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Occurrence of aflatoxins in brazil nuts commercialized in the northeast of Brazil

Ocorrência de aflatoxinas nozes no brasil comercializadas no nordeste do Brasil

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ABSTRACT
The objective of this study was to detect the presence and levels of aflatoxins in Brazil nuts. The results showed that of a total of 84 Brazil nut samples (25 kg), 11 (13.1%) were found to be positive for aflatoxin using thin layer chromatographic analyses. Of the aflatoxins found, aflatoxin B1 was the most commonly found mycotoxin (n = 6, 7.2%) at the collection points evaluated. With respect to the samples evaluated by the enzyme linked immunosorbent assay, 35 (41.7%) were shown to be positive. The average level of contamination of the Brazil nuts by total aflatoxins was 6.24 µg/kg ± 5.29, the lowest value being 0.7 µg/kg and the highest 28 µg/kg, and three samples were shown to be above the limit permitted by Brazilian and European legislation. Regarding the influence of the variables of temperature and air humidity on the production of aflatoxins according to the seasonal variations of the dry and rainy periods, no statistically significant correlation could be found. The results obtained indicated the need to monitor the contamination of nuts by aflatoxins and develop strategies to guarantee the adoption of good handling practices as a contribution to assuring the quality and safety of these foods.

Keywords: Brazil nuts, aflatoxins, food safety.

RESUMO
O objetivo deste estudo foi detectar a presença e os níveis de aflatoxinas na castanha do Brasil. Os resultados mostraram que de um total de 84 amostras de castanha do Brasil (25 kg), 11 (13,1%) foram consideradas positivas para a aflatoxina usando análises cromatográficas em camada fina. Das aflatoxinas encontradas, a aflatoxina B1 foi a micotoxina mais encontrada (n = 6, 7,2%) nos pontos de coleta avaliados. No que diz respeito às amostras avaliadas pelo ensaio imunossorvente ligado à enzima, 35 (41,7%) mostraram-se positivas. O nível médio de contaminação da castanha-do-pará por aflatoxinas totais foi de 6,24 µg / kg ± 5,29, sendo o valor mais baixo 0,7 µg / kg e o mais alto 28 µg / kg, e três amostras mostraram-se acima do limite permitido pelo Brasil e Legislação europeia. Em relação à influência das variáveis temperatura e umidade do ar na produção de aflatoxinas, de acordo com as variações sazonais dos períodos seco e chuvoso, não foi encontrada correlação estatisticamente significante. Os resultados obtidos indicaram a necessidade de monitorar a contaminação das nozes por aflatoxinas e desenvolver estratégias para garantir a adoção de boas práticas de manuseio como contribuição para garantir a qualidade e a segurança desses alimentos.

Palavras-chave: castanha do Brasil, aflatoxinas, segurança alimentar.

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1 INTRODUCTION

The Brazil nut tree (*Bertholletia excelsa* H.B.K.) is one of the most important species of the Legal Amazon, and its nut is a non-timber extractive product of great socioeconomic and environmental importance[1,2]. Brazil stands out as being the second largest Brazil nut exporting country, producing about 38,805 tons/year and generating a revenue of 68.4 million Real from their I nternal and external commercialization[1,3]. For this reason, the Brazil nut is one of the most important products for Brazilian agribusiness, particularly in the northern part of the country[4].

In addition to its economic potential, the Brazil nut is well known for its nutritional properties, such as elevated protein (15 – 20% by weight) and sulfur amino acid contents, lipid content (60 – 70% by weight) including essential fatty acids, and antioxidant properties due to the presence of selenium and vitamin E[5,6]. Despite its socioeconomic importance and elevated nutritional value, contamination of the nuts by mycotoxins has been one of the major problems for its consumption and exportation[1]. This contamination is favored by a low technological level in the productive chain of the nuts, principally when associated with variations in the climatic conditions and the absence of good handling practices[7].

Considering the wide range of known mycotoxins, the aflatoxins are evaluated as those of greater importance in Brazil due to their toxic effects and the incidence and magnitude of their contamination of foods[8]. Aflatoxins B1, B2, G1 and G2 represent the main group of these toxins and are produced by the fungi *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*[9]. They are considered to be carcinogenic, mutagenic, immunosuppressive and teratogenic[10]. In addition the aflatoxins have become the mycotoxins most studied in Brazil nuts since the reduction in exports in 2003 when the European Union rejected batches of the nuts due to the high frequency of elevated levels of these toxins[9]. Hence the objective of this study was to detect the presence and levels of aflatoxins in Brazil nuts commercialized in São Luis, MA, Brazil.

