Aflatoxins in raw Brazil nut (*Bertholletia excelsa* H.B.K.)

Ariane Mendonça Kluczkovski (Corresponding Author)

Federal University of Amazonas, Av. Gen. Rodrigo O. J. Ramos, 6200, Manaus-AM-Brazil

E-mail: ariane@ufam.edu.br

Heloisa Barros

Federal University of Amazonas, Brazil

E-mail: heloisalbarros20@gmail.com

Janaína Barroncas

Federal University of Amazonas, Brazil

E-mail: janainabarroincas@gmail.com

Cibele Viana

Federal University of Amazonas, Brazil

E-mail: cibele.dsv@gmail.com

Emerson S. Lima

Federal University of Amazonas, Brazil

E-mail: eslima@ufam.edu.br

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Abstract

The Brazil nut is a nutritionally rich food, produced and consumed in tropical regions. On the other hand, it may be suitable to toxigenic fungi and consequently contamination by aflatoxins (AFL), toxic substances to consumers. In this context, the objective of this work was to evaluate the occurrence of AFL in raw Brazil nuts, that is, before being subjected to industrial drying stages. Of 23 samples were purchased at retail in the city of Manaus-Am-Brazil and evaluated by high performance and liquid chromatography for total AFL (B1+b2+G1+G2). From the samples, 8.6 % were positive for total AFL and. Only AFG1 and AFG2, were detected in the LOD (0.8 µg/kg) and met to the limit of <10 µg/kg of current legislation in Brazil and European Union. In conclusion, despite the contaminated samples met the legal limits, that seems the AFL contamination could happen in raw Brazil nut, as it has been reported in dried seeds. In this sense, some effort in the initial steps of the Brazil nut chain must be applied to mitigate AFL contamination before reach the factory processing.

Key words: HPLC, moisture content, water activity

1. Introduction

The seed of the Brazil nut tree (*Bertholletia excelsa*), popularly known as Brazil nut, plays an important social and economic role in northern Brazil and has become the main non-timber product in the Amazon region (Rego et al., 2021; Santana et al., 2017). In general, the Brazil nut is commercialized in the dehydrated form, however in the tropical regions, where the seeds extractive areas are located, the raw Brazil nut is used in several culinary preparations. It has an exotic flavor, it is rich in unsaturated fatty acids and proteins of high biological value, and bioactive compounds such as selenium and vitamin E (Alcantara et al., 2022; Cardoso et al., 2017). Since the Brazil nut is an extractive product of the rainforest with extreme social and economic value, it was necessary to study the risks associated with consumption, as some embargo was applied to Brasil nut by European Union in the past, due to the presence of aflatoxins (AFL), in unacceptable level. The AFL is a potential carcinogenic substance produced as secondary metabolites of some fungi, such as *Aspergillus* and they can cause toxic effects to humans and animals (Navale et al., 2021; IARC, 2016). For the fungus to produce mycotoxins, such as AFL, it needs favorable conditions for its growth, such as: water activity, moisture content, pH, chemical composition of the food and temperature, as well as gene expression (Yunes et al., 2019), conditions easily found in tropical regions where Brazil nuts are collected. The extractive activity to collect the Brazil nuts in the forest is an element of biodiversity conservation in Amazonian conservation units that meet the needs and rights of traditional and indigenous people (Silva et al., 2020). The Brazil nut chain starts with the seed’s extractive in the forest, and it is storage and transported for processing plants with different drying stages in a complex production chain (Taniwaki et al., 2019). Despite several works evaluated AFL in Brazil nut for export, the contamination data for unprocessed (raw) Brazil nuts are scarce, as the occurrence of AFL is usually monitored in the different forms of the finished product, dehydrated or granulated seeds (Alves et.al., 2020; Maturova, 2019). In Brazil, the government states the factories to export commercial the Brazil nut in the dehydrated form. In a practical away, the natural moisture content (*mc*) of the seeds directly from the forest will allow the seed degradation and enhance the chance of AFL contamination. Once the seeds become contaminated by AFL the factory drying steps will not be able to remove the contaminant due to the thermal stability of the toxin. In this context, the objective of this work was to evaluate the risk inherent in the presence of AFL in raw Brazil nuts, i.e., before industrial processing, to evaluate the risk for consumption, especially in tropical regions, where they are frequently used in daily food of the local population.

