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Selenium and Aflatoxin Levels in Raw Brazil Nuts from the Amazon Basin

ARIANE M. PACHECO AND VILDES M. SCUSSEL*

Food Science and Technology Department, Center of Agricultural Sciences, Federal University of Santa Catarina, Post Office Box 476, Rod. Admar Gonzaga, 1346, Itacorubi, 88.304-001, Florianopolis, SC, Brazil

Whereas selenium (Se) is an important antioxidant in human metabolism to prevent cancer, aflatoxins are highly carcinogenic. Brazil nuts from Eastern and Western Amazon regions were evaluated to find any relationship between Se and aflatoxins levels. A total of 80 (in-shell and shelled) nuts samples were collected directly from different forest sites and analyzed for Se by atomic emission spectrometry and aflatoxins by liquid chromatography tandem mass spectrometry. The limit of quantitation (LOQ) for Se was 2.0 mg/kg, and LOQ for total aflatoxins was 0.390 μg/kg. Nut Se levels from the Eastern region were higher than the Western, in addition to the aflatoxins. The moisture content (mc) and water activity (a_w) of the raw nuts from the two regions did not present a significant difference, for either in-shell or shelled. The mc was 24.5% (minimum of 20.1% and maximum of 30.4%) and 22.1% (minimum of 14.6% and maximum of 28.9%) and a_w of 0.85 for both regions. Further studies need to be carried out to discover the role of Se on fungi growth stress and aflatoxin production mechanisms.

KEYWORDS: Aflatoxins; selenium; LC−MS/MS; Brazil nuts; Amazon basin; moisture content; tandem liquid chromatography

INTRODUCTION

Brazil nuts (*Bertholletia excelsa* H.B.K.) are one of the most important nutritional examples of food with high content and quality protein. They have high levels of essential amino acids, including the sulfur containing methionine and cysteine (*1*, *2*). In addition, Brazil nuts are richer in selenium (Se) than other nuts (*3*–*5*). Se plays an important role as an antioxidant and is also involved in thyroid metabolism and cancer prevention. It was shown that the inclusion of Brazil nuts in the diets of rats that received intragastric administration of the mammary carcinogen dimethylbenz(*a*)anthracene (DMBA) reduced the number of tumors by up to 72.7% (*6*). Because Se has been reported as an important micronutrient and Brazil nuts are a source, there has been an increasing interest by consumers in that commodity (*7*–*9*). Brazil nuts have been recommended to be consumed, either as is or as an ingredient in processed food. Because Se has been considered as an excellent antioxidant, it has been recommended as antiaging and is consumed by athletes, the elderly, and those that desire a healthy life (*8*). The Se daily intake (DI) varies among different countries from 6 (New Zealand) to 224 μg/day (Canada) (*10*, *11*). For European countries, the Se DI have been reported from 32.3 to 70 μg/day (*12*). The Se recommended dietary allowance (RDA) was established by the National Research Council in 2000 as 55 μg/day, and the tolerable upper intake level (TUL) for adults was set at 400 μg/day (*13*).

Brazil nuts may vary in size, shape, and flavor when grown in different areas of the Amazon basin (Gribel, personal communication), despite the time of harvest; thus, their composition may also vary. Most of them grow in areas surrounded by rivers, and some grow far from them, in drier soil. The Amazon basin is divided into Western and Eastern regions (*14*), and both have the Amazon River and its tributaries intersecting them. Some studies on Se levels in Brazil nuts have been carried out (*1*, *4*, *7*, *8*, *15*–*17*), and one correlated Se with the tree nuts growing areas (*16*). The authors reported different Se levels in nuts from two areas divided for that study into West (States of Acre and Rondônia only) and Central (States of Amazonas and Para). They collected processed nut samples in the retail market and reported Se levels of 3.06 and 36.0 mg/kg for each region, respectively, in individual nuts. However, the commercial snack products are prepared by the factories using raw nuts from different Amazon sites, and it is difficult to determine their exact origin.

On the other hand, some aflatoxins and fungi have been detected in processed in-shell Brazil nuts (*18*–*26*). In fact, these toxins are produced by *Aspergillus flavus*, which finds good conditions for its proliferation in the Amazon forest, with high temperatures > 25 °C and relative humidity (RH) > 80% (*23*, *25*). The fungus species may grow on nuts during the extractive activities that are carried out under artisan conditions in the indigenous communities (*23*). It also can grow during the raw nut transport, performed mostly by boats, to the factory site, where they are going to be processed and have their moisture content (mc) reduced (*25*, *26*).

