

## RISK ASSESSMENT OF AFLATOXIN IN BRAZIL NUT BY PRODUCT CONSUMPTION IN THE AMAZON REGION

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### ABSTRACT

**Objective:** Evaluate exposure to aflatoxins in processed Brazil nut (chopped and sliced) products marketed in Amazonas State.

**Methods:** The samples were purchased during the 2017 harvest at the local retail in the city of Manaus/AM/Brazil in the form of sliced and chopped. Moisture content (MC) and water activity (aw) were verified, aflatoxins (AFB1, AFB2, AFG1, AFG2) were quantified by liquid chromatography. To characterize the risk of exposure to genotoxic use the population margin of exposure (MOE).

**Results:** Chopped and sliced Brazil nut samples analyzed here presented an MC average of 1.62% and water activity of 0.26. These values indicate that samples are safe, according to physical-chemical baselines. Regarding aflatoxins presence, 8% of the samples presented aflatoxins total levels >10 µg/kg. A risk evaluation was performed in which exposure of the population to these substances is observed and, once found; the MOE was 1036 ± 793 (<10 000).

**Conclusion:** Regarding the risk assessment, it was possible to observe that there is a possibility exposure of the population to these substances since the average of MOE found was 1036±793, or <10 000, characterizing this possible risk.

**Keywords:** Mycotoxin, *Bertholletia excelsa*, margin of exposure.

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### INTRODUCTION

The Brazil nut (*Bertholletia excelsa*, H.B.K) is one of the most important non-timber species in the Amazon region. It is collected in the forest, industrially improved, and consumed in a dehydrated form or as an ingredient in culinary preparations or products. Despite its exotic flavor and being rich in nutrients, it is associated with aflatoxin (AFL) contamination, a fungal metabolite that is carcinogenic and hepatotoxic to human health [1]. Studies related to AFL occurrence have reported that both the shell and nut are susceptible to contamination and that mycotoxigenic strains may be in the defective fractions of the damaged nuts and peels [2]. Due to environmental conditions in Northern Brazil (temperatures between 30 and 35°C and relative air humidity from 80% to 95%), toxin strains, such as *Aspergillus flavus*, can produce AFLs [3]. Brazil nuts are collected in the forest and transported for drying, which has several stages. However, Brazil nuts can be contaminated at any stage of processing, making them a public health problem since the ingestion of these metabolites may cause adverse health effects [4]. The export of Brazil nuts occurs in the Amazon region and for all countries, and the increasingly dynamic changes in food habits and production worldwide have increased the importance of analyzing the risks associated with their consumption by calculating the exposure, not only by the presence of AFL. Several studies have evaluated the risk of raw materials for food and feed products [5,6]. Such risks can be managed to mitigate negative effects to the consumer and, together with scientific support, help properly communicated for health prevention. In Brazil, in addition to the whole exported almond, the nut can be used in the chopped and sliced format to be used in cooking preparations or consumed as snacks. In this context, it is important to evaluate the risks inherent in the presence of AFL in Brazil nut derivatives to contribute to public policies and promote consumer health since it is susceptible to environmental factors that affect contamination. Da Costa *et al.* observed the effects of adequate drying

on reducing the presence of fungi, demonstrating that it is fundamental that humidity is at safe levels and avoid AFL production [7]. In Northern Brazil, despite Brazil nuts and its derivatives being consumed in the daily diet of the population, studies relating AFL levels and Brazil nuts are scarce, as research generally focuses on nuts obtained in forests or already dehydrated and destined for export [8,9]. In view of this, the objective of this study was to evaluate exposure to AFL in processed Brazil nut (chopped and sliced) products marketed in Amazonas State. Thus, it will be possible to understand/estimate the impact of the presence of these substances on the consumer diet, contribute to monitoring contamination, provide data to help prevent diseases, and evaluate the relationship of moisture content (MC) and water activity of this sample with AFL concentration.

### METHODS

#### Sampling

Brazil nut samples are presented in Figs. 1 and 2. The samples were purchased during the 2017 harvest at the local retail in the city of Manaus/AM/Brazil, thus simulating the acquisition of products derived from nuts. The samples were purchased in the form of sliced (n=15, Fig. 1) and chopped (n=15, Fig. 2). Although they were acquired in different places, most samples came from the same industry. All samples had a shelf life of 6 months.