2 MATERIAL AND METHODS

Sampling

The Brazil nut samples were collected from retail establishments in two large nut commercialization centers in the city of São Luis, State of Maranhão (MA), Brazil in the period from August 2013 to July 2014. Samples of shelled nuts were included, commercialized on the retail market and in permanent establishments. This was a non-

probabilistic sample, where seven retail establishments were selected from two commercial centers, four being located in the Central Market and three in the Reviver Market (RM). Brazil nut samples weighing 300 g were collected from each retail establishment, selected once a month in the morning. The samples were placed in hermetically sealed bags and identified with the month, salesman and collection point and transported in thermal boxes to the Toxicological Analyses Laboratory of the Federal University of Maranhão (UFMA).

Standards and reagents

The total aflatoxin standards were acquired from Sigma-Aldrich (St. Louis, MO, USA) and the stock and working solutions prepared according to the Association of Official Analytical Chemists (AOAC) methodology, as adapted by the Adolfo Lutz Institute[11]. Milli-Q water (Millipore, Brazil) was used to prepare the standards. The immunoassays were carried out using Veratox kits (total aflatoxins B1+B2+G1+G2 – direct competitive) acquired from the Neogen Corporation®, tested by AOAC (License nº 050901). All the reagents and solvents used in the analyses were from Vetec, Brazil and of analytical grade.

Aflatoxin determinations

Determination of aflatoxins by Thin Layer Chromatography (TLC)

The lipids were first extracted from the nuts using the Soxhlet method with petroleum ether as the extractor solvent, and the aflatoxins then extracted based on the adapted methodology of the Adolfo Lutz Institute[11]. To extract the aflatoxins, 30 g of defatted sample were homogenized with 10 mL distilled water and heated to 60 ºC in a conical flask with the aid of a glass rod. A 100 mL aliquot of chloroform was then added, the flask stoppered with cotton wool and shaken manually for 30 seconds, followed by a further 30 minutes of shaking in a mechanical shaker. The chloroform extract was then filtered through 8 g of Celite placed in qualitative filter paper contained in a glass funnel, and 50 mL of extract collected in another conical flask and the chloroform evaporated off in a water bath at 80 ºC. The residue was transferred to a 0.5 mL Eppendorf microtube, identified and stored at a temperature between 0 and 8 ºC until analyzed.

Twelve 20 x 20 cm silica gel 60 F254 plates (Merck), previously activated for 15 minutes in an incubator at 100 ºC, were used for the TLC identification of the aflatoxins. A 5 µL aliquot of the standard aflatoxin solution was first applied to each plate followed by 5 µL of sample, applying ten samples to each plate. Each plate was developed in chambers previously prepared with the mobile phase composed of toluene – ethyl acetate – formic

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acid (50:40:10). The plates were removed after 12 cm of development of the mobile phase and placed in a drying oven. The dry plates were analyzed under an ultraviolet (UV) lamp at a wavelength of 350 nm. The samples were interpreted as positive for aflatoxins if they possessed fluorescent bands similar to those of the aflatoxin standards.

Determination of aflatoxins by the Enzyme Linked Immunosorbent Assay (ELISA)

The Veratox total aflatoxin kit (B1+B2+G1+G2) direct competitive of the Neogen Corporation®, tested by AOAC (License nº 050901) was used to quantify the aflatoxins in all the samples analyzed in this study. The aflatoxins were first extracted by homogenizing 10 g of previously ground sample with 50 mL 70% (v/v) methanol for 2 minutes in a blender. The mixture was left to rest for 3 minutes and then filtered through qualitative filter paper (Whatman nº 1, USA), and 5 mL of the filtrate collected for the ELISA analysis.

In the ELISA analysis, 100µL of the controls (0, 5, 15 and 50 ppb) and of the samples were first added to the microwells, to which 100µL of conjugated reagent had previously been added. The contents of the microwells were then mixed with a multichannel pipette and 100µL of the mixture (conjugate, sample and controls) transferred to antibody sensitized microwells. After incubation for 2 minutes, the microwells were emptied and washed five times with distilled water. After removing the water, 100µL of substrate was added and incubated for 3 minutes, followed by the addition of 100µL of blocking solution. Quantification was by absorbance using a 650 nm filter in the plate reader (Thermo Plate TP READ). The aflatoxin concentrations were given by the Neogen Veratox software and compared with the RDC nº 07 of February 18th 2011, which establishes an upper limit of 10 µg/kg of total aflatoxins in shelled Brazil nuts destined for direct consumption.