* To whom correspondence should be addressed. Telephone/Fax: +55-48-3721-5386. E-mail: vildescussel_2000@yahoo.co.uk.

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Despite the decrease of Brazil nuts export to the European Union (EU), because of a more restricted regulation of aflatoxin levels, the nut antioxidant properties have increased the consumer interest. Whereas Se is an important antioxidant in human metabolism to prevent cancer, aflatoxins are highly carcinogenic. The Se concentration in individual nuts varies greatly, ranging from 0.20 to 253 mg/kg (*4*), depending upon the soil composition and tree absorption. The aflatoxin association with Se in Brazil nut has not been studied nor related to the different Amazon regions. Therefore, this study was carried out to evaluate the Se content, the possible presence of aflatoxin, and any correlation between them in raw Brazil nuts using atomic spectrometry and tandem mass spectrometry. The study compared the raw Brazil nuts from the Western and Eastern regions of the Brazilian Amazon basin.

MATERIALS AND METHODS

Material. *Samples.* Samples consisted of raw, medium-size, in-shell Brazil nuts, year 2006 harvest, from the Eastern (oriental) and Western (occidental) regions of the Brazilian Amazon basin. We used and referred for this study to the official geographic location of the two main Amazon regions as follows: the Eastern Amazon region comprises the states of Para, Amapa, Northern part of Maranhao, and the Eastern part of Amazonas (starting from Manaus city to the East); the Western Amazon region comprises the states of Acre, Rondônia, Roraima, and the Western part of Amazonas (starting from Manaus city to the West). **Figure 1A** shows details of the whole Amazon basin with the Brazil nut trees distribution and the Northern Brazilian states with the location of the cities of sample collection.

Chemicals. Ammonium acetate, ammonium sulfate, hydrochloric acid, nitric acid, and anhydrous sodium sulfate (Analar grade) were provided by Vetec, Rio de Janeiro, Brazil. Methanol, acetonitrile, benzene (HPLC grade) were provided by Carlo Erba, Rodano, Italy. Ultrapure water (MilliQ system) was provided by Millipore. Selenium standard: from NIST (certificate number SRM 3149) as sodium selenite (Na_2SeO_3) acidified aqueous solution prepared with nitric acid). Aflatoxin standards: AFB_1, AFB_2, FG_1, and AFG_2, from Sigma, Germany.

Apparatus and Equipments. For Se analysis, we used a spectrophotometer (Hitachi) and atomic emission spectrophotometer [inductively coupled plasma (ICP)], Otima 2000, Perkin-Elmer, Toronto, Canada. For aflatoxin analysis, we used a liquid chromatograph (LC) 1100 (Agilent) and mass/mass detector (MS/MS) API 4000 triple-quadrupole (Applied Biosystems) equipped with either atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) interface; ZORBAX C_8 column (4.5 × 150 mm, 5 μm particle size), Hicrom.

Other Materials. Disk mill, Metvisa, São Paulo, Brazil; commercial Brazil nut-crackers, CIEX, Manaus, Brazil. a_w: Pawkit, Decagon.

Methods. *Sampling.* A total of 80 samples were collected (from April to May) from two cities that represent the main extractive communities of each Amazon region (Western region, cities of Amaturá and Boca-do-Acre; Eastern region, cities of Itacoatiara and Autazes; **Figure 1B**). These are converging points to which the nuts collected in the forest around that location are gathered and also where nuts are stored (second storage) prior to boat loading to be transferred to the factories for processing. The sampling method used was that required by the EU (*27*). The samples were representatively collected from silos (capacity of 400–800 kg) of each city, weekly, and homogenized in bags, and final portions of 30 kg were taken, packed, and immediately sent to the laboratory. These amounts were divided into two portions of in-shell and shelled nuts for Se and aflatoxins analysis (considered to be representative for <0.1 ton).