#### MC and water activity

MC was determined by AOAC and water activity (aw) was verified by AquaLab series 4TE by DECAGON at room temperature (25°C) [10].

#### Aflatoxin quantification

Aflatoxins (AFB1, AFB2, AFG1, and AFG2) were quantified by liquid chromatography using the AOAC Official Method 994.08 [10]. In a 50 g sample, the AFLs were extracted with 100 mL of acetonitrile:water

solution (90:10 v/v) and shaken at high speeds for 5 min with subsequent filtering using filter paper. Then, 3mL of the filtrate was transferred to a 10 mL culture tube with the application through MYCOSEP 226 (Romer Labs) cleaning column for extract purification. For derivatization, 0.2 mL of purified extract and 0.7 mL of water:glacial acetic acid:trifluoroacetic acid (35:10:5 v/v) were subjected to 65 °C heating for 8.5 min to derivatize the AFB1 and AFG1. The resulting solutions were applied and quantified in a high-efficiency liquid chromatography system with mobile phase – acetonitrile, methanol, and ultra-pure water (1:1:4), waters X-Terra column, 150 mm×4.6 mm, flow of 1.0 mL.min<sup>-1</sup> eluting in isocratic mode, with fluorescence detector: λ excitation –360 nm, and λ emission – 440 nm; volume of injection of 50 µL; race time of 20 min. Three pools of AFL standards B1, B2, G1, and G2 (Sigma Aldrich) were used. The limit of detection (LOD) and limit of quantification (LOQ) for each toxin (AFB1/AFB2/AFG1/AFG2) were 0.136/0.136/0.250/0.250 and 0.410/0.410/0.750/0.750 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 aflatoxin (AFB1, AFB2, AFG1, and AFG2) were 94.5, 73.5, 97.8, and 99.1%, respectively.

**Risk assessment diet**

To estimate exposure to chemicals in food, the calculation proposed by Jardim and Caldas was used using aflatoxin concentration in the food (ng/kg), food consumption (kg), and body weight (kg)

(individual or study population) [11]. Equation 1 defines the exposure estimation in mg/kg:

$$EE = \frac{\text{Compound concentration} \times \text{Food consumption}}{\text{Corporal weight}} \tag{1}$$

The calculation was performed with samples that presented AFB1 contamination. Fractions of AFB1 below LOQ were not considered. Food consumption used was 10.57 g/day for high consumption of Brazil nuts [12]. The value of body weight used was 60 kg [13].

**Exposure to carcinogenic and genotoxic substances**

Risk assessors to characterize the risk of exposure to genotoxic use the population margin of exposure (MOE) and carcinogenic substances that can be found in food or feed [14]. Equation 2 was performed for measures of MOE. The MOE can be calculated from the BMDL (benchmark dose lower confidence limit) value, preferably BMDL10, identified as the most appropriate toxicological reference point for estimation:

$$MOE = \frac{BMDL_{10}}{EE} \tag{2}$$

According to EFSA, the BMDL<sub>10</sub> value of 170 ng/kg/day is based on carcinogenicity data in rats exposed to AFB1 [14]. The higher the value found for the MOE, the lower the risk of health damage. Values below 10 000 may indicate possible problems to human health. The MOE approach provides an indication for the level of safety concern about the presence of a substance in food but does not quantify the risk as such [14].

**Statistical analysis**

The relationship between AFLs and aw and MC was analyzed by simple linear regression [15,16]. Sample normality was tested using the Shapiro-Wilk test [17,18]. For comparisons between two groups and the Wilcoxon test was chosen as an alternative to the Student's t-test when the data presented asymmetry [19-21].

**RESULTS AND DISCUSSION**

**Aflatoxins versus MC and aw**

A significant relation of AFL (total) and aw and MC (p<0.0004) was observed, as well as for AFG1 with these two parameters (p<0.0001). The same did not occur with AFB1 (p>0.0633). There was no relationship between AFB1 and aw (p>0.0731) and between AFB1 and MC (p>0.0633). Data regarding AFL (total), AFG1, and AFB1 did not present normal distribution (p<0.0024) (Fig. 3). The chopped and sliced nuts were obtained by cutting the almond and differentiated only in the finished product format and by the Wilcoxon test. No effect of the cut shape was observed on aw content (p=0.9591), MC (p=0.9999), AFB1 (p=0.3152), AFG1 (p=0.8244), and AFL (total) (p=0.9350). Both the mean and range of MC levels in this work (Table 1) were below acceptable limits.