Climatic data

The climatic data for air temperature and relative humidity in São Luís, MA, Brazil during the period from August 2013 to July 2014 were provided by the Geo-environmental Nucleus (NUGEO), located in the State University of Maranhão (UEMA).

Data analysis and statistics

The results were presented in a descriptive way, tabulated and organized with the aid of a spreadsheet constructed using the Microsoft Excel program 2010®. The data were expressed in tables in the form of absolute and relative frequencies for the categorical variables (samples contaminated by aflatoxin detected by TLC or ELISA) and in mean plus

standard deviation for the quantitative variables (aflatoxin concentrations determined by ELISA). The climatic seasons were classified according to the seasons that occur in São Luís, MA, Brazil, that is, dry (July to December) and rainy (January to June). The Fisher's Exact test or the Chi-squared test was used to verify the hypothesis of the association of the ELISA result and that of TLC with the collection point and climatic season. The Mann Whitney test was used to check for any difference in the aflatoxin concentration dosed according to the origin and collection season. The Data Analysis and Statistical Software program (STATA®) version 12.0 was used to process and analyze the data and the tests were carried out considering a significance level of 5% ($p < 0.05$).

3 RESULTS AND DISCUSSION

General characterization of the samples analyzed

A total of 84 (25 kg) Brazil nut samples was collected during the period of the study, of which 48 (14.4 kg) samples were acquired from the Central Market and 36 (10.8 kg) from the Reviver Market. All the samples were analyzed by the TLC and ELISA methods, and there were no losses during the analyses.

With respect to the sample analyses carried out by TLC, of the 84 samples, 11 (13.1%) were positive for aflatoxins of the types B1, B2, G1 and G2, and of the aflatoxins found, aflatoxin B1 (AFB1) (n = 6; 7.2%) was the most commonly identified mycotoxin at the collection points. Table 1 presents the results of the TLC analysis for aflatoxins in detail.

The Brazil nut is one of the most important Brazilian agro-business products, and is commercialized on the internal and external markets in the pharmaceutical and derma-cosmetic areas, and in a more accentuated way in the food area, due to its elevated nutritive value[7]. However, the Brazil nut has presented high levels of contamination by aflatoxins, especially when shelled and commercialized on the retail market[12,1,9,13]. Thus the contamination by aflatoxins of these products represents a high risk for the health of the exposed population, since these toxins promote carcinogenic, immunosuppressive and hepatotoxic effects[7,14].

In the present study, the presence of aflatoxins in Brazil nuts as found by TLC presented higher results than found by other researchers. Evaluated 120 samples of Brazil nuts collected in Amazonas from different sectors along the productive chain and found that 5 (4.1%) of the samples commercialized on the retail market were contaminated by these toxins[15]. Also in contrast with the present study, detected 14 (8.2%) samples contaminated

by aflatoxins on analyzing 171 Brazil nut samples coming from Amazonas[16]. Such results are of concern due to the wide occurrence of these toxins in nuts frequently consumed by the population, principally if one considers that the aflatoxins are capable of accumulating along the productive chain, since they are highly stable, heat-resistant molecules in different biotic and abiotic media, as well as being potentially dangerous to the health[10].

The greater incidence of the aflatoxin B1 (AFB1) in the nuts should also be highlighted, in consonance with the results obtained, who also showed the predominance of AFB1 when evaluating samples from three brands of shelled Brazil nuts and commercialized on the retail market in the city of Rio Branco, AC, Brazil[9]. For their part, reported that 22% of the 200 samples of Brazil nuts harvested in the Brazilian states of Acre, Amapá, Amazonas and Pará, were contaminated with AFB1, a result superior to that obtained in the present study[13]. Although there is variation in the number of samples contaminated by AFB1, the reporting of the presence of these toxins in foods is of fundamental importance, since they are classified as carcinogenic to humans (Group 1) according to the International Agency for Research on Cancer (IARC)[14].

In addition AFB1 shows a synergistic action with other toxic compounds, principally the hepatitis virus, and this association can be a strong inducer of hepatocellular carcinomas[7,17]. For this reason the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations do not consider the ingestion of any dose of aflatoxin B1 to be safe, since these compounds have high carcinogenic and genotoxic potentials for human beings[7,18]. It is interesting to point out that current Brazilian legislation has not established any maximum tolerable limits specific for this toxin.