Sample Preparation. From the two portions of in-shell nuts, one was deshelled using a special commercial Brazil nutcracker. The samples, still frozen, were finely ground (particle size < 100 μm) in the disk mill and homogenized, and portions of 500 g were transferred to polyethylene containers with stoppers and stored in a freezer. Portions of 50 and 25 g were used for Se and aflatoxin analysis, in duplicate.

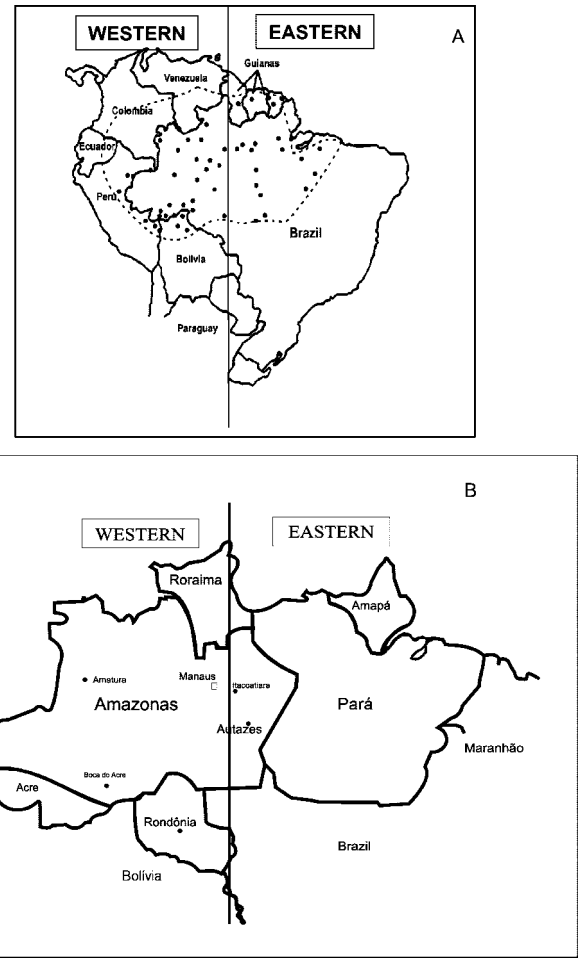

Figure 1. (**A**) Brazil nut tree distribution in the South American Amazon basin, Western (occidental) and Eastern (oriental) regions. (- - -) Delimited area of the Brazil nut tree distribution. (●) some of the major extractive areas of Brazil nuts. (**B**) Northern Brazilian States comprising the Amazon basin and the sites of sample collection (Eastern region, Itacoatiara and Autazes cities; Western region: Boca do Acre and Amaturá cities).

Selenium Analysis. Selenium analysis was performed with ICP, optical emission spectrometry (OES), using the atomic emission method (*28*). The digestion of the samples (0.4 g) was carried out using 5 mL of concentrated HNO_3. The limit of detection (LOD) was 2.00 mg/kg, and the limit of quantification (LOQ) was 3.50 mg/kg. LOQ was defined as the lowest point of the calibration curve with high repeatability, axial view. The recovery was 92% ($n = 3$).

Aflatoxins Analysis. Aflatoxins analysis was performed with LC−MS/MS with APCI in the positive mode (*29*). The LC conditions (C_8 column) were mobile-phase methanol/water gradient [45% water/55% methanol (3 min); from 3 to 5 min, the gradient was changed to 30% water/70% methanol] and flow rate of 1 mL/min. For MS/MS, parent and the two daughter ions (*m*/*z*) were selected for each toxin as follows: AFB_1, *m*/*z* 313.1 (241.10 and 285.10); AFB_2, *m*/*z* 315 (259.09 and 287.20); AFG_1, *m*/*z* 329.1 (200.05 and 243.05); and AFG_2, *m*/*z* 331.2 (245.07 and 231.20). The LOD and LOQ for LC−MS/MS of AFB_1, AFB_2, AFG_1, AFG_2 were 0.04, 0.045, 0.05, and 0.06 μg/kg and 0.08, 0.09, 0.1, and 0.12 μg/kg for each aflatoxin, respectively. The LOD and LOQ for total aflatoxin were 0.195 and 0.390 μg/kg. To obtain those parameters, the finely ground Brazil nuts were homogenized and spiked prior to extraction with aflatoxins at five concentrations ranging from 1 to 10 μg/kg. Portions of 25 g were taken for extraction adding 100 mL of acetonitrile/water (80:20, v/v) to the sample, mixed for 2 h, and filtered. The LOD method was defined by 3 times the signal-to-noise ratio, and the LOQ method was defined by 6 times the signal-to-noise ratio. Five points were used to build an analytical curve, to obtain the *R* values for LOD and LOQ. Each point corresponded to a