With the dehydration processes in which the nut passes through processing, MC decreased from the raw material to the finished product. This was observed in samples of Brazil nuts in pieces by other authors who found an average MC of 2.2±0.3 [24] and 2.00–3.12% [25]. For Arrus *et al.*, peeled nuts must be kept with MC around 4.5% to avoid the growth of *A. flavus* and AFL production [26]. The environmental



Fig. 1: Sliced Brazil nut



Fig. 2: Chopped Brazil nut

Table 1: Aw and MC levels

Parameters	Results		Maximum acceptable limit <sup>a</sup>
	Mean±SD	Range <sup>b</sup>	
MC %	2.19±0.004	1.62–2.88	15%
Aw	0.40±0.10	0.26–0.54	0.70

<sup>a</sup>Limits established according to MC and Aw [22,23]; <sup>b</sup>(Minimum–Maximum).  
MC: Moisture content, Aw: Water activity

conditions of the Amazon rainforest, from the period of processing and storage to the consumption of the product, may also influence aw and MC content, which favors AFL production. According to the *Codex Alimentarius* Commission, aw <0.7 is recommended as safe [23]. Regarding AFL (total), the range was 1.24–20.29 µg/kg and two samples (8%) were above the legal limit of a max of 10 µg/kg (Table 2). The two analyzed samples that exceeded the limit established by legislation were sliced with AFL (total)=13.82 µg/kg and a sample of chopped with AFL (total)=20.28 µg/kg. It is important to emphasize that, unlike other authors, our samples were from retail and considered “discardable” by the Brazil nut factory. Chopped nuts are quickly consumed in local markets as they have lower prices compared to the whole Brazil nut.

It possibly has lower levels of AFL because they were from production regions of Brazil nuts, generally of the same harvest. On the other hand, Andrade *et al.* found the average AFL values of 36.9 µg/kg when analyzing retail samples in Distrito Federal State in Brazil [12]. This is the region that has no Brazil nut factories and buys Brazil nuts from other regions, including Amazon State, and stores them with nuts from past harvests under conditions that may favor mycotoxin production.

Other authors also analyzed cashew nuts and used liquid chromatography as a method of analysis, although they did not specify whether the nut was sliced or chopped. Álvares *et al.* (n=3) collected cashew nuts in Acre State (Brazil), AFL (mean of AFL [total=0.86 µg/kg])

