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Occurrence and exposure assessment to aflatoxins in peanuts commercialized in the northwest of Parana, Brazil

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ABSTRACT: Peanuts are a nutritious food consumed worldwide. Some species of the genus Aspergillus, producers of aflatoxins, colonize peanuts. Human exposure to aflatoxins occurs by ingesting contaminated foods. The objective of this paper was to assess the occurrence and dietary exposure of the population to aflatoxins in peanuts commercially available in Maringa, Brazil, from May 2013 to April 2017. A total of 104 peanut samples were evaluated by thin-layer chromatography and confirmed by derivatization with trifluoroacetic acid. The contamination rate detected was 24.0% in average concentration of 13.4µg/kg. Twenty positive samples (19.2%) exceeded the maximum level permitted in Brazil for the sum of four aflatoxins. Estimated probable daily intake was 1,28 µg/kg body weight/day, exceeding the Provisional Maximum Tolerable Daily Intake (0.001µg/kg body weight/day). According to the high levels of aflatoxins found in peanuts, there is need for further monitoring the presence of aflatoxins in peanuts in natura to reduce the levels of contamination.

Key words: aflatoxins, Aspergillus flavus, peanuts, risk assessment, public health.

Ocorrência e avaliação da exposição as aflatoxinas em amendoim comercializado na região noroeste do Paraná, Brasil

RESUMO: O amendoim é um alimento nutritivo e muito consumido no mundo todo. Algumas espécies de fungos do gênero Aspergillus, produtores de aflatoxinas, colonizam o amendoim. A exposição humana as aflatoxinas ocorre por meio do consumo de alimentos contaminados. O objetivo deste estudo foi avaliar a ocorrência e exposição da população as aflatoxinas em amendoim comercializado no município de Maringá, Brasil, durante o período de maio de 2013 a abril de 2017. Foram avaliadas 104 amostras de amendoim por análise cromatográfica e confirmadas por derivação com ácido trifluoroacético. A frequência de contaminação foi de 24,0% em concentração média de 13,4µg/kg. Vinte amostras positivas (19,2%) apresentaram-se acima do limite máximo permitido no Brasil para a somatória das quatro aflatoxinas. A ingestão diária provável média encontrada foi de 1,28µg/kg peso corpóreo/dia, superando a ingestão diária máxima tolerável provisória (0,001µg/kg peso corpóreo/dia). De acordo com os elevados teores de aflatoxinas encontrados em amendoim, há necessidade de um maior monitoramento da presença de aflatoxinas em alimentos, tendo em vista a redução nos níveis de contaminação do amendoim in natura. Palavras-chave: aflatoxinas, Aspergillus flavus, amendoim, avaliação de risco, Saúde Pública.

INTRODUCTION

Peanut (*Arachis hypogaea*) is a staple food of many people on earth. It is a low-cost source of fat, proteins, vitamins and minerals in the diet (BARBOUR et al., 2014). Peanut cultures are widely sensible to infection by fungi, mainly species producer of toxins, which mainly occurs during harvest and post-harvest, affecting quality and food safety (SOUZA et al., 2014). Mycotoxins are responsible for major economic losses that occur worldwide, due to problems to human and veterinary population, as damages in agricultural production (CAMILETTI et al., 2017). Aflatoxins of peanuts and derived assumes

outstanding importance in public health, given the high consumption of these products, especially for children (MARTINS et al., 2017).

Occurrence of aflatoxins observed in peanuts in Latin America can be largely explained by the traditional practices of harvesting, drying and storage adopted by producers. In Brazil, climatic conditions behalf the development of toxin-producing fungi, as *Aspergillus* and aflatoxins. Worsening of this situation occurs during the Summer or conditions of high proliferation of insects (CAMILETTI et al., 2017; MARTINS et al., 2017).

Data on the occurrence of aflatoxins in Brazil revealed a high incidence. RODRIGUEZ-

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AMAYA (2001) demonstrated that 27 and 47% of the samples of peanuts from South and Southeast of Brazil were contaminated with aflatoxins, respectively. These samples were at levels above 20μg/kg, maximum limit for the presence of aflatoxins in peanuts in Brazil (ANVISA, 2011). MALLMAN et al. (2005) analyzed 664 samples of peanuts and their derivatives, 31.33% were contaminated with aflatoxins in Rio Grande do Sul. MAGRINE et al. (2010) analyzed samples of peanut and their derivatives commercialized in Paraná State. Of the 100 samples, 50 (50%) had aflatoxin and 13 (13%) with values higher than 20μg/kg.

Human exposure to aflatoxins at level measured in nanograms and micrograms per day occur mostly through the consumption of maize and peanuts, staple foods in tropical countries. The aim was to assess the ocurrence and dietary assessment of the population to aflatoxins in peanuts commercially available in the northwest of Parana State, Brazil, from May 2013 to April 2017.