Table 1. Selenium and Aflatoxin Levels in Raw Brazil Nut Samples from Western and Eastern Regions of the Amazon Basin

Amazon basin		Brazil nut	selenium[a] (mg/kg)			$AFB_1 + AFB_2 + AFG_1 + AFG_2$[b] (μg/kg)				mc[c] (%)			a_w[d]		
region	city	type	mean	range	RSD[e] (%)	mean	range	RSD (%)	>EU limit[f] (%)	mean	range	RSD (%)	mean	range	RSD (%)
Eastern															
	Itacoatiara	in-shell[g]	20.5[h]	11.1–34.7	9.3	6.1[h]	2.4–11.5	2.0	7 (7.9)	26.7	21.6–30.4	3.3	0.88	0.76–0.95	0.06
		shelled	43.7[i]	23.7–61.0	10.6	4.5[i]	1.7–11.9	3.0	3 (9.5)	21.1	14.6–25.4	3.6	0.89	0.86–0.90	0.02
	Autazes	in-shell	29.2[h]	12.9–38.6	5.2	3.8[h]	2.0–9.2	2.0	5 (6.7)[j]	25.9	23.4–28.3	1.7	0.89	0.82–0.94	0.04
		shelled	43.9[i]	20.7–69.7	17.1	3.0	3.3–8.0	2.0	3 (7.9)	22.5	15.6–27.8	3.7	0.88	0.84–0.91	0.03
Western															
	Boca do Acre	in-shell	13.5[h]	9.7–18.5	3.4	1.5[h]	1.8–4.5	1.0	1 (4.5)	21.2	20.1–23.4	1.1	0.84	0.80–0.90	0.04
		shelled	25.3[i]	13.8–35.1	7.1	1.5[i]	2.2–5.5	2.0	1 (5.5)	24.3	20.6–20.4	2.8	0.84	0.80–0.89	0.03
	Amaturá	in-shell	11.9[h]	9.2–16.7	2.8	2.5	1.2–3.7	1.4	0	23.2	20.9–28.0	2.3	0.82	0.79–0.84	0.02
		shelled	21.8[i]	8.5–29.1	6.2	3.0	1.9–6.6	1.3	2 (5.4)	26.2	23.7–28.9	1.9	0.83	0.80–0.86	0.02

[a] LOQ = 2.00 mg/kg. [b] Total aflatoxins = LOQ of 0.39 μg/kg. [c] Moisture content. [d] Water activity. [e] Relative standard deviation. [f] Number of samples with aflatoxin levels higher than the European Union limit of total aflatoxin = 4.0 μg/kg. [g] Shell included. [h] Same letters presented significant difference, for in-shell samples, concerning the two regions (Eastern and Western). [i] Same letters presented significant difference, for shelled samples, concerning the two regions (Eastern and Western). [j] Levels of total aflatoxin in the samples.

mean of five injections of each extract. The recoveries for each aflatoxin (AFB_1, AFB_2, AFG_1, AFG_2) were 92.4, 72.5, 99.8, and 97.1%, respectively. The shell/nut ratio used for calculation was that reported by de Mello and Scussel (*30*) of 60:40 (60% shell/40% nut) with the factor of 1.5, considered the standard ratio for normal healthy whole Brazil nuts.

Moisture Content (mc). mc was performed by the gravimetric method (*31*).

Water Activity (a_w). a_w was performed by the water activity kit (Pawkit) and calibration salt slush (*31*).

Statistical Analysis. Statistical analysis was performed by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The data obtained on the Se and aflatoxins levels in the raw Brazil nuts collected from the Eastern and Western regions of the Amazon basin, as well as mc and a_w, are summarized in **Table 1**. Differences in the Se and aflatoxin levels were observed both, from the two regions and nut type (in-shell or shelled).