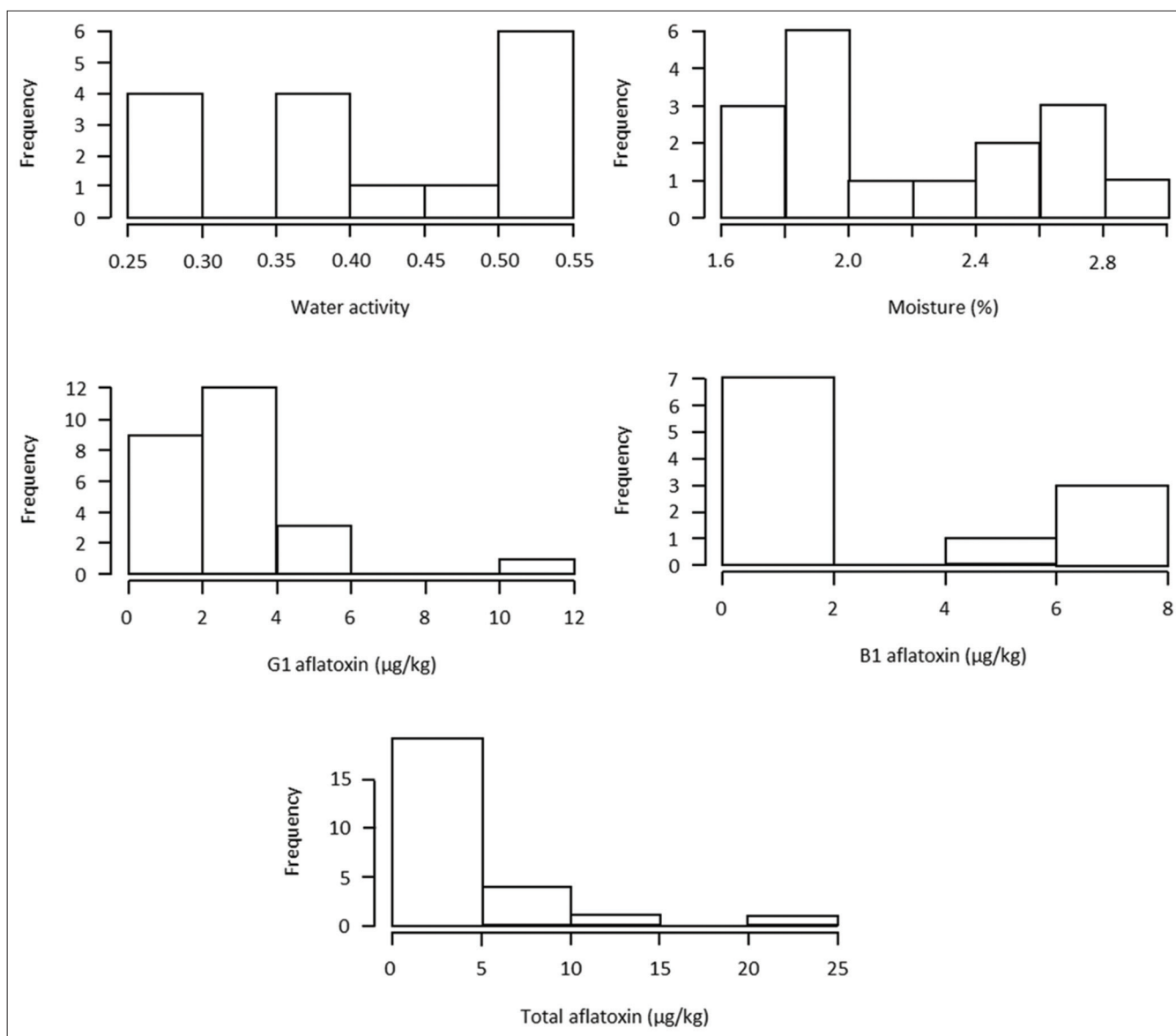


Fig. 3: Aflatoxin versus water activity and moisture content in sliced and chopped Brazil nut samples

Table 2: Aflatoxin levels in Brazil nuts – sliced and chopped

Samples	Results µg/kg (mean±SD) <sup>a</sup>					% samples >10 <sup>b</sup>
	AFB1	AFB2	AFG1	AFG2	AF total	
30	2.7±2.87 (0.5–7.86)	1.19±0.036 (1.16–1.21)	2.85±2.17 (0.75–11.32)	1.34± 0.54 (0.96–1.73)	4.25± 4.29 (1.24–20.29)	8%

<sup>a</sup>Results are expressed in average ± standard deviation (range); the limit of quantification were: AFB1=0.410, AFB2=0.410, AFG1=0.750, and AFG2=0.750; <sup>b</sup>Limit established in legislation up to 10 µg/kg of AFL and up to 2.0 µg/kg of AFB1 [27]

Table 3: Characterization of the risk

Exposure ng/kg/day <sup>a</sup> (mean±SD) <sup>c</sup>	MOE <sup>b</sup> (mean±SD) <sup>c</sup>
0.48±0.51 (0.09–1.38)	1036±793 (123–1977)

Results are expressed in average ± standard deviation. <sup>a</sup>Calculation of the exposure based on high consumption of 10.55 g of Brazil nuts per day [12].

<sup>b</sup>Based on a toxicological reference (BMDL<sub>10</sub>) of 170 ng/kg/day [14]. <sup>c</sup>Results are expressed in average±standard deviation (range)

was also found, but they met the limits of the legislation [25]. Iamanaka *et al.* evaluated samples sold at a supermarket (n=21) and found a mean total AFL of 0.24 µg/kg and maximum of 0.98 µg/kg [28]. In our study, the highest AFB1 content found was 7.86, which exceeded the maximum limit allowed by the legislation of 2 µg/kg for AFB1, the other three samples analyzed also exceeded this limit. Cunha *et al.* evaluated multiple toxins in Brazil nuts purchased in Portugal and reported that it was possible to associate the data of occurrence and consumption, evaluate the exposure, and characterize the risk to the consumption of nut tree products by the population [29]. Therefore, a promising field for future studies is the study of other mycotoxins in samples from retail in the Amazon region. Another important aspect to consider is that these products did not present adequate packaging, that is, low-density plastic, with passage to sunlight and that may accelerate the degradation process of the lipid fraction of the chestnut. Silva *et al.* already considered this by analyzing processed nuts and observed negative effects of processing in fat and amino acids that decrease product quality [30].