2. Material and Methods

Sampling

The samples (*n*=23) of Brazil nuts, fresh and shelled, were purchased from retailers located in the South and Center-South zone of the city of Manaus-AM-Brazil, in 2019-2020. In original packaging (plastic), Brazil nuts were immediately transported for laboratory analysis that were carried out during the second half of 2019 and the first half of 2020. The sample is illustrated in Figure 1.

1.a The Brazil nut fruit (pod) with the seeds in kernel

1.b The shelled raw Brazil nuts packaged

Figure 1. Raw Brazil nuts

Assays

The Brazil nut raw samples were evaluated for total AFL (B1+B2+G1+ G2) by high performance liquid chromatography (HPPC) according to AOAC (2016). The AFL extraction step was carried out with 50g of sample, stirred with 100mL of acetonitrile: water (90:10 v/v), for 5 min. The extract was filtered through filter paper and applied to the Multisep 228 cleaning column (Romer Labs). From the extract, about 2 ml of purified filtrate was collected for derivatization. The solution for analysis was obtained by adding a derivatizing solution to the purified extract - water: trifluoroacetic acid: acetic acid 70:20:10 (v/v) - in a water bath at 65ºC for 8.5min. The solutions were applied and quantified in HPLC in: Mobile phase - acetonitrile, methanol, and ultrapure water; flow of 1.2mL/min. eluting in gradient mode, with fluorescence detector: λ ex- 364 nm and λ em- 440nm; injection volume 10µL; 15 min. running time. The AFL standards (Sigma Aldrich®) with 2 µg/ml AFB1 and AFG1 and 0.5 µg/ml AFB2 and AFG2 were used. The limit of detection (LOD) and limit of quantification (LOQ) for each toxin (AFB1/AFB2/AFG1/AFG2) were 0.140/0.140/0.260/0.260 and 0.440/0.440/0.780/0.780 µg/kg, respectively. The LOD method was defined by 3 times the signal/noise ratio and LOQ by 6 times the signal/noise. Five points were used to build an analytical curve to obtain the correlation coefficient (R) values for LOD and LOQ. Each point corresponded to a mean of five injections of each extract. The recoveries for each AFL were: 95.0 (AFB1), 75.5 (AFB2), 98.5 (AFG1), and 99.0% AFG2)

The moisture content (*mc*) level was evaluated by the AOAC (2016) method, in which approximately 2g of Brazil nut samples, *in natura*, were weighed, and subsequently subjected to moisture quantification using of the GEHAKA IV3100 instrument, performing the drying in a standard way with constant temperature.

The Water activity (Aw) was carried out by means of direct measurement, in triplicate, using the instrument Aqualab series 4TE from DECAGON, with internal temperature control at 25°C, dew point method (AOAC, 2005).

For the Statistical analysis, it was used the student's t test was used to compare the levels of contamination between the samples and the comparison between the data performed through analysis of variance (ANOVA).

3. Results and Discussion

3.1 a_w and Mc%

The table 1 shows the results of the a_w and *mc* analysis. All samples exceeded the maximum recommended limit of 15% for the *mc* of the finished product according to Brazilian legislation (Brazil, 2010), with a range of 4.09 - 30.5%. Da Costa et al. (2017) also analyzed *mc*% of nuts before drying and found an average content of 26.91%, while Hauth et.al (2017) reported a maximum, *mc* content of 30%. The data suggest that efforts are needed to better control the *mc* before processing, since this variable can interfere with AFL production. For a_w, we found an average content of 0.96, like that of Da Costa et al. (2017). Silva & Marsaioli-Junior (2003) reported the a_w content of 0.79, and in another work, Kluczkovski et al. (2020) analyzed Brazil nuts directly from extractive communities in different harvests, 2019 and 2020, with a range of 0.88-0.99, and 0.98-0.99, respectively. Based on data from this study, and from other authors, raw seed, regardless of the region where the samples were obtained, is above the recommended limit of 0.70 (CAC, 2006).

Table 1. Water activity and moisture content % level in raw Brazil nut samples

N	a_w[1]		Mc[2]	
	Mean ± SD[3]	Min-Max	Mean ± SD	Min-Max
23	0.96 ± 0.20	0.83-0.98	19.18 ± 0.49	4.09-30.55

[1]a_w: Water activity; [2]Mc: Moisture Content %;[3]SD= Standard deviation.