With respect to the samples analyzed by ELISA, 35 (41.7%) gave positive results, of which 25 (29.8%) had come from the Central Market and 10 (11.9%) from the Reviver Market. The mean contamination level by total aflatoxins (Dp) found in the Brazil nuts was 6.24 µg/kg (± 5.29 µg/kg), the minimum level being 0.7 µg/kg and the maximum 28 µg/kg (Table 2). On comparing the aflatoxin concentrations with the values permitted by Brazilian legislation, it can be seen that three samples were above the permitted limit (13, 20 and 28 µg/kg).

It should be pointed out that the majority (n = 32; 38%) of the contaminated samples from the Central Market had total aflatoxin concentrations in the range from 0.7 to 4 µg/kg, and those from the Reviver Market from 5 to 9 µg/kg.

Regarding the use of the ELISA method, an elevated number of samples contaminated with total aflatoxins was observed. This high incidence of nuts contaminated by aflatoxins is of concern, since the IARC has evidence of the carcinogenic effect in humans of the natural production and mixture of aflatoxins B1, B2, G1 and G2 in low concentrations[17,18].

An analysis of the aflatoxin concentrations in the samples showed that three samples were above the limits permitted by both Brazilian legislation (RDC nº 07 of February 18th 2011) and European legislation (EU nº 165, February 26th 2010) which establishes an upper limit of 10 µg/kg of total aflatoxins in shelled Brazil nuts destined for human consumption[19,20]. Found that 119 samples of Brazil nuts contaminated by aflatoxins showed levels above those established by the legislation (10.2 to 60.3µg/kg). Such contaminations in nuts compromise food safety and consumer health, considering that the exposure to elevated aflatoxin concentrations can cause acute intoxication[15].

Although always associated with chronic syndromes, such as carcinogenic effects, aflatoxins can cause acute and subacute effects in human beings. The acute effects are characterized by rapid perception and by the ingestion of elevated doses of aflatoxins, and can cause irreversible damage. The literature has reported that the commonest clinical manifestations of acute intoxication are alterations in the metabolism of carbohydrates, lipids and proteins, alterations in the hematological parameters, the presence of blood in the feces, acute hepatitis and muscle tremors, and can lead to death between 12 and 14 hours after ingestion. On the other hand, subacute effects are the result of the ingestion of less elevated doses, which cause disorders and alterations in the organs of men and animals, especially in the liver, as well as causing discomfort, mainly nausea. It should be pointed out that the appearance of these clinical signs depends on other factors, such as the nutritional status of the exposed individual, the composition of the diet, the sex and the age[21-23].

In addition, a lack of conformity with the legislation can cause considerable disquiet amongst the Brazil nut exporters, since a high incidence of aflatoxins in these oily nuts can significantly decrease exportation, and consequently cause a great impact on marketing of this product[7,9]. 518 notifications of contamination by aflatoxins in oleaginous products were emitted by the General Directory of the European Commission of Health and Consumers by way of the “Rapid alert system for Human and Animal Food” (RASFF), and of these 16 were for Brazil nuts[7]. This demonstrates the need to adopt new strategies for the monitoring

and control of contamination by aflatoxins throughout the whole productive chain of Brazil nuts, in addition to developing more sensitive and specific methods for the detection and quantification of these toxins[24].

When the relationship between the samples analyzed by the TLC method and the collection points (Central Market and Reviver Market) was tested, no statistically significant ($p = 0.075$) association could be observed between these variables, as can be seen in Table 3.

However, on analyzing the association between the presence of aflatoxins as determined by the ELISA method and the sample collection points, a statistically significant ($p = 0.025$), Table 3) was found, and the Central Market presented a larger number of samples (n = 25, 52.08%) contaminated by aflatoxins. These results were similar to those found by[25], who carried out a study to determine the presence of aflatoxins in peanut samples coming from two districts (Busia and Homa Bay) in Kenya, and found a significantly significant difference ($p < 0.0002$) in the number of positive samples when comparing the two collection points[26]. Some authors have suggested that different contamination levels found in samples collected from distinct points are frequently the result of a lack of good handling practices, such as prolonged storage periods and a lack of monitoring of the temperature and humidity of the commercialization locality, amongst others[27,2,9].

