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IDENTIFICATION OF AFLATOXINS IN PEANUT BUTTER CANDY MARKETED IN CAMPOS GERAIS, MG, BRAZIL

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ABSTRACT

Aflatoxins are the mycotoxins that can cause the more significant harm to humans and animals, due to its high toxicity, wide occurrence, and carcinogenic, mutagenic, teratogenic and immunosuppressive properties. In this perspective, the present study aimed to verify the presence of aflatoxins in peanut butter candy samples "paçocas" marketed in the city of Campos Gerais - MG. The technique used for the separation and identification of substances was the thin layer chromatography prior to liquid-liquid extraction. The results showed that the peanut butter candy samples used in this study were no contamined by aflatoxins. It is important to perform control analysis in the products more susceptible to be contamined by aflatoxins as they may cause serious health problems to the human and animals health.

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INTRODUCTION

A contaminant is any substance in a given food that is not considered a natural constituent. These substances may become part of the food during its production, processing and storage, leading to food characteristics alterations (Midio; Martins, 2000; rocha *et al.,* 2008). Among the diverse factors that compromise food quality, the contamination by mycotoxins, especially aflatoxins deserve attention.

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Aflatoxins are toxic metabolites produced by fungi such as Aspergillus flavus and Aspergillus parasiticus, and represent a severe public health problem in different world regions (Dilkin, 2002; Kwiatkowski; De Faria Alves, 2007). This mycotoxins group exhibited significant impact in the development of research in the mycotoxicology field, due to its occurrence and cause of more than 100,000 dead birds fed contaminated feed in England, around the 60's decade (Sabino *et al.,* 1997). Aflatoxins are the mycotoxins that can cause the more significant harm to humans and animals, due to its high toxicity, wide occurrence, and carcinogenic, mutagenic, teratogenic and immunosuppressive properties (De Sylos; Rodriguez-Amaya; Carvalho, 1996; Nordin; Luchese, 1998). The effects caused by aflatoxins may be influenced by the

nutritional status, gender, age, exposure to chemical agents, toxin dosage and exposure time, species, frequency and diet composition (Amado, 2002; Freitas; Ferreira; Moreira, 2015). The biological effects triggered by these mycotoxins vary from carbohydrate and lipid metabolism alterations to impairment of mitochondria function, steroids synthesis and protein and nucleic acids biosynthesis. The mycotoxins damage to DNA has been the most harmful effect on living organisms (Sabino *et al.,* 1997). Thus, it is relevant to study the aflatoxins presence in food such as grains (peanut and derivatives) that constitute the ideal substrate for fungi growth (De Oliveira; De Castilho Koller, 2011; Santos; Lopes; Kosseki, 2001; Silva *et al.,* 2013). The present study aimed to verify the aflatoxins presence in peanut butter candy samples in the city of Campos Gerais, Minas Gerais, Brazil.

MATERIALS AND METHODS

The present study was performed according to the AOAC guidelines, Cap. 26, 13ª ed. 1980 (Jarvis, 2003).

Samples: Samples from different peanut butter candy "paçoca" brands were randomly acquired in 22g packaging in Campos Gerais, MG, from September to December 2013. Visually, the samples collected did not present contamination sings and were stored at room temperature.

Reagents: Methanol (Química Fina); Potassium chloride solution 4% (Química Contemporânea Ltda); Chloroform (Dinâmica); Copper sulphate solution 10% (Dinâmica); Toluene (Merck); Ethyl acetate (Cromoline); Formic acid (Pro Analyai) and Aflatoxin standards (AFs standards).

Equipments: Chromatographic plates (Agela Technologies); Ultraviolet lamp (Boitton Instruments); Chromatographic Cube and capillaries.

Samples preparation

Initially, a pilot study with 50 samples (25 of each brand, named brand A and brand B) was performed. For each analysis, 20 peanut butter candies were used, in quadruplicates, totalizing 80 samples. Three analyzes of each brand were performed, totalizing 480 samples. The peanut butter candy samples were crushed and processed into a homogeneous paste and then were divided into parts where subsamples were taken for routine analysis.

Extract purification: 150 mL of copper sulfate solution 10% and 5g of celite was added to the sample. The mixture was stirred with a glass stick and filtered through filter paper.

Liquid-liquid extraction: 150 mL of the purified extract was transferred to a separation funnel. 150 mL of distilled water was added, and two extractions (3 min each) were performed following chloroform addition. Ten mL of the extract was transferred to a 50 mL Erlenmeyer and evaporated to dryness in a water bath at 80 ^{o}C. The evaporated material was covered in aluminum paper and stored in the fridge.