3.2 AFL Level

The table 2 shows the AFL occurrence in the samples. Only 8.6% were positive, and AFG1 e AFG2 were detected. For AFB1 and AFB2 the samples showed <LOD.

Table 2. Aflatoxin in raw Brazil nut

N	**Positive samples (%)**	**AFL** (µg/kg)					
		B1[a]	**B2**[a]	**G1**		**G2**	
				Mean±SD[b]	Min-Max	Mean±SD	Min-Max
23	02	N.D.	N.D.	0.35±0.23	0.09-2.65	0.09-.12	1.28±0.46

[a] Not detected in LOD: AFB1= 0.260 µg/kg; AFB2=0.260 µg/kg; [b] SD=Standard Deviation

Table 3 shows the results of the samples that were positive for contamination by AFL, in which only AFG1 and AFG2 were detected. However, the values were obeying the limits of <10 µg/kg established by EU (2006). In a previous work Kluczkovski et al. (2020), evaluated raw and processed brazil nut seeds, and reported the AFL content in their raw and shelled Brazil nut samples (<10 µg/kg) met the legal standards in force in Brazil. Da Costa et al. (2014) analyzed Brazil nut seed before processing and detected all AFL with a content of 8.228 (µg/kg) of total AFL. In view of the data, it is necessary to reinforce the controls and monitoring of contamination before processing, as the stability of the AFL in the face of thermal drying processes indicates that if the raw material arrives already contaminated, the finished product will also be contaminated. Pacheco & Scussel (2007) also detected AFL in raw Brazil nuts, including relating samples from different Amazonian regions and the selenium content, and identified that seed Se levels from the Eastern region were higher than the Western, in addition to the AFL. Therefore, several factors, both environmental and gene expression, need to be studied, in addition to the occurrence of contamination, to apply preventive actions.

4. Conclusion

Raw Brazil nut samples were analyzed for moisture content %, water activity and AFL. It was possible to conclude that the samples acquired *in natura*, in the retail stores of the city of Manaus-Brazil, presented a_w and mc% levels above the recommended limits, which may contribute to the metabolism of toxigenic fungi to produce mycotoxins, such as AFL. Despite the AFL content, showed levels below the recommended limit in Brazil and European union, it brings the alert about contamination in the previous steps in the Brazil nut chain. In this sense we suggest to government to monitor food in retail stores to prevent fungal and/or mycotoxin contamination, as well the Brazil nut factories applies prevention program in the extractive areas to avoid environmental factors in the Brazil nut seeds storage, to allow the AFL contamination for example.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest in publishing this research article.

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References

Alcantara, D. B., Dionísio, A. P., Artur, A. G., Silveira, B. K. S., Lopes, A. F., Guedes, J. A. C., … & Zocolo. G. J. (2022). Selenium in Brazil nuts: An overview of agronomical aspects, recent trends in analytical chemistry, and health outcomes. *Food Chem., 15*, 372, 131207. https://doi:10.1016/j.foodchem.2021.131207

Alvares, V. S., De Castro, I. M., Da Costa, D. A., De Lima, A. C., & Madruga, A. L. S. (2012). Qualidade da castanha-do-brasil do comércio de Rio Branco, Acre. (2012). *Acta Amaz., 42*, 269-274. https://doi.org/10.1590/S0044-59672012000200013

Alves, C. M. S., Sousa, W. R., Cândido, C. J., Neto, J. A. B., Sampaio, R. M., Santana A. A., Belford, I. K. P., Polisel, C. G., & Monteiro, S. C. M. (2020). Occurrence of aflatoxins in brazil nuts commercialized in the northeast of Brazil. *Braz. J. of Health Review, 3,* 8129-8144. https://doi.org/10.34119/bjhrv3n4-073

AOAC-Association of Official Analytical Chemists (2016). Official methods of analysis, 20th edition. AOAC International, Gaithersburg, MD, USA.

BRAZIL. Ministry of Agriculture, Livestock and Supply. Instruction Normative nº 11, 22 march (2010) Criteria and Procedures for the control hygiene and health the Brazil nut and its byproducts. Diário Oficial da República Federativa do Brasil, Brasília, DF.

CAC- Codex Alimentarius Commission. (2010). Proposed draft maximum level for total aflatoxins in brazil nuts. ALINORM 10/33/41 Appendix V, 47. Joint FAO/WHO Food Standards Program, FAO, Rome.