Despite the elevated number of samples contaminated by total aflatoxins according to the ELISA method (n = 35, 41.67%), there was no statistically significant difference in the concentrations of these toxins between the collection points.

The present study allows one to affirm there was an important difference in the results of the TLC and ELISA techniques with respect to the number of samples contaminated with aflatoxins. Such results were also found in other studies, such as that of autor who analyzed for the presence of aflatoxins in 123 samples of corn-based products from the state of Paraná in Brazil and found seven positive samples using TLC and 16 using ELISA[28]. It has been reported in the literature that the elevated detection of aflatoxin in samples analyzed by ELISA is due to the high sensitivity and specificity of this technique[28,29]. In addition ELISA has been widely used in aflatoxin analyses as a valuable supplement to chromatographic techniques. Thus the joint use of the two techniques is crucial to optimize the results[25].

Regarding the association between the dry and rainy seasons existing in the city of São Luís, MA, Brazil and the presence of aflatoxins detected by the TLC and ELISA methods, and also with the concentrations of toxins quantified by ELISA, no statistically significant difference could be found between these variables (Table 4).

Based on the information provided by NUGEO – UEMA, São Luís showed a mean temperature of 26.8 ºC and a relative air humidity of 82% during the development of this study. No correlation was found between the production of aflatoxins in the samples, as analyzed by the ELISA method, and the temperature and relative air humidity observed during the dry and rainy seasons in São Luís, MA, Brazil.

However, other factors should be taken into consideration which could permit the production of aflatoxins in the Brazil nuts, such as a low technological level in the production chain, the moisture content of this food, the temperature, the relative air humidity, the climatic season, the amount of fungal spores and the fungal strains[1,30]. In addition the interaction between these factors can provide different results for aflatoxin production, even in identical foods[30], as can be seen in the present study.

Amongst these factors, the temperature and relative air humidity have a great influence on fungal development and the production of aflatoxins[30]. Affirmed that the ideal temperature range would be from 25 ºC to 40 ºC, and relative air humidity above 85%. In the present study the temperatures and humidity observed in São Luís, MA, Brazil were constantly high throughout the whole year, similar to the optimum conditions for fungal contamination and aflatoxin production as reported in the literature, and could explain the presence of contaminated samples during the whole collection period[15].

Similar results can be found in other studies. Attributed contamination by aflatoxins to the tropical climate in Malaysia, whilst suggested that the temperature in the State of Piaui, Brazil (25 ºC to 35 ºC) was responsible for the fungal growth and aflatoxin production, since the relative air humidity was below the value reported in the literature[31]. Regions with tropical and subtropical climates are more susceptible to the production of these toxins[32].

It should be pointed out that the temperature and air humidity, according to the seasonal variations of drought and rain, did not correlate with the aflatoxin concentrations. These results are different from those reported in the literature, which report the probability of fungal proliferation and aflatoxin production increasing during the rainy season[12,33,5,30]. The absence of such correlation between the seasonal variations and the aflatoxin

concentrations probably occurred due to the constancy of the elevated temperature and air humidity values observed during both seasons.

In addition this suggests that other factors could have contributed to the contamination of the samples, such as the low technological level in the Brazil nut productive chain. This factor has constantly resulted in the colonization of the internal tissues of these nuts by aflatoxicogenic fungi during the pre-harvest, harvest and post-harvest steps[7,34]. On account of this the literature has reported the isolation of seventeen fungal species from Brazil nuts, of which *Aspergillus flavus* was the most frequently found, standing out as that responsible for deterioration of the nuts and the production of aflatoxins[7,1]. It is important to note that the toxins produced throughout the production chain remain in the nuts even after the industrial shelling process[15].

The water activity of the nuts is another very important factor[27]. The majority of aflatoxicogenic fungi require a relative air humidity above 85% and a water activity between 0.78 and 0.80 as their ideal growth conditions[13,15]. However, aflatoxins are only produced when the water activity is above 0.85[13]. The elevated water activity of the nuts associated with bad storage conditions, high temperature and high humidity are the main parameters affecting fungal development and the production of these toxins[13]. Due to the considerable occurrence of aflatoxins in the nuts analyzed in the present work, the study of other variables such as the nut microbiota, water activity and storage conditions, amongst others, is recommended.

4 CONCLUSIONS

In general, the presence of aflatoxin was detected in 13.1% of the Brazil nut samples using the TLC method. However, a higher index of samples contaminated by aflatoxin was verified using the ELISA method, and some samples presented levels above those permitted by Brazilian and European legislation. An association was also found between the presence of aflatoxin as verified by ELISA and the sample collection points. These data demonstrate a lack of conformity with food safety and expose nut consumers to serious health risks.