#### Exposure evaluation

According to EFSA, the probable risk is characterized when the MOE value found is below 10,000 [14]. The mean value of the exposure and MOE values is shown in Table 3, where the highest value was 1977, that is, <10,000, which already indicates a likely risk to human health. All Brazil nut samples that presented AFB1 contamination analyzed in our study presented MOE <10,000, which demonstrates a possible exposure of the population to this toxic compound, even if they are present at a level below the allowed by the legislation (max 2 µg/kg). These data are important in the area of public health within the monitoring of food to support decision-making with public policies for Brazil nuts by competent bodies.

A study by Andrade *et al.* evaluated AFL in products consumed in Brazil (peanuts, rice, nuts, and corn), including Brazil nuts without peels, and found a total exposure value of 16.3–27.6 ng/kg/day, and the values found for Brazil nuts without peels were 4.3–4.7 ng/kg/day [12]. The values found in our study ranged from 0.09 to 1.38 ng/kg/day. For the MOE values found in the study by Andrade *et al.*, values between 25.0 and 25.8 were found, which are values <10 000, characterizing the possible risk [12,14]. According to Jardim and Caldas, the MOE is not a quantification of risk, but its value is used to classify substances, indicates the level of concern, and establishes the priorities of action for the risk managers to reduce the risk to the health of the population consuming these products [11].

#### CONCLUSION

The samples of chopped and sliced Brazil nuts used in this study had a mean of 2.19% MC and aw 0.40, whose values, because they are below acceptable maximum limits, guarantee the stability of the nut samples for these parameters. All analyzed samples presented AFL, and two samples (8%) had total AFL above the maximum limit allowed by the 10 µg/kg legislation. As regards for the specific contamination by AFB1, which is the most toxic and carcinogenic, 16% of the analyzed samples presented AFB1 concentration above the limit of 2 µg/kg of AFB1. Regarding the risk assessment, it was possible to observe that there is a possibility exposure of the population to these substances since the average of MOE found was 1036±793, or <10 000, characterizing this

possible risk. In view of the results obtained, greater monitoring of the presence of AFLs during the processing/commercialization of these samples is necessary to prevent contamination and reduce the risk.

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#### AUTHORS' CONTRIBUTIONS

Dr. Ariane M. Kluczkovski analyzed the data and wrote the manuscript, whereas Arine Lopes, Samir Pinto, and Diana Coelho carried out the laboratory work. Dr. Augusto Kluczkovski-Junior was responsible for produce the jam samples. Both the authors read and approved the final manuscript.

#### CONFLICTS OF INTEREST

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

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#### REFERENCES

- International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. In: Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Vol. 56. Lyon, France: International Agency for Research on Cancer; 1993. p. 1-599.
- Vargas EA, Santos EA, Whitaker TB, Slate AB. Determination of aflatoxin risk components for in-shell Brasil nuts. Food Addit Contam Part A 2011;28:1242-60.
- Luo J, Taniwaki MH, Iamanaka BT, Vogel RF, Niessen L. Application of loop-mediated isothermal amplification assays for direct identification of pure cultures of *Aspergillus flavus*, *A. nomius*, and *A. caelatus* and for their rapid detection in shelled Brazil nuts. Int J Food Microbiol 2014;172:5-12.
- Bando E, Goncales LN, Tamura NK, Machinski M Jr. Biomarkers for assessment of human exposure to mycotoxins. J Bras Patol Med Lab 2007;43:175-80.
- Nugraha A, Ivonne K, Rietjens MC. Risk assessment of aflatoxin B1 exposure from maize and peanut consumption in Indonesia using the margin of exposure and liver cancer risk estimation approaches. Food Chem Toxicol 2018;113:134-44.
- Van der Fels-Klerx JH, Adams P, de Jong J, Hoogenboom R, de Nijs M, Bikker P. A model for risk-based monitoring of contaminants in feed ingredients. Food Control 2017;72:211-8.
- Da Costa DA, Alvares VS, Kusdra JF, Nogueira RM, Maciel VT, Miqueloni DP. Quality of in-shell Brazil nuts after drying using a pilot natural convection oven in the state of Acre, Brazil. Braz J Food Technol 2017;20:2015104.
- Baquião AC, Zorzete P, Reis TA, Assunção E, Vergueiro S, Correa B. Mycoflora and mycotoxins in field samples of Brazil nuts. Food Control 2012;28:224-9.
- Pacheco AM, Scussel VM. Aflatoxins evaluation on in-shell and shelled dry Brazil nuts for export analysed by LC-MS/MS-2006 and 2007 harvests. W Mycotoxin J 2010;2:295-304.
- Association of Official Analytical Chemists. AOAC Official Methods of Analysis. 20<sup>th</sup> ed. Gaithersburg, MD, USA: Association of Official Analytical Chemists International; 2016.
- Jardim AN, Caldas E. Chemical dietary exposure and the risks to human health. Quim Nova 2016;32:1898-909.
- Andrade PD, de Mello MH, França JA, Caldas ED. Aflatoxins in food products consumed in Brazil: A preliminary dietary risk assessment. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2013;30:127-36.
- Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008-2009. Raw Data. Rio de Janeiro, Brazil: Instituto Brasileiro de Geografia e Estatística; 2011.
- European Food Safety Authority. Opinion of the scientific committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA 2005;282:1-31.

