parts and its Variations among Site of Collection

Levels of Se for each nut part increased in the following order: *shell* < *brown skin* < *edible part* reaching 0.44 < 20.58 < 44.13 mg/kg and 0.31 < 6.12 < 19.25 mg/kg for the two Amazon site of nut collection, respectively. Thus being Manaus' (C-W) with the highest levels.

Shell, Brown Skin & Edible Part Se Distribution: The Se level of the Brazil nuts factories waste - *brown skin* - was 3.5 fold higher for C-W (22.58 mg/kg) than the E (6.12 mg/kg) city of collection. The values obtained for the *shells*, despite the nuts origin, were inferior to those of the other nuts components (C-W: 0.44 and E: 0.31 mg/kg), showing that shells do not add to the whole Brazil nut Se content, but the *brown skin*. Therefore, the *brown skin* of Manaus city, C-W region (20.58 mg/kg) could be consider a possibility of Se extraction for use in food fortification and/or in supplements, followed by that of Cameta city, Eastern regions, however, with a lower concentration (6.12 mg/kg). As expected the *edible part*, already reported by several authors (Barclay et al. 1995; Pacheco and Scussel, 2007; Furr et al. 1979; Secor and Lisk, 1989; Chang et al. 1995; Vonderheide et al. 2002; Coutinho et al. 2002; Chunhieng et at., 2004; Kannamkumarath et al. 2002) showed to be rich in Se with its content detected more than 2 fold higher in the C-W (44.13 mg/kg) than the E (19.25 mg/kg) site of collection.

Central-Western & Eastern Brazil Nuts Se Variation: Whole and/or edible part (despite if with or without *brown skin* on) Brazil nuts Se variation throughout the Amazon region has been reported in the literature (Barclay et al. 1995; Pacheco and Scussel, 2007; Furr et al. 1979; Secor and Lisk, 1989; Chang et al. 1995; Vonderheide et al. 2002; Coutinho et al. 2002; Chunhieng et at., 2004). That variation is dependent of several factors as the soil Se concentration; the chemical Se form in different Amazonian soil (acid or alkaline rivers); presence of heavy metal (Hg, mining); rain intensity and the genetic characteristics of the plant on metabolizing the Se absorbed from the soil and also the forms that Se is present in the nut (SeMet and SeCys) (Pacheco and Scussel, 2007). It has been reported that the soil of Eastern Amazon is rich in Se with concentrations ranging from 5.2 to 9.4 mg/kg which are much higher than the Western region (1.0 to 2.4 mg/kg) (Nogueira, 2002). In fact, the soil Se concentrations of Itacoatiara and Autazes cities (which are located closer to the border between W and E regions) are higher with 9.4 and 8.1 mg/kg, respectively. FAO in 1993 reported that the reason that Brazil nut tree concentrates Se is due to the fact that Se is very similar chemically to sulfur, an essential nutrient for the nut seed amino acids (Met and Cys) and protein formation. Sulfur is often deficient in Amazon soils, especially after decades of Brazil nut harvesting and export. If the soil contains significant amounts of Se, it may be used by the plant instead of sulfur (FAO, 1993). Despite the Amazon location and soil type, Brazil nut trees also have special absorption capacity due to their long roots system which reaches dipper soil layers finding nutrients, different of the well-known sandy/rather poor surface Amazon soil.

Effect of Temperature on Brazil Nuts Se Levels: During this study, questions raised whether temperature would have some effect on Brazil nuts Se level, which was checked. The data on nuts submitted to 60°C and 121°C in oven (temperature) and autoclave (temperature + moist pressure), respectively are in **Table 1**. Temperature did not affect Se levels, despite the method applied. In fact, it increased by ±0.3%, due to moisture evaporation, leading to a false level increment, reaching at 60 and 121°C, levels of 20.65 and 6.13 mg/kg for C-W nut *brown skin* and 22.64 and 6.15 mg/kg for E, respectively. As Se forms in Brazil nuts are mainly water soluble (Fairweather-Tait et al. 2011), the inclusion of any water step probably would have interfered on its loss during processing. However that does not occur throughout the whole Brazil nuts drying process in the factory. Other food processing could lead to Se loss though, such as boiling. In addition, the high lipid (65- 70%) content in nuts cells can play a role on reducing Se extraction through moist.

