

## The Occurrence of Aflatoxin B<sub>1</sub> Contamination in Peanuts and Peanut Products Marketed in Southern Brazil

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### ABSTRACT

*This study investigated the occurrence of aflatoxin B<sub>1</sub> in peanuts and peanut products marketed in the State of Rio Grande do Sul, Brazil. One hundred one samples of peanuts and peanut products were collected and analyzed by thin-layer chromatography with a charge-coupled device system. Aflatoxin B<sub>1</sub> was present in 14% of the samples analyzed, in concentrations ranging from 24.0 to 87.5 µg/kg in the peanut samples and from 22.0 to 84.6 µg/kg in the peanut-product samples. These values exceeded the Brazilian regulatory limit (20.0 µg/Kg for aflatoxins B<sub>1</sub>+G<sub>1</sub>+B<sub>2</sub>+G<sub>2</sub>). These results suggest that although aflatoxin contamination in peanuts marketed in southern Brazil is lower than in other Brazilian regions, it is still a serious problem for human health and the economy.*

**Key words:** peanuts, peanut products, aflatoxin B<sub>1</sub>, Brazil

### INTRODUCTION

Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are secondary metabolites produced by toxigenic strains of *Aspergillus flavus* Link. ex Fries, *A. parasiticus* Speare and *A. nomius* Kurtzman, B.W. Horn and Hesselt. These mycotoxins have been found in different foods, such as corn, peanuts, rice and walnuts (Sulaiman et al. 2007). Peanuts, in particular, are known to be a major substrate for these fungal species (Juan et al. 2008).

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most toxic mycotoxin for mammals and exhibits hepatotoxic, teratogenic and mutagenic properties (Nakai et al. 2008). Together, aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are classified as a class 1 carcinogen for humans by the International Agency for Research on Cancer (IARC 2002).

Commodities, such as peanuts, may be contaminated in the field or after harvest during storage, processing or transport, resulting in not only a public health hazard, but also a financial loss (Craufurd et al. 2006; Hussein and Brasel 2001; Sabino et al. 1999).

In Brazil, peanut production is concentrated in the southeast region. The State of São Paulo is the greatest peanut producer, with a peanut output estimated to be 80.9 thousand tons for the 2008/2009 harvest, representing 71% of the Brazilian national peanut production (CONAB, 2010). The quality of peanuts and their derivatives consumed in southeastern Brazil is frequently monitored (Nakai et al. 2008; Rodriguez-Amaya and Sabino 2002; Sabino et al. 1999; Freitas and Brigido 1998). However, these results cannot be

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generalized to other States in Brazil where climate conditions for peanut production are considerably different.

Rio Grande do Sul is the southernmost state of Brazil and contributes approximately 3.7% of the national peanut production (CONAB 2010). In this region, peanuts are cultivated on a small scale by rural producers. Raw peanuts and peanut-containing foods, such as candies, are marketed at local fairs or sold to local industries. However, most peanuts and peanut products marketed in the state come from the southeast region of the country.

The Brazilian regulatory limit for peanuts for the total concentration of aflatoxins B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub> and G<sub>2</sub> combined is 20.0 µg/kg (ANVISA 2011), in agreement with the regulations for aflatoxins in MERCOSUR (Southern Common Market), a trading bloc consisting of Argentina, Brazil, Paraguay and Uruguay (MERCOSUL/ GMC, 2002). However, in practice and as a precaution, a lower value should be implemented because of the high variability of aflatoxin occurrence in different peanut products and the importance of international trade (Fonseca 2002).

In general, aflatoxin regulations vary between different countries, with limits ranging from 0 to 35.0 µg/kg. In the European Union, for example, the maximum levels allowed in food for AFB<sub>1</sub> and for the sum of aflatoxins are 2.0 µg/kg and 4.0 µg/kg, respectively (Koe 1999).

It is highly unlikely that commodities will contain aflatoxins B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> and not AFB<sub>1</sub> (Yabe and Nakajima, 2004), and the concentration of the sum of aflatoxins B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> is generally less than the concentration of AFB<sub>1</sub> alone. For this reason, analysis of one target component (AFB<sub>1</sub>) appears to be sufficient, efficient, and more practical (FAO 2004).

Although the extent of aflatoxin contamination and the levels of aflatoxin detected have been generally low, the presence of aflatoxins in peanuts and peanut products continues to be an alarming problem in Brazil (Rodriguez-Amaya and Sabino 2002). In part, the problem is due to climatic conditions, which are conducive to fungal development, and to a deficiency of good agricultural practices, but the principal cause of the problem is the inefficiency of the government agencies responsible for aflatoxin monitoring.

Because peanuts are an important food commodity and AFB<sub>1</sub> is toxic for humans and animals, the present investigation studied the occurrence of this

mycotoxin in peanuts and peanut products marketed in the State of Rio Grande do Sul, Brazil, to evaluate the potential risk to consumers.