Thin layer chromatography

The evaporated material was eluted in 500 µL of chloroform and 10µL of each sample was applied in 0.25mm thick Silica gel plaques. The samples were evaluated in UV-364nm.

RESULTS INTERPRETATION

The results were compared to the Tolerable Maximum Limit (TML) taken for aflatoxins in Brazil, according to the RDC nº 274/02 resolution – ANVISA, Ministry of health (Resol. Mercosul nº 56/94 modified Mercosul/GMC nº 25/02) and ordinance MAARA nº 183/964. The TML is 20µg/Kg for the aflatoxins B_1, B_2, G_1 and G_2 sum (peanuts, corn and their products) (Mie Kawashima; Valente Soares, 2006).

Table 1. Retention factors comparison among standard plate and samples A and B – replicate 1

Standard plaque	Brand A	Brand B
0.27	0.69	0.60
0.31	0.65	0.65
0.31	0.65	0.64
0.31	0.70	0.65

Table 2. Retention factors comparison among standard plate and samples A and B – replicate 2

Standard plaque	Brand A	Brand B
0.27	0.70	0.80
0.31	0.70	0.79
0.31	0.65	0.80
0.31	0.70	0.80

Table 3. Retention factors comparison among standard plate and samples A and B – replicate 3

Standard plaque	Brand A	Brand B
0.27	0.80	0.80
0.31	0.79	0.79
0.31	0.80	0.80
0.31	0.80	0.80

RESULTS AND DISCUSSION

Thin-layer chromatography uses a solvent system which is used in a combined manner, where the solvent polarity is critical. For the aflatoxins analysis of peanut butter candies, the solvent system was applied, which is already well established in the literature for sample elution. The solvent system was composed by toluene: ethyl acetate: formic acid (60:30:10), which efficiently separates the compounds following a visual comparison detection of the spots fluorescence intensity and the retention factors (Rf) on a UV-365nm light sample with the standard reference plate. The distance marked previously for the solvent run was 10 cm. This distance measures the relationship between the distance traveled by the substance and the distance traveled by the solvent and is called the Retention Factor (Rf) that is determined using the expression:

$$Rf = \frac{\text{Distance traveled by the substance.}}{\text{Distance traveled by the solvent.}}$$

After the chromatography run, the following results were obtained in the replicates

The Tolerable Maximum Limit (TML) for aflatoxins in Brazil is 20µg/Kg for aflatoxins B_1, B_2, G_1 and G_2 sum (peanuts, corn, and derivatives) according to the RDC nº 274/02 – ANVISA resolution (Ministry of Health (Resol. Mercosul nº 56/94 modified Mercosul/GMC nº 25/02)) (De Mello; Para, 2005; Hinton *et al.,* 2003).

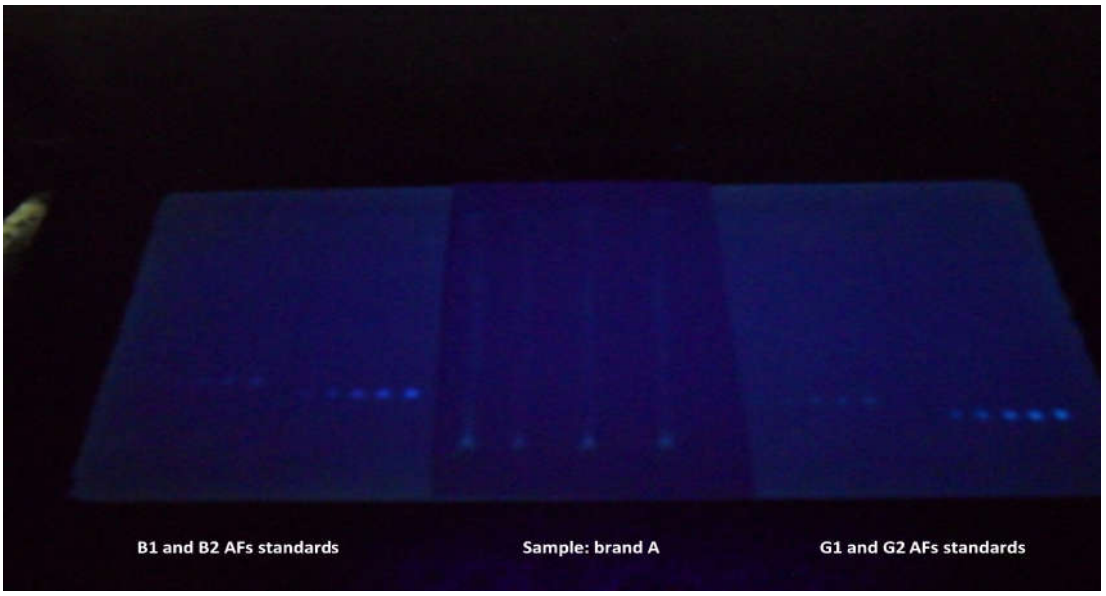

Figure 1. TLC analysis of the peanut butter candy brand A under Ultra-violet light. From left to right the application was: Aflatoxin standard B1 and B2; Sample; Aflatoxin standard G1 and G2