Selenium Levels in Raw Brazil Nuts. *Se in the Raw Nuts from Eastern and Western Amazon Regions.* The Se levels detected in all nuts samples surveyed, from both regions, ranged from 8.5 to 69.7 mg/kg (RSD = 11.2%). However, they had a clear difference with respect to the Amazon region, where the nuts originated. The Western region, either the shelled or in-shell nuts samples, presented lower Se levels than the Eastern region nuts. The mean Se levels of shelled nuts were 23.6 and 43.8 mg/kg, respectively, for both regions. Similarly, for the in-shell nuts, it was 12.7 and 24.8 mg/kg, respectively. The Se levels were ca. 2-fold lower in the nuts from the Western region than those from the Eastern region, respectively. The Se difference among the two nut types was probably due to the shell being included in the analytical sample portion of the in-shell nuts for Se analysis, thus diluting it. From our data, the shell might have less Se than the edible part because the results were lower than shelled nuts. We did not analyze the shell. Data in the literature for in-shell and shelled nut Se levels report that in-shell nuts have more Se than shelled (*15–17*). However, that is not because the authors analyzed the Se in the shell and in the edible part separately but because of their snack packs label information on the type and product origin (i.e., nuts commercialized shelled are from the West and nuts commercialized in-shell are from the East). On the other hand, when Vonderheide et al. (*17*) analyzed the shell, they found very small amounts (2 μg/g). No significant difference was observed for the Se levels of nuts collected in the two cities, the nut converging points from the same Amazon region. Either Boca do Acre and Amaturá (Eastern Amazon) or Itacoatiara and Autazes cities (Western Amazon) presented similar Se levels of 12.7/23.6 or 24.8/43.8 mg/kg (in-shell/shelled), for each region, respectively. Considering that the Se levels detected in the nuts are a consequence of its absorption by the Brazil nut trees from the soil could be the reason why the individual nut batches that came from the several extractive communities surrounding each city and from the same region had similar levels. As far as the Se levels reported in the literature for Brazil nuts are concerned (*1–5*, *15–17*), they vary quite widely, especially the data reported by Chang et al. (*16*), by a fluorometric method. These authors reported Se levels, using a range from 0.02 to 512 μg/g, being 0.03–31.7 and 1.25–512 mg/kg for West and Central areas divided for study purposes. Despite the levels detected, the Amazon regions that were indirectly derived from the information obtained from the packing label cannot indicate the real precedence of the nuts. Usually the factories that produce those snack packs receive the nuts already dried from wholesale factories. Those factories in turn receive the raw nuts from several extractive areas for processing. Thus, the nuts analyzed by the authors may come also from the Amazonas state, which is included in the official Western region.

Se in the Amazon Basin Soil. The Se distribution in Brazil nut is dependent upon the tree absorption ability according to factors, such as the soil Se concentration, the chemical Se form in different Amazonian soil (acid or alkaline rivers), the presence of heavy metal (Hg, mining), the rain intensity, the genetic characteristics of the plant upon metabolizing the Se absorbed from the soil (*32*), and also the forms that Se is present in the nut (selenomethionine and selenocysteine) (*17*). It has been reported that the soil of the Eastern Amazon region is rich in Se, with concentrations ranging from 5.2 to 9.4 mg/kg, which are much higher than the Western region (1.0−2.4 mg/kg) (*33*). In fact, the soil Se concentrations of Itacoatiara and Autazes cities, which are located in the Eastern region are higher, with 9.4 and 8.1 mg/kg, respectively. On the other hand, soils of Boca do Acre and Amaturá (Western region) are lower in Se, with 2.4 and 1.2 mg/kg (*33*). Therefore, the Se in the nuts of the two regions may also vary in proportion to those soil compositions. The Food and Agriculture Organization (FAO) in 1993 reported that the reason that the Brazil nut concentrates Se is probably due to the fact that Se is very similar chemically

to sulfur, an essential nutrient for the nut seed amino acids (methionine and cysteine) 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 (*34*).