15. Zar JH. Biostatistical Analysis. 5<sup>th</sup> ed. Upper Saddle River: Prentice-Hall/Pearson; 2010. p. 944.
16. Hoffmann R. Análise de Regressão: Uma Introdução à Econometria. 5<sup>th</sup> ed. Piracicaba: Rodolfo Hoffmann; 2016. p. 393.
17. Noughabi HA, Arghami NR. Monte Carlo comparison of seven normality tests. J Stat Comput Simul 2011;81:965-72.
18. Yap B, Sim C. Comparisons of various types of normality tests. J Stat Comput Simul 2011;81:2141-55.
19. Blair CR, Higgins JJ. A comparison of the power of Wilcoxon's rank-sum statistic to that of student's t statistic under various non-normal distributions. J Educ Stat 1980;5:309-35.
20. Sawilowsky SS. Abelson's paradox and the Michelson-Morley experiment. J Modern Appl Stat Meth 2005;4:352.
21. Fay MP, Proschan MA. Wilcoxon-Mann-Whitney or T-test? On assumptions for hypothesis tests and multiple interpretations of decision rules. Stat Surv 2010;4:1-39.
22. Ministry of Agriculture, Livestock and Supply. Criteria and procedures for the control hygiene and health the Brazil nut and its by products. In: Diário Oficial da República Federativa do Brasil. Brasília, DF: Ministry of Agriculture, Livestock and Supply; 2010.
23. World Health Organization. Proposed draft maximum level for total aflatoxins in Brazil nuts. In: Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission. Rome: World Health Organization; 2010.
24. Pacheco AM, Martins M. Brazil nut sorting for aflatoxin prevention: A comparison between automatic and manual shelling methods. Food Sci Tech 2013;33:369-75.
25. Álvares VS, Castro IM, Costa DA, Lima AC, Madruga AL. Quality of Brazil nuts marketed in Rio Branco, Acre. Acta Amazonica 2012;42:269-74.
26. Arrus K, Blank G, Abramson D, Clearc R, Holley RA. Aflatoxin production by *Aspergillus flavus* in Brazil nuts. J Stored Prod Res 2005;41:513-27.
27. Agência Nacional de Vigilância Sanitária. Provides for Maximum Tolerated Limits (LMT) for Mycotoxins in Food. Brazil: Agência Nacional de Vigilância Sanitária; 2011.
28. Iamanaka BT, Nakano F, Lemes DP, Taniwaki M. Aflatoxin evaluation in ready-to-eat Brazil nuts using reversed-phase liquid chromatography and post-column derivatization. Food Addit Contam Part A 2014;31:917-23.
29. Cunha SC, Sa VM, Fernandes JO. Multiple mycotoxin analysis in nut products: Occurrence and risk characterization. Food Chem Tech 2018;114:260-9.
30. Silva RF, Ascheri JL, Souza JM. Influence of Brazil nut processing on the quality of nuts. Sci Agro Technol 2010;34:445-50.