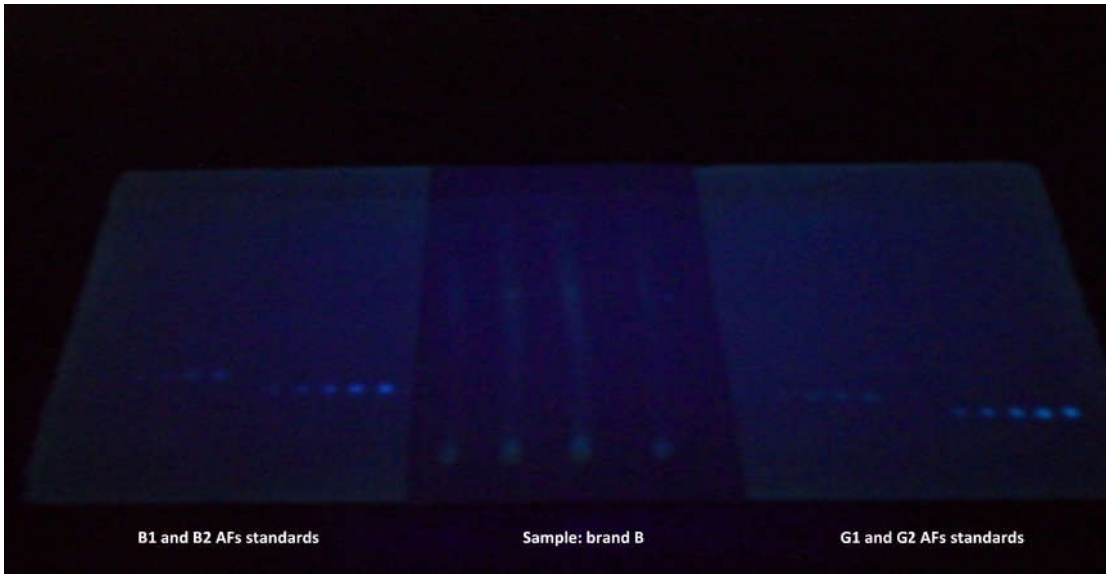

Figure 2. TLC analysis of the peanut butter candy brand B under Ultra-violet light. From left to right the application was: Aflatoxin standard B1 and B2; Sample; Aflatoxin standard G1 and G2

In the analyzes performed, if the samples had the same Rf and staining as the standards, it would indicate the aflatoxins presence. According to the results presented, the peanut butter candy samples were negative for aflatoxin contamination (Figure 1 and 2). A similar result was reported by Ferreira in 2011, in the determination of AFs in peanut butter candies marketed in the city of Lavras-MG. The literature reports peanut contamination with a relatively high incidence. Peanuts naturally benefit fungi growth, especially when the peanut is beaten, bagged and stored in high humidity environment. This observation was demonstrated by Oliveira and Koller (2011), which shows the presence of Aspergillus spp and aflatoxin in the grains, but not in peanut butter candy (Amado, 2002; De Oliveira; De Castilho Koller, 2011). The thin layer chromatography combined with liquid-liquid extraction has been satisfactory to determine aflatoxins concentration in peanut grains and peanut butter candies. The TLC is the gold standard in many Brazilian laboratories, as it is a safe method and do not require expensive equipment (Soares; Rodriguez-Amaya, 1989).

This technique also allows adequate compounds separation, which makes this method useful in the aflatoxins characterization (Baggio, 2006). This method was also used by Amaral and Junior, 2006, for the aflatoxins determination in corn and its derivatives. Rocha *et al.* (2008), also used chromatography as a separation method to determine aflatoxins incidence in peanut grains and peanut butter candy marketed in the city of Alfenas-MG. In this work, eight positive samples (38%) were obtained in 21 analyzed peanut samples and two positive samples (13%) in 15 peanut butter candy samples (Rocha *et al.,* 2008). The contaminants fungi species identification is also an important step to determine mycotoxins presence in substrates and may be used as a preventive method.

Conclusion

The present work suggests that peanut butter candy samples marketed in Campos Gerais, MG, Brazil, are negative regarding aflatoxins contamination. According to the

calculated RF and fluorescence intensity, the samples analyzed do not present contamination signs. To confirm the aflatoxins presence, samples spots should have the same intensity as the standard plates. This result association confirms the contamination absence in the analyzed lots. The negative results confirmation is an essential indication of quality in storage, grain processing as well as the care in the production of the consumed product in Campos Gerais, MG, Brazil. The result is also beneficial for the population that is consuming the traditional product.