Se RDA per Brazil Nut. Considering the current data obtained, Brazil nuts can reach high levels of Se; its daily intake needs to be evaluated. Although Se has and can be used because of its anticancer activity and it has been recommended to be used as a cancer-preventive element; however, Se doses must be tested to establish its safety for long-term high-dose supplementation using organic Se compounds (*35*, *36*). A Se safe dose is the amount that is considered enough to supply the needs and does not cause any toxicological effects. That intake can be achieved naturally in the diet, by ingestion of different types of nuts that are rich in Se, including the Brazil nuts. Because its concentration in Brazil nuts, from our data, depends upon the extractive regions where they originate, it is important to follow the recommended Se RDA of 55 μg/day or at the most TUL of 400 μg/day (*13*). The Eastern region had Se levels higher (20.7–69.7 μg/g) than the Western region (8.5–35.1 mg/kg). The concentration would lead to an amount of Se in an individual edible nut (medium size, shelled, 3.8 g edible) of 78.6–264.8 and 32.3–133.3 mg/kg for nuts from Eastern and Western regions, respectively. With the regard to the amount of nut to be consumed to accomplish the RDA or TUL, a person should ingest 1 nut/day. From the data obtained in this study, it seems that the nuts with the Western Se level would supply the RDA and the Eastern Se level would supply both RDA and TUL.

Toxicity and Se Stress. As far as Se toxicity is concerned, it has been reported that high Se levels can be either cytotoxic or possibly carcinogenic. The cytotoxic effect has been suggested to be associated with oxidative stress (*36*). Therefore, supranutritional doses, as presented in some Brazil nuts, could be considered a warning factor if the consumption per day exceeds the dietary doses especially in a long-term consumption. The Brazil nuts that have high Se levels could be used by the food industries as ingredients, in small quantities, in several products. A work carried out by Lima et al. reported that high levels of Se were detected from fish of an Amazon region area studied (*37*). The authors reported the reason being the environmental exposure to high Se levels in the soil and plants. On the other hand, studies with *Drosophila melanogaster* have suggested that Se could inhibit aflatoxin B_1 and its toxic effects (*38*). In preliminary experiments to evaluate the selenium toxicity of a Brazil nut meal in rats, it was evident that the selenium action was extremely dependent upon the doses (9 versus 300 mg/kg) to affect as a nutritional element to increase weight or to be toxic to cause damage (*39*). Although,, in another study, it was evident that *A. flavus* growth was increasingly inhibited as the concentrations of selenate in the culture solution increased, suggesting the influence of some selenium form in the fungal metabolism (*40*).

Aflatoxin Levels in Raw Brazil Nuts from Eastern and Western Amazon Basin. Although the aflatoxin levels detected were not very excessive in the raw Brazil nuts, they were present in some samples and varied with the nut type and region from where they were collected.

Raw In-Shell Brazil Nuts and Aflatoxin. Because the LC−MS/MS method is very sensitive, able to detect aflatoxins at parts per trillion (ppt) levels, they were found in 29 and 26 nuts samples from each region, respectively. Levels of total aflatoxins ranged from 1.2 to 11.5 μg/kg. The Eastern region samples contained more numbers of samples and levels of aflatoxins (2.0–11.5 μg/kg) than the Western region (1.2–4.5 μg/kg). Although those nuts samples showed some aflatoxins, most of the levels were lower than the limit established by the EU of 4.0 μg/kg for total aflatoxins, a more restrictive international regulation. Only 1 (4.5%) and 12 (14.6%) nut samples from Western and Eastern regions contained levels higher than that regulation. No Brazil nut surveyed presented levels higher than the Canada, Mercosur, or U.S.A. limit (15, 20, and 20 μg/kg) (*41*, *42*). The aflatoxin B_1 levels found in the in-shell nut samples (1.22–1.40 μg/kg) were lower than the EU limit of 2.0 μg/kg in all samples. As far as raw Brazil nuts and aflatoxins levels reported in the literature are concerned, there are only two studies using nuts at that stage and they are mainly on fungi infestation and their toxigenic strains. Arrus et al. (*24*) carried out some analysis on raw nuts taken from the pods collected when they were still on the trees. They isolated *A. flavus* strains, but no aflatoxins were detected. The methodology used was enzyme-linked immunosorbent assay (ELISA) with a LOD of 1.75 μg/kg total aflatoxins. Our data was obtained using a more sensitive methodology (LOQ of 0.39 μg/kg), which can give a better view of aflatoxin presence in the nuts, but it is important to emphasize that the data obtained were from an intermediary stage of the Brazil nut chain (i.e., after pods/nuts collection from the forest transferred to the cities and before the boat transport to the factories). These raw nuts after transport to the factories are going to be submitted to the drying process and part of those aflatoxin levels may be reduced. The reduction could happen during the two sorting steps: prior to drying in heaters and after they pass through a conveyor, where the spoiled/cracked and moldy nut will be discarded (*31*). Concerning transport by ship abroad, some containers have monitoring/controlling of systems for RH and temperature. Containers without those systems may provide favorable conditions for fungal growth.

