EUROPEAN ACADEMIC RESEARCH
Vol. X, Issue 5/ August 2022

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Occurrence of aflatoxins in Brazil nut oil

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Abstract

This study aimed to quantify carcinogenic agents as aflatoxin for fungi in Brazil nut oil. In 21 samples, the mean value found was 92.34 (0.42–818.69) μg/kg. These data are relevant regarding the risk of finding consumption mechanisms at the date of consumption in good occurrence or the discarding of data and the most frequent risk of discarding to evaluate Brazil nuts.

Keywords: *Bertholletia excelsa*, lipids, mycotoxin

1. INTRODUCTION

The Brazil nut (*Bertholletia excelsa*) is marketed for its excellent nutritional composition, and Brazil nut products have space for research in the market. However, there is a need to evaluate the safety, conservation, and stability of the oil to increase its commercial value. Among the non-timber extractive products for northern Brazil, the nut is known for its nutritional quality, such as proteins (15–20% by weight) and sulfur amino acid and lipid content (60–70% by weight). The oil includes fatty acids and antioxidant properties and methods and can be extracted separately (ALVES et al., 2020), the most common being mechanical cold pressing. This method involves placing whole seeds in a hydraulic press and applying pressure or extracted oil. The oil can be obtained by centrifugation, filtration, decantation, or fractional distillation to separate it from residual parts from the shipment (SCHONS et al., 2017). The procedures used for extracting and refining edible vegetable oils can effectively reduce aflatoxins, varying according to the type of oil and the

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method of oil refining. However, studies have reported a high incidence of aflatoxin contamination in edible oils worldwide since the raw material for edible oil production is usually stored for long periods under conditions that can promote fungal growth and mycotoxin production. In Brazil, among the numerous causes of this contamination is, for example, the relative humidity and ambient temperature of the food production and storage environments.

Hence, the Brazil nut can be affected by the climate of the Amazon region with its high temperatures (>30 °C) and high relative humidity (>70%) throughout the year. Additionally, other factors include the low level of technology adopted by natives and long periods in which the Brazil nut is in contact with the soil microbiota during the rainy season, constituting the primary factors for the contamination of seeds by aflatoxin-producing fungi. This condition can lead to severe losses in the agricultural economy and international trade.

As for other tree nut oils, in China, one study analyzed aflatoxin occurrence in peanut oil samples randomly collected from family workshops in western Guangdong during 2016–2017, and the authors found that contamination was season dependent, with the worst case in spring (QI et al., 2019). For instance, there are controversies regarding solubility in peanuts, and market research has shown that aflatoxins do not occur in the oil. Thus, the use of peanut oil in human food is often overlooked as a source of aflatoxin exposure, although artisanal extraction of oil from contaminated peanuts in artisanal facilities, for example, results in the transport of these mycotoxins into the oil. Consequently, these peanut oils can have high contamination levels, as reported in peanut oil studies in China, Nigeria, and Portugal (SHEPARD, 2018).

Considering that Brazil nut oil is also extracted manually by mechanical pressing for culinary purposes, there is no scientific data to guarantee that there is no aflatoxin contamination, making it necessary to evaluate the possibility of aflatoxin occurrence from the chestnut kernel from which the oil is extracted. Despite the nutritional properties of the nut that make it an interesting raw material for the industry, the association with aflatoxin contamination is frequently reported (TANIWAKI et al., 2018). In this context, it is necessary to study the presence of aflatoxins in Brazil nuts used in oil production and the possibility of contamination transfer.

2. MATERIALS AND METHODS

2.1 Sampling and raw material

The samples (n = 21) were obtained after pressing residual Brazil nuts (rotten, flat, and yellowish) acquired in processing plants in Amazonas State (Brazil) and submitted to pressing in a mechanical press (TECNAL®).

2.2 Testing

2.2.1 Aflatoxin extraction

The aflatoxins (B1, B2, G1, and G2) were analyzed by liquid chromatography using the AOAC method (2016). The oils were accurately weighed (5 g ± 0.1 mg) and transferred to 50-mL centrifuge tubes. Then, 5 g of NaCl and ethanol/water (7:3, v/v) were added to give a final volume of 20 mL. The mixture was extracted using a mechanical shaker for 2 min. After filtration, 15 mL of extract was collected and diluted with 30 ml of high-performance liquid chromatography (HPLC) grade water to ensure complete elimination of the filtrate, and the mixture was filtered before starting purification. The AflaStarTM R immunoaffinity column attached to a 10 mL glass syringe was used. Then, 15 mL of the sample extract was injected into the syringe. With the aid of a vacuum pump, the extracts were passed through the immunoaffinity column at about 6 mL min^{-1}. The column was washed twice with HPLC grade water (10 mL). Aflatoxins were eluted off the column with methanol (1 mL). The solvent should be kept in contact with the column for at least 1 min to ensure elution of aflatoxins from the column. The extract was collected in Eppendorf tubes and stored in the freezer for further analysis by HPLC.

