OCCURRENCE OF AFLATOXIN IN BRAZIL NUTS OIL

ABSTRACT

A food processing product for processing materials such as Brazil nuts. Therefore, the objective of the work was quantified as aflatoxin, carcinogenic agents 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 regarding the discarding of data for evaluation of Brazil as well as the most frequent risk of discarding for evaluation of Brazil.

INTRODUCTION

The Brazil nut (Bertholletia excelsa) is marketed for its excellent nutritional composition. Brazil nut products have space for studies in the market, however, there is a need to evaluate the safety, conservation and stability of the oil, in order to increase its commercial value. Among the non-timber extractive products for the northern region of Brazil, the nut is known for its nutritional quality, such as protein (15 - 20% by weight), sulfur amino acid content and lipid content (60 - 70% by weight). The oil includes fatty acids and antioxidant properties and methods (ALVES et al, 2020) and can be extracted separately, the most common being mechanically by cold pressing. This method consists of placing whole seeds in a hydraulic press and applying pressure or extracted oil. The oil can by centrifugation, filtration, decantation or fractional distillation to separate it from residual parts from the shipment (SCHONS, 2017).

Brazil nuts are not associated with the presence of aflatoxins (AFL), which are carcinogenic to humans.

The procedures used for extracting and refining edible vegetable oils can be effective in reducing aflatoxins, varying with the type of oil and the method of oil refining. However, studies have reported a high incidence of aflatoxin contamination in edible oils worldwide as the raw material for edible oil production is usually stored for long periods under conditions that can promote fungal growth and mycotoxin production. al, 2014). In Brazil, among the causes for this contamination is, for example, the relative humidity and ambient temperature of the places of production and storage of food.

The Brazil nut can then be affected by the climate of the Amazon region, with high temperature (>30 C) and high humidity (>70%) during most of the year; in addition to the low level of technology adopted by extractivists and the long period in which the Brazil nut fruit is in contact with the soil microbiota, during the rainy season, constituting the primary factors for the contamination of almonds by aflatoxin-producing fungi. (Araujo, 2018). This condition can lead to severe losses in the agricultural economy and international trade. With respect to other tree nut oils, in China, the occurrence of AFL in peanut oil samples randomly collected from family workshops in western Guangdong during the period 2016-2017 was analyzed.

Contamination was season dependent in western Guangdong, with the worst case in spring (QI et al, 2019). In peanuts, for example, there are controversies regarding solubility and market research has been carried out that AFL does not occur in oil. Thus, the use of peanut oil in human food is often overlooked as a source of aflatoxin exposure, but 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 levels of contamination as found in peanut oil studies in China, Nigeria and Portugal (SHEPARD, 2018).

Considering that in Brazil Brazil nut oil is also extracted manually by mechanical pressing for culinary purposes, there is no scientific data to guarantee that there is no contamination by AFL, so it is necessary to evaluate the possibility of the occurrence of AFL 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 AFL 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.

MATERIALS AND METHOD

2.1 Sampling and raw material

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

2.2 Test

2.2.1 Aflatoxins Aflatoxin extraction

The AFL (B1, B2, G1 and G2) were analyzed by liquid chromatography using the A.O.A.C 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) was added to give a final volume of 20 ml. The mixture is extracted on a mechanical shaker for 2 min. After filtration, 15 ml of extract was collected and diluted with 30 ml of HPLC grade water to ensure complete elimination of the filtrate, the mixture was refiltered 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 a rate of about 6 ml.min-1. The column was washed twice with 10 ml of HPLC grade water. Aflatoxins are eluted off the column with 1 ml of methanol. 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 and stored in the freezer for further analysis on HPLC.

Quantification: samples were quantified for AFLs B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2) by liquid chromatography using the A.O.A.C 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 0.2ml of the purified extract was passed to a derivatization vial with 0.7ml of derivatizing solution with the aid of a 1ml syringe and a filter for a Nylon syringe with a porosity of 0.45µm, this vial was closed and heated at 65°C for 8.5 minutes in a water bath (time required to complete the derivatization of AFL B1 and G1), this procedure was repeated for the 24 samples.

The resulting solutions were applied and quantified in a High Performance Liquid Chromatography (HPLC) system, with: Mobile phase - acetonitrile, methanol and ultra pure water (1:1:4), column: X-Terra by Waters, 150x4, 6mm, flow of 1.0mL/minute eluting in isocratic mode, with fluorescence detector: λ ex- 360 nm and λ em- 440 nm; injection volume 50µL; 20 minute run time. Four pools of AFL B1, B2, G1 and G2 standards were used - Sigma Aldrich®, with different concentrations of AFL B1, B2, G1

and G2, 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, in order to obtain the chromatogram of the standards at different concentrations. The chromatogram obtained from the tapioca gum samples is then compared 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 Aflatoxin standard obtained from the reading in HPLC of different concentrations of the aflatoxin pool.

The detection limit and quantification limit for each aflatoxin AFB1/AFB2/AFG1/AFG2) are 0136/0.136/0.250/0.250 and 0.410/0.410/0.750/0.750 µg/kg respectively. The recoveries in each AFLA (AFB1/AFB2/AFG1/AFG2) are 94.5, 73.5, 97.8 and 99.1% respectively. As for oils, the same detection and quantification limits were found for AFL, being these, 0136/0.136/0.250/0.250 and.410/0.410/0.750/0.750 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

2.2.1 Statistical analysis

Student's t test will be used to compare the levels of contamination between the samples and the comparison between the data performed by means of analysis of variance (ANOVA), and the results will be compared to safety standards.

RESULTS AND ANALYSIS

The AFL content in Brazil nut oil presented in table 1, demonstrating that all AFL were detected in all samples. The mean was 92.34 (0.42-818.69) µg/Kg. The Brazilian sanitary legislation (Brazil, 2021) does not have maximum limits allowed for AFL in Brazil nut oil. But the normative instruction N° 88, of March 26, 2021, establishes the maximum tolerated limits (LMT) of contaminants in food (0,05) µg/Kg). There are several studies on contamination in Brazil nut seeds but research on oil is scarce, with data being found on oils from other tree nuts/legumes such as peanuts.

Table 1. Aflatoxin content (B1, B2, G1 and g2) 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

*LOQ=0.410/0.410/0.750/0.750 µg/kg (AFB1; B2; G1;G2)

CONCLUSIONS

MAHONEY (2021) evaluated the concentration of AFL in highly consumed vegetable oils (including peanut, olive, corn, soybean, linseed, sesame, palm, canola, sunflower and coconut), according to him, the highest and lowest levels of AFB1 and total PA were related to sunflower oil (2.64µg/kg) and sesame oil (43, 60µg/kg).

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