With respect to the air temperature and humidity in the city of São Luís, MA, Brazil, these variables showed characteristics that propitiate fungal development and aflatoxin production. However, no correlation was observed between the climatic seasons (dry and rainy) and aflatoxin production. Considering the results obtained, monitoring of the contamination of Brazil nuts by aflatoxins is required, and the development of strategies

that guarantee the adoption of good handling and commercialization practices of these nuts, assuring quality and safety and hence consumer health.

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A Brazil nut has high nutritional value and is one of the most important extractive species in the Amazon rainforest region, being exported to several countries. However, low technological levels, presents its production chain and inadequate conditions for the manufacture of raw materials, favoring the occurrence of contamination by fungi produced by aflatoxins. This problem is caused by a product marketed mainly in the overseas market due to the strict control of European countries and the United States regarding the levels of toxins present in food. Under these conditions, the present work aimed at study was to detect the presence and levels of aflatoxins in Brazil nuts, since, as aflatoxins are toxic and potentially carcinogenic compounds to humans.

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Table 1. TLC analysis for the presence of aflatoxins in the Brazil nut samples – São Luís, MA, Brazil, 2013-2014

Collection point	**Samples n (%)**	**Contaminated samples n (%)**	**Aflatoxins present (n; %)**
CM[1]	48 (57.1)	7 (8.3)	AFB1[3] (3; 3.6) AFG1[4] (2; 2.4) AFG2[5] (1; 1.2) AFB1+AFG1 (1; 1.2)
RM[2]	36 (42.9)	4 (4.8)	AFB1 (2; 2.4) AFB2[6] (1; 1.2) AFG2 (1; 1.2)
Total	84 (100)	11 (13.1)	11 (13.1)

[1]CM: Central Market; [2]RM: Reviver Market; [3]AFB1: Aflatoxin B1; [4]AFG1: Aflatoxin G1; [5]AFG2: Aflatoxin G2; [6]AFB2: Aflatoxin B2.

Table 2. Incidence of total aflatoxins in Brazil nuts as determined by the ELISA method; São Luís, MA, Brazil, 2013-2014

Collection point	**Samples n (%)**	**Contaminated samples n (%)**	**Aflatoxins (µg/kg)**	**Mean (µg/kg)**
CM[1]	48 (57.3)	25 (29.8)	0.7 – 28.2	6.85
RM[2]	36 (42.7)	10 (11.9)	1.3 – 9.1	4.71
Total	84 (100)	35 (41.7)	0.7 – 28.2	6.24

CM: Central Market; 2RM: Reviver Market.

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Table 3. Association between the presence and the concentration of aflatoxins and the collection points of the Brazil nut samples (Central Market and Reviver Market)

VARIABLES	GENERAL		COLLECTION POINTS				p-value
			Central Market		Reviver Market		
	n	%	N	%	n	%	
TLC result							0.075[a]
Aflatoxin-negative	73	86.9	41	85.42	32	88.89	
Aflatoxin-positive	11	13.1	7	14.58	4	11.11	
ELISA result							**0.025[b]**
Aflatoxin-negative	49	58.33	23	47.92	26	72.22	
Aflatoxin-positive	35	41.67	25	52.08	10	27.78	
Positive ELISA results							0.387[c]
Mean ± standard deviation	6.24 ± 5.29		6.85 ± 6.02		4.71 ± 2.34		

[a]Fisher's exact; [b]Chi-squared; [c]Mann-Whitney

Table 4. Association between the presence and concentration of aflatoxins and the dry and rainy seasons in São Luís, MA, Brazil (2013-2014)

VARIABLES	CLIMATIC SEASONS				p-value
	Dry		Rainy		
	n	%	n	%	
TLC result					0.520[a]
Aflatoxin-Negative	35	83.33	38	90.48	
Aflatoxin-Positive	7	16.67	4	9.52	
ELISA result					0.268[b]
Aflatoxin-Negative	22	52.38	27	64.29	
Aflatoxin-Positiv	20	47.62	15	37.71	
Positive ELISA results					0.309[c]
Mean ± standard deviation	5.09 ± 3.14		7.76 ± 7.09		

[a]Fisher's exact; [b]Chi-squared; [c]Mann-Whitney