Samples were quantified for the aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) by liquid chromatography using the AOAC method (2005). For the derivatization of this purified extract, a derivatizing solution composed of water:glacial acetic acid:trifluoroacetic acid (35:10:5 v/v) was used, where 0.2 mL of the purified extract was passed to a derivatization vial with 0.7 mL of derivatizing solution using a 1 mL syringe and a filter for a Nylon syringe with a porosity of 0.45 µm; this vial was closed and heated at 65 °C for 8.5 min in a water bath (time required to complete the derivatization of AFB1 and AFG1) This procedure was repeated for the 24 samples. The resulting solutions were applied and quantified in an HPLC system with: mobile phase - acetonitrile, methanol, and ultra-pure water (1:1:4), column: X-Terra by Waters, 150 x 4, 6 mm, flow of 1.0 mL/min eluting in isocratic mode with a fluorescence detector: λ ex - 360 nm and λ - 440 nm; injection volume 50 µL, and 20 min run time. Four pools of AFB1, AFB2, AFG1, and AFG2 standards were used — Sigma Aldrich®, with different AFB1, AFB2, AFG1, and AFG2 concentrations prepared from a pool of stock solution (ng/mL) containing: AFB1 = 300; AFB2 = 50; AFG1 = 150, and AFG2 = 50. The pools of the standards were subjected to derivatization and analyzed by HPLC to obtain the chromatogram of the standards at different concentrations. The chromatogram obtained from the tapioca gum samples was then compared to each peak with the peak and retention time obtained by each standard (AFB1, AFB2, AFG1, and AFG2). The quantification of the samples was

performed from a curve of each AFL standard obtained from the reading in HPLC of different concentrations of the aflatoxin pool. The detection and quantification limits for each aflatoxin (AFB1, AFB2, AFG1, and AFG2) were 0.136, 0.136, 0.250, 0.250 and 0.410, 0.410, 0.750, 0.750 µg/kg, respectively. The recoveries in each aflatoxin (AFB1, AFB2, AFG1, and AFG2) were 94.5, 73.5, 97.8, and 99.1%, respectively. As for the oils, the same detection and quantification limits were found for aflatoxins, being 0.136, 0.136, 0.250, 0.250 and 0.410, 0.410, 0.750, and 0.750 µg/kg. Therefore, recovery values were obtained at three levels of tested concentrations: 2.5, 5.0, and 10.0µg/kg, with recovery values of 95.7, 72.8, 96.9, and 98.8%, respectively.

2.2.2 Statistical analysis

The Student t test was used to compare the contamination levels between the samples; the comparison between the data was performed by analysis of variance (ANOVA). The results were compared to safety standards.

3. RESULTS AND ANALYSES

The aflatoxin content of the Brazil nut oil is listed in Table 1, and our findings revealed that all aflatoxins were detected in all samples; the mean was 92.34 (0.42–818.69) µg/kg. The Brazilian sanitary legislation does not have maximum limits for aflatoxins in Brazil nut oil (BRASIL, 2021). There are various studies on contamination in Brazil nut seeds, although research on oil is scarce, with available data being limited to oils from other tree nuts/legumes, such as peanuts.

Table 1. Aflatoxin content in oil samples extracted from Brazil nuts

Aflatoxins	RESULTS (µg/kg)*	
	Mean	Min–Max
B1	3.18 ± 2.02	0.84–352.83
B2	5.32 ± 3.42	0.42–163.83
G1	5.49 ± 3.30	1.26–818.69
G2	9.16 ± 8.69	1.27–125.06

*Limit of quantification = 0.410, 0.410, 0.750, and 0.750 µg/kg (AFB1, AFB2, AFG1, and AFG2)

Regarding aflatoxins in other tree nuts, MAHONEY (2021) evaluated aflatoxin concentrations in highly consumed vegetable oils (e.g., peanut, olive, maize, soybean, linseed, sesame, palm, canola, sunflower, and coconut); the author reported that the highest and lowest AFB1 levels were related to sunflower and sesame oils.

Brazil nut oil has been evaluated to solve the problem of damaged seeds by the industries' food facilities. To justify the occurrence of aflatoxins in Brazil nut oil, it is pivotal to emphasize the presence of aflatoxins in damaged seeds as they seem to be transferred to the oil fraction. Thus, aflatoxin-contaminated waste must be properly disposed of and not released

into the environment. Another way to use discarded seeds is as a source of protein and calories for animal feed (RAMOS et al., 2022; FOUTZ et al., 2020). Nevertheless, the clinical problems in these animals are rarely evaluated regarding aflatoxin contamination in their metabolism and yield. RUFINO et al. (2018) included Brazil nut oil in diets given to breeder cocks and concluded that it was an energetic additive promoting better reproductive performance despite altering the biochemical serum profile of the birds. This is just one example, and further research is necessary to shed more light on Brazil nut oil and its effects on metabolism.

In other tree nuts, one study attempted to solve this problem of environmental contamination by aflatoxins by utilizing contaminated peanuts and converting them into biodiesel (JUNG et al., 2021). In addition to the methods to solve the problems with contaminated seeds of tree nuts and the environment, new methods have been developed, such as electrochemical immunosensor to detect AFB1 in vegetable oil (WANG et al., 2022; LI et al., 2022). In this context, the occurrence of aflatoxins in Brazil nuts is preliminary data for further investigation of methods to prevent and detect aflatoxin occurrence for cosmetic and food industry safety and public health protection.

4. CONCLUSIONS

This study identified the presence of aflatoxins in oil samples obtained from Brazil nut residue. All analyzed samples showed the presence of AFB1, AFB2, AFG1, and AFG2. The data demonstrate that it seems possible to transfer the aflatoxins to the oil obtained in manual pressing, and our findings indicate that the quality of the raw material, especially as raw material for food industries, requires further attention. Given the results reported herein, greater monitoring of aflatoxin presence is necessary during the processing/commercialization of vegetable oil raw materials to ensure safe levels of aflatoxins.

Acknowledgments: Capes Foundation – Procad Amazonia 2018; FAPEAM

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