MATERIALS AND METHODS

Sampling

Sampling was determined according to the annual per capita food acquisition of the Southern Region of Brazil on the Family Budget Survey (IBGE, 2008-2009). A total of 104 samples of three different brands of whole peanuts *in natura* were acquired from three supermarkets located in the city of Maringa, Parana State, Brazil, from May 2013 and April 2017. The three brands chosen for analysis stood out among the most sold in the region and were available to consumers during the period of sampling. A total of 2-3 samples were acquired per month. The selected supermarkets were located in different areas of the city of Maringa and represented the northwest region of Parana State. Weight of each sample was 500g. Samples were stored at -20°C for a maximum of 30 days.

Quality management

Peanuts were analyzed in duplicates. Each analysis of approximately 11 samples was accompanied by one sample spiked with $20\mu g/kg$ of aflatoxins B_1 (AFB₁) and G_1 (AFG₁) and $5\mu g/kg$ of aflatoxins B_2 (AFB₂) and G_2 (AFG₂). Recovery rate between 70 and 140% was used as the analytical criterion, suggested by HORWITZ et al. (1993).

Aflatoxins standards

Aflatoxin standards were acquired from Sigma Chemical Co. (St.Louis, MO, USA). Standards

solutions were made according to the official procedure AOAC method 971.22 (AOAC, 2005). Storage solutions were analyzed and quantified by ultraviolet spectrophotometer (Shimadzu UV-1601P, Tokyo, Japan) at 350nm. Using solutions were realized in benzene-acetonitrile (98:2, v/v), containing 5µg/ml AFB₁ and AFG₁ and, 1.5µg/ml AFB₂ and AFG₂.

Determination of aflatoxins in peanuts for thin-layer chromatography (TLC)

The extration of aflatoxins was performed according to the method described by SOARES & RODRIGUEZ-AMAYA (1989) using methanol, potassium chloride, copper sulfate, celite and chloroform. The chloroform phase was evaporated to dryness in a water bath at 80°C. The residue thus obtained was resuspended in 400µl of benzene-acetonitrile (98:2, v/v) for analysis.

For screening and quantification, the mobile phase consisted of toluene-ethyl acetate-chloroform-formic acid (35:25:25:10, v/v/v/v) for TLC, according to GIMENO (1979). Aflatoxins were visualized under UV light at 366nm and quantified by fluorescence comparison by applying known volumes of samples and aflatoxin standards onto plates. All calculations were carried out according to the AOAC (2005). Identity of the aflatoxins was confirmed by chemical derivatization reactions with trifluoroacetic acid, as described by PRZYBYLSKI (1975).

PDI_M was calculated by multiplying the average concentration of the sum of the four aflatoxins (B₁+B₂+G₁+G₂) reported in the samples analyzed in this study by the average consumption of peanut-derived products in Paraná State (6.7g/person/day), based on the data reported by MAGRINE et al. (2011), divided by the average body weight of an adult (70kg).

RESULTS AND DISCUSSION

The limit of quantification of the method was 2µg/kg for AFB₁, AFB₂, AFG₁ and AFG₂ for TLC. The average recovery obtained by the method used was showed in table 1. According to the European Community the acceptable levels for recovery would be between 70-140%, so the average recoveries remain within the acceptable range. Results reported in this research are similar to others studies (MAIA & SIQUEIRA, 2002; SEKIYAMA et al., 2005; AMARAL et al., 2006). ROCHA et. al (2008) achieved a mean recovery for TLC method of 93 % for the sum of the four aflatoxins in peanut.

Table 1 - Recovery values (%) for aflatoxins in spiked peanuts (n = 10).

			Mean				
Aflatoxins	μg/kg	1-2	3-4	5-6	7-8	9-10	Recovery (%)
		$M^a \pm SD^b$	M ±SD	M ±SD	$M \pm SD$	$M \pm SD$	
AFB_1	20	90 ±14	140 ± 85	107 ±38	107 ±38	107 ± 38	106.0
AFB_2	5	140 ±38	90 ±14	140 ± 14	80 ±0	120 ± 57	120.0
AFG ₁	20	80 ±0	90 ±14	120 ±57	107 ±38	107 ± 38	106.7
AFG ₂	5	90 ±14	80 ±0	140 ±38	80 ±0	90 ±14	90.0

^aMean. ^bStandard deviation.

Results reported for the occurrence of aflatoxins in peanuts commercially available in northwest of Parana are presented in table 2. Data revealed a high frequency of aflatoxins in the samples. Of the 104 samples analyzed, 24.0% were contaminated by aflatoxins, and 20 samples (AFB₁+AFB₂+AFG₁+AFG₂) above 20µg/kg, maximum limit for the presence of aflatoxins in peanuts in Brazil (ANVISA, 2011). In the present study, three of the samples were reported to be contaminated with detectable levels of AFG₁ or AFG₂.