Table 1: Selenium Distribution in the Brazil Nut (*Berthollethia excelsa* H.B.K.) Parts Collected from the Central-Western and Eastern Brazilian Amazon Basin

Brazil nut[a]		From amazon basin regions*					
Name	Botany	C-W (Manaus)[b]		E (Cameta)[b]		T°C effect on Se (mg/kg)	
		Weight (g)	Se level (mg/kg)*	Weight (g)	Se level (mg/kg)*	40°C (C-W/E)	121°C (C-W/E)
Nut Parts							
Shell	Tegument	4.2±1.21	0.44±0.02 (0.71%)	4.0±3.13	0.31±0.03 (1.21%)	NA**	NA
Brown Skin	Testae	1.2±3.05	20.58±0.32 (30.7%)	1.2±2.03	6.12±0.05 (23.8%)	20.65/6.13	20.64/6.15
Edible	Endosperm	3.8±4.15	44.13±0.25 (68.6%)	3.6±3.21	19.25±0.11 (75.0%)	44.26/19.32	44.26/19.35
In-shell Nut							
Whole***	Seed	8.03±3.01	32.65±0.07	8.124±3.2	12.28±0.06	NA	NA

C-W: Central-Western E: Eastern Se: selenium [a] medium size Type, weight average 8 g/width 19.9-26.3 for face ABC, S/N ratio 1.2 (Classif. by De Mello; Scussel *(13)* length 40-50 mm, [b]nuts collected from each region converging to the factory site C-W and E, respectively *standard deviation (n=5) T°C temperature
** not analysed *** analysed whole nut (shell+brown-skin+edible-part) all together

Brazil Nuts Parts Proximate Composition and Nut Ratio

Despite of the large amount of information regarding Brazil nut *edible part* proximate composition, nothing has been published regarding the factories dry shelled nut discarded products: *shell* and *brown skin*. In this study we were able, apart from, Se quantification, to determine their proximate composition (**Table 2**). Although the *shell* and *brown skin* showed low level of lipids (7.3/7.23 and 15.83/16.04%) and proteins (3.83/3.26 and 8.34/7.91%) for C-W/E sites of collection, respectively, the fiber content was quite high for those factory wastes especially the *brown skin*, reaching 57.17% of dietary fiber which is quite promising on the food fiber enrichment point of view. Regarding the mc and a_w of the Brazil nut components of C-W/E Amazon origin utilized in the current work, they were for *edible nut*, *shell* and *brown skin* of 4.20/4.21; 3.32/4.12; 4.81/3.48% and 0.65/0.65; 0.70/0.62; 0.32/0.45 for mc and a_w, respectively.

Table 2: Proximate Composition of Brazil Nut (*Berthollethia excelsa* H.B.K.) Shell, Brown Skin and Edible Parts from Different Amazon Regions

Brazil nut	C-W[a]/E[b] Amazon Region						
	Lipid (%)	Protein (%)	Carbohydrates		Ash (%)	mc[c] (%)	a_w[d]
			Starch (%)	Dietary Fiber (%)			
Nut Parts							
Shell	7.30/7.23	3.82/3.26	3.34/.3.53	32.25/22.91	5.80/6.77	3.32/4.12	0.70/0.62
Brown Skin	15.83/16.04	8.34/7.91	3.80/3.902	57.13/60.02	4.25/4.32	4.81/3.48	0.32/0.45
Edible Part	68.00/66.43	15.90/16.34	2.41/2.37	5.91/4.05	3.59/3.70	4.20/4.21	0.65/0.65
In-shell Nut							
Whole	31.15/32.90	9.96/10.02	24.60/25.32	22.7/23.03	2.45/2.64	5.3/6.02	0.61/0.55

[a]Central-Western [b]Eastern [c]moisture content [d]water activity

Brazil nut Parts Ratio

The ratio of the three components (shell/brown skin/edible part) of the Brazil nuts did not differ much from that established in the literature (De-Mello and Scussel, 2007) for shell/edible part of 4.2/3.8 or 53/47% for a medium size nut (8 g) with a Shell/Nut ratio of 1.2. The amount of brown skin obtained from each nut did not reach 2 g, i.e., average of 1.2±3.05 g thus not interfering to that ratio.

Conclusion

Se plays an important role in human's metabolism and health. Brazil nuts are Se rich, mainly the *edible part* however; the *brown skin* holds a quite high portion of that element, apart from being rich in fiber.

Currently the factories that produce shelled dry Brazil nuts consider *brown skins* as waste, thus discarding or sending them together with the nut shell residue for fuel. Considering the importance of Se and the need of human body Se intake, *brown skin* could be consider a possibility of utilization either in supplements or to be added in food for Se and fiber enrichment (shakes, ice creams, cheese) (Pereira et al. 2010a; 2010b). For those use, *brown skin* should pass through quality and safety analyses, as currently is applied for the *edible part* of Brazil nuts (either in-shell or shelled). Other studies need to be carried out regarding differences on Brazil nuts varieties that growth in the soils of the Amazon rain forest.

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