Raw Shelled Brazil Nuts and Aflatoxin. When nuts are processed shelled, apart from the rotary heating step, they still pass through a steam vapor step, with the temperature reaching 105 °C for 5–15 s (to facilitate shell removal) apart from two sorting steps, at deshelling and classification tables, thus discarding the spoiled nuts prior to vacuum packing. As far as aflatoxin in shelled nuts are concerned, although the raw shelled nut samples were prepared from the same main nut composite samples used for in-shell nut samples, it was observed that 6 nut samples from the Eastern region ones presented slightly higher levels (1.7–11.9 μg/kg), i.e., above the EU regulation, and none higher than the Canada, Mercosur, or U.S.A. limit (15, 20, and 20 μg/kg) (*41*, *42*).

Moisture Content and Water Activity versus Fungi and Aflatoxins in Raw Brazil Nuts. As expected, the mc mean of the raw Brazil nuts were high and so was the a_w, because they were collected directly from the forest with no treatment or processing at all. The a_w values obtained were similar to those commonly reported for *A. flavus* growth in different commodities (*43*). The a_w for shelled nuts were 0.83 for Western and slightly higher (0.88) for Eastern (Table 2). The mc mean for in-shell samples was 30.4% (Eastern) and 28.0% (Western). In a more recent study carried out by Arrus et al. (*24*) specifically for processed Brazil nuts, it was showed that the optimal conditions for *A. flavus* growth on those nuts, after 30 days of storage, were mc 8.6 and a_w 0.91 at a RH of 97% and 30 °C. They reported also the limiting mc and a_w of 4.5 and 0.68 and 5.0 and 0.75 for shelled and in-shell nuts fungal growth at those same temperatures and times. However, in practice, nuts in the factory do not stay stored longer than 2 days prior to processing. Despite

the conditions mentioned above, those data were obtained from store-dried nuts and our findings are from raw nuts. The high mc and a_w obtained in the raw nuts indicate the need for good management practices to be applied previously, in the extractive (forest and city storage) and transport activities prior to their arrival to the factory. It is necessary to develop a more efficient system of drying in the forest prior to river transport and also the improvement of aeration on the boats. This should also be applied at the factory raw nut reception to select the healthy ones with lower mc, fungi, and aflatoxins. If the raw Brazil nuts that get to the factory are already contaminated with aflatoxins or with a high load of fungal deterioration, the final product (in-shell nuts) may still have some contamination. The drying temperature (two steps with ca. 70 °C) is not enough for aflatoxin degradation, even if fungi can be reduced and surface-shell-spoiled nuts sorted. The situation could be different if the nuts are to be sold shelled dry, because they pass through two sorting steps, at deshelling and classification tables, thus discarding the spoiled nuts prior to vacuum packing.

Se versus Aflatoxins. Whereas Se is an important antioxidant in human metabolism to prevent cancer, aflatoxins are highly carcinogenic. Data showed that the raw Brazil nuts were rich in Se and presented some aflatoxins levels. Although aflatoxin levels were not very high, they were detected, especially because of the high sensitivity of the methodology of detection used (MS/MS). In addition, aflatoxin have also been reported as contaminating processed dry nuts (*44*). Se content in Brazil nuts from the two nut-producing Amazon regions and their aflatoxin levels were higher in the Eastern region than those in the Western region. High Se content has been reported, causing oxidative stress to organisms (*36*). These data lead to a question: Is there a relation between the Se level and aflatoxin production in the Brazil nuts? These high Se content might also cause oxidative stress on fungus strains, activating the mechanisms for secondary metabolite production. Dependient upon the levels, Se could activate *A. flavus* aflatoxigenic strains to aflatoxin production, thus contaminating Brazil nuts, at the first and second storage stages in the forest and after processing, as long as spores and optimum conditions for fungi growth are present. Another factor for aflatoxin production could be the interaction and competition with different strains of *Aspergillus* to affect aflatoxin production as well (*45*).

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