REFERÊNCIAS

Amado, M. A. 2002. Métodos imunológicos na detecção e determinação de aflatoxinas em alimentos: vantagens e inconvenientes. Millenium,

Baggio, E. C. R. 2006. Determinação de aflatoxina M1 em leite pasteurizado pelos métodos de CCD e CLAE utilizando coluna de imunoafinidade.

De Mello, D. R. and Para, R. T. D. C. M. 2005.Resolução De Diretoria Colegiada-RDC Nº. 275, DE 22 DE Setembro DE 2005.

De Oliveira, L. D. S. F. and De Castilho Koller, F. F. 2011. Ocorrência DE Aspergillus spp. E Aflatoxinas EM Amostras De Amendoim In Natura E Paçocas. Revista de Ciências Ambientais, v. 5, n. 1, p. 57-68.

De Sylos, C. M., Rodriguez-Amaya, D. B. and Carvalho, P. R. 1996. Occurrence of aflatoxin M1 in milk and dairy products commercialized in Campinas, Brazil. *Food Additives and Contaminants*, v. 13, n. 2, p. 169-172.

Dilkin, P. Micotoxicose suína: aspectos preventivos, clínicos e patológicos. Biológico, v. 64, n. 2, p. 187-191, 2002.

Freitas, D. F., Ferreira, M. C. and Moreira, E. A. 2015. Identificação de aflatoxinas em paçocas de amendoim comercializadas na cidade de Lavras-MG. Revista de Ciências Farmacêuticas Básica e Aplicada, v. 35, n. 4.

Hinton, D. M., *et al.,* 2003. Immunotoxicity of aflatoxin B1 in rats: effects on lymphocytes and the inflammatory response in a chronic intermittent dosing study. Toxicological Sciences, v. 73, n. 2, p. 362-377.

Jarvis, B. B. 2003. Analysis for mycotoxins: the chemist's perspective. Archives of Environmental Health: An *International Journal,* v. 58, n. 8, p. 479-483.

Kwiatkowski, A. and De Faria Alves, A. P. 2007. Importância da detecção e do controle de aflatoxinas em alimentos. SaBios-Revista de Saúde e Biologia, v. 2, n. 2.

Midio, A. and Martins, D. 2000. Agentes tóxicos naturalmente presentes em alimentos. AF Midio & DI Martins, Toxicologia de alimentos (cap. 2; pp. 31-47). São Paulo: Livraria Varela.[Links],

Mie Kawashima, L. and Valente Soares, L. M. 2006. Incidência de fumonisina B1, aflatoxinas B1, B2, G1 e G2, ocratoxina A e zearalenona em produtos de milho. Ciência e Tecnologia de Alimentos, v. 26, n. 3.

Nordin, N. and Luchese, R. 1998. Detecção de Aflatoxinas e Zearalenona em milho (Zea mays) destinado à alimentação animal. Bol. Soc. *Brás. Ciênc. Tecnol*., Aliment, v. 32, p. 35-39.

Rocha, M. D. D., *et al.,* 2008. Incidência de aflatoxinas em amostras de amendoim e paçoca comercializadas na cidade de Alfenas-MG, *Brasil. Rev Bras Toxicol.*, v. 21, n. 1, p. 15-19.

Sabino, M., *et al.,* 1997. Evaluation of the efficiency of two immunoassay kits for detection of aflatoxin B1 in corn, fish feed, peanuts and its products. Ciencia e Tecnologia de Alimentos (Brazil).

Santos, C. D., Lopes, M. D. R. V. and Kosseki, S. Y. 2001. Ocorrencia de aflatoxinas em amendoim e produtos de amendoim comercializados na regiao de Sao Jose de Rio Preto/SP. Revista do Instituto Adolfo Lutz, v. 60, n. 2, p. 153-157.

Silva, R., *et al.,* 2013. Detecção E Quantificação DE Aflatoxinas EM Amostras De Grãos De Amendoim E Derivados Comercializados Na Região De Marília–SP, 2002-2009 Detection and quantifi cation of afl atoxins in samples of peanut grains and derivatives traded in the. Alimentos e Nutrição Araraquara, v. 24, n. 1, p. 64.

Soares, L. and Rodriguez-Amaya, D. 1989. Survey of aflatoxins, ochratoxin A, zearalenone, and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method. *Journal-Association of Official Analytical Chemists*, v. 72, n. 1, p. 22-26.

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