Levels of aflatoxins ($B_1+B_2+G_1+G_2$) in the positive samples analyzed ranged from 8-832µg/kg. A comparison of the results reported in this study with other studies realized in Brazil demonstrated that the occurrence of aflatoxins in the analyzed samples continue to be a risk for health of Brazilian population (Table 3).

However, levels of aflatoxins reported in this study were higher than those reported in other countries. IQBAL et al. (2013) evaluated 198 samples of peanuts and derivatives commercialized in Punjab, Pakistan and related that 93 (47%) samples were positive for aflatoxins, of which 16% (32/98) exceeded 4µg/kg, maximum limit set by the European Union (EC, 2006). BANKOLE et al. (2005) reported that 64.2% of dry-roasted ground peanuts commercialized

in Nigeria were contaminated with AFB₁, average concentration of 25.5µg/kg.

The PDI $_{\rm M}$ reported in this study was 1.28µg/kg body weight (b.w.)/day. This intake was higher than that estimated by other authors. JAGER et al. (2013) related a PDI $_{\rm M}$ of 0.0137µg/kg b.w./day for the sum of the four aflatoxins. MAGRINE et. al. (2011) determined PDI $_{\rm M}$ for aflatoxin B $_{\rm I}$ and reported a value of 0.010µg/kg b.w./day for high consumption of peanuts.

Despite the lack of consensus on the Acceptable Daily Intake (ADI) for aflatoxins, KUIPER-GOODMAN (1998) proposed a Provisional Maximum Tolerable Daily Intake (PMTDI) of $0.001\mu g/kg~b.w./day$ of AFB $_{\rm l}$ for adults and children without a diagnosis of the Hepatitis B virus and of $0.0004\mu g/kg~b.w./day$ of AFB $_{\rm l}$ for carriers of the Hepatitis B virus. The PDI $_{\rm M}$ for AFB $_{\rm l}$ (3.99 $\mu g/kg~b.w./day) obtained in the present study exceeded the PMTDI values, demonstrating the importance of new studies to assess the risks to human health through exposure to aflatoxins in Brazil.$

CONCLUSION

According to the high levels of aflatoxin reported in the analyzed samples, there is a need of

Table 2 - Aflatoxin levels in samples of peanuts commercialized in Maringa, Brazil (May 2013 to April 2017).

Aflatoxins	N° positive samples	Occurrence (%)	Total samples		Positive samples	
	- Posterior surrepress		Mean (μg/kg)	Range (µg/kg)	Mean (μg/kg)	Range (µg/kg)
\mathbf{B}_1	25	24.0	41.7	2-720	163.4	8-720
B_2	21	20.2	7.0	2-112	27.6	2.4-112
G_1	3	2.9	2.8	2-53.3	28.4	16-53.3
G_2	3	2.9	2.1	2-4.8	4.5	4-4.8
$B_1 + B_2 + G_1 + G_2$	25	24.0	13.4	2-832	79.7	8-832

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Table 3 - Occurrence and levels of aflatoxins in peanuts in the Brazil.

Year	State	Occurrence (%)	$\begin{array}{c} B_1 + B_2 + G_1 + G_2 \\ \text{Medium (}\mu\text{g/kg)} \end{array}$	$B_1+B_2+G_1+G_2$ Higher (µg/kg)	References
2017	Sao Paulo	12/119	ND^a	100.9	MARTINS et al., 2017
2012	Sao Paulo	8/23 (35)	8.5 ± 12.7	36.7	JAGER et al., 2013
2011	Federal District	29/359 (8)	14.2 ± 85.1	1496	ANDRADE et al., 2013
2011 2010	Sao Paulo	ND^a	ND^a	260 52	INAMURA et al., 2014
2010	Rio Grande do Sul	7/12 (58)	16.2	126.2	OLIVEIRA & KOLLER, 2011
2009	Sao Paulo	12/75 (16)	ND^a	ND^a	SILVA et al., 2013
2007	Parana	5/10 (50)	3.4	21.9	MAGRINE et al., 2011
2007	Sao Paulo	19/48 (40)	12.8 ± 2.42	ND^a	OLIVEIRA et al., 2009
2007	Minas Gerais	8/21 (38)	64.0	ND^a	ROCHA et al., 2008
2002	Parana	1/6 (17)	ND^a	3422	EIZENDEHER et al., 2005
2001	Sao Paulo	56/87 (64)	306	1659	SHUNDO et al., 2003
2000	Federal District	26/53 (49)	107	1421	CALDAS et al., 2002

aNot determined.

monitoring the presence of aflatoxins in peanuts *in natura* in order to reduce the levels of contamination in food commercialized in northwest of Parana, Brazil. Risk of hepatocellular carcinoma present in the diet of the studied population has shown to be greater than thated report in other countries and may represent a health problem for this population. Other researches need to becarried out to assess the risk of human exposure to aflatoxins, with a view to PDI_M reported.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish results.

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