Cardoso, B. R., Duarte, G. B. S., Reis, B. Z., & Cozzolino, S. M. (2017). Brazil nuts: nutritional composition, health benefits and safety aspects. *Food Research International. 100*, 9-18. https://doi.org/10.1016/j.foodres.2017.08.036

Da Costa, D. A., Álvares, V. S., Kusdra, J. F., Nogueira, R. M., Maciel, V. T., & Miqueloni, D. P. (2017). Quality of in-shell Brazil nuts after drying using a pilot natural convection oven in the state of Acre, Brazil Braz. *J. Food Technol., 20*, e2015104. https://doi.org/10.1590/1981-6723.10415.

Hauth, M. R., Botelho, S. C. C., Tonini, H., & Botelho, F. M. (2016). Tempo de permanência da Castanha-Do-Brasil Na Mata Após A Queda: contaminação por Aflatoxinas. Jornada Científica Da Embrapa Agrossilvipastoril, 5, Sinop. Anais. Sinop, MT: Embrapa, 93-97.

IARC -International Agency for Research on Cancer. (2016). Agents classified by the IARC Monographs, Volumes1-116.

Kluczkovski, A. M. (2019). Fungal and mycotoxin problems in the nut industry. *Current Opinion in Food Science, 29*, 56-63. https://doi.org/10.1016/j.cofs.2019.07.009

Kluczkovski, A. M., Viana, C., Barroncas, J., Lima, E., Valentim, C., Xavier, L., Campelo, P., & Kluczkovski, A. (2020). Risk Assessment of Aflatoxin In Brazil Nut By Product

Consumption In The Amazon Region. *Asian. J. Pharm. Clin. Res., 13*, https://doi.org/10.22159/ajpcr.2020.v13i8.37622

Kluczkovski, A., Silva, A., Barroncas, J., Lima, J., Pereira, H., Mariosa, P., & Vinhote, M. L. (2020). Drying in Brazil Nut Processing as Tool for Prevention of Contamination by Aflatoxins. *J. of Agricultural Studies, 8*, 70-81. https://doi.org/10.5296/jas.v8i4.17387

Maturová, H. (2019). Determination of the presence of mycotoxins in nuts in stages of post-harvest handling and storage. *Mitteilungen Klosterneuburg, 69*, 208-215.

Navale, V., Vamkudoth, K. R., Ajmera, S., & Dhuri, V. (2021). Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology Reports, 8*, 1008-1030. https://doi.org/10.1016/j.toxrep.2021.04.013

Pacheco, A. M., & Scussel, V. M. (2007). Selenium and Aflatoxin Levels in Raw Brazil Nuts from the Amazon Basin. *J. Agric. Food Chem., 55,* 11087-11092. https://doi.org/10.1021/jf072434k

Rego, L. J. S., Soares, N. S., Isbaex, C., Silva, S., Januncio, J. C., Silva, M. L., & Romero, F. (2021). Brazil nuts a non-timber potential: Uncertainties and investment. *Research, Society and Development*, *10*, e22101521868. https://doi.org/10.33448/rsd-v10i15.21868

Santana, A. C., Santana, Á. L., Santana, Á. L., & Martins, C. M. (2017). Valuation and sustainability of Brazil nuts in the Amazon. *Rev. Cienc. Agrar. Amazonian J. of Agric. and Env. Sciences, 60*, 77-89. https://doi.org/10.4322/rca.60101

Silva, F. A., & Marsaioli Junior, A. (2003). Atividade de água em amêndoas de castanha do Brasil (*Bertholletia excelsa*) secas por microndas e convencionalmente. *Rev. Cienc. Exatas e Naturais*, *5*, 23-32.

Taniwaki, M. H., Pitt, J. I., Copetti, M. V., Teixeira, A. A., & Iamanaka, B. T. (2019). Understanding Mycotoxin Contamination Across the Food Chain in Brazil: Challenges and Opportunities. *Toxins*, *11*, 411. https://doi.org/10.3390/toxins11070411

Yunnes, N. B. S, Oliveira, R. C, Reis, T., Baquião, A. C., Rocha, L. O., & Correa, B. (2020). Effect of temperature on growth, gene expression, and aflatoxin production by *Aspergillus nomius* isolated from Brazil nuts. *Mycotoxin Res., 36*, 173-180. https://doi.org/10.1007/s12550-019-00380-w