## MATERIALS AND METHODS

### Samples

A total of 101 peanut containing samples from products ready for consumption were analyzed for AFB<sub>1</sub>. Fifty-eight peanut samples and 43 peanut product samples (e.g., peanut candies) from several brands were collected at random from supermarkets and rural fairs in five cities of the State of Rio Grande do Sul, Brazil: Porto Alegre, Santa Cruz do Sul, Santa Maria, São Leopoldo and Ijuí.

The sampling period was from May through August, 2009 and 2010. The supply and consumption of peanuts increases considerably from May through August because of Brazilian festivals (Fechino and Netto 2004).

### Determination of aflatoxin B<sub>1</sub>

Samples weighing approximately 1 kg were ground and homogenized, and 50 g sub-samples were removed for analysis in duplicate.

AFB<sub>1</sub> was determined according to the method described by Soares and Rodriguez-Amaya (1989). Briefly, 50 g of each sample was extracted with 270 mL methanol and 30 mL 4% potassium chloride and blended at high speed for 5 minutes. The samples were then filtered, 150 mL of the filtrate was collected, and 150 mL of 10% copper sulfate and 50 mL of diatomaceous earth were added, followed by moderate stirring and filtration. The filtrate was again collected (to 150 mL) and transferred to a separation funnel, and AFB<sub>1</sub> was extracted twice with 10 mL chloroform. Chloroform extracts were collected and subjected to solvent evaporation with a 60°C water bath.

The extracts were resuspended in 100 µL chloroform and separated by thin-layer chromatography (TLC). Different concentrations of extracts and an AFB<sub>1</sub> standard were applied on pre-coated silica gel plates (Merck, Darmstadt, GFR). The plates were developed in a saturated chamber with chloroform:acetone (99:1, v/v). Aflatoxin spots were observed under long-wavelength ultraviolet light ( $\lambda = 366$  nm) and identified by comparison with an AFB<sub>1</sub> standard. The concentration and purity of the aflatoxin

standard were determined according to the method of the Association of Official Analytical Chemists (Scott 1990). Confirmatory tests for AFB<sub>1</sub> were carried out using trifluoroacetic acid (Przybylski 1975).

Quantitative analyses were performed as described by Hoeltz et al. (2010). The quantification of fluorescence intensities from the UV lamp was performed with a charge-coupled device (CCD) camera (Sony, Tokyo, Japan). The TLC plate was positioned in the system, and a CCD camera was aligned to obtain the optimal pixel resolution for the resulting CCD images. Five images were taken of each experiment and were analyzed using ImageJ 1.40 software (<http://rsb.info.nih.gov/ij/>).

#### Validation of the analytical method

The validation of the analytical method used in this study was based on the following parameters: linearity, percent recovery, limits of detection (LOD) and limits of quantification (LOQ).

Linearity was determined with an analysis of five-point calibration curves using the intensity of aflatoxin fluorescence versus aflatoxin concentration. Recovery and precision were determined by analyzing 3 peanut and 3 peanut-products samples spiked with 16.0, 20.0 and 32.0 µg/kg of AFB<sub>1</sub>. The LOD was derived from the AFB<sub>1</sub> fluorescence with the lowest detectable signal on the TLC plate with decreasing concentrations of AFB<sub>1</sub> standard solution (1.0, 0.8, 0.6, 0.4 and 0.2 ng per spot).

Because the limit for AFB<sub>1</sub> in food is 2.0 µg/kg in the European Union, the LOQ was obtained by

spiking samples with decreasing concentrations of AFB<sub>1</sub> standard solution (2.4, 2.0, 1.6, 1.2 and 0.8 µg kg<sup>-1</sup>). The LOQ value was considered the smallest amount of AFB<sub>1</sub> in samples that can be quantitatively determined with accuracy and precision.

## RESULTS AND DISCUSSION

The method used for AFB<sub>1</sub> determination was efficient. AFB<sub>1</sub> recovery rates, obtained by spiking peanut samples with 16.0, 20.0 and 32.0 µg/kg AFB<sub>1</sub> in duplicate, were 94%, 97% and 102%, respectively, with relative standard deviations (RSD) of 4.24, 4.03 and 5.65%, respectively. AFB<sub>1</sub> recovery rates obtained by spiking peanut product samples with 16.0, 20.0 and 32.0 µg/kg in duplicate were 87.5%, 84.7% and 97.5%, respectively, and the relative standard deviation (RSD) for repeatability was 10.2, 7.0 and 7.9%, respectively. The limit of detection was 0.4 ng per spot, and the limit of quantification was determined to be 1.2 µg/kg. Linearity was determined by analyzing ten AFB<sub>1</sub> calibration standards with a concentration range from 0.8 ng to 4.8 ng. The correlation coefficient for the linearity analysis was 0.998.

AFB<sub>1</sub> was not detected in 86% of the 101 samples analyzed, whereas 14% showed AFB<sub>1</sub> levels > 20.0 µg/kg (Table 1). These high AFB<sub>1</sub> levels are in violation of current Brazilian regulations (ANVISA, 2011).

**Table 1** - Levels of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) found in peanut and peanut-products samples marketed in the State of Rio Grande do Sul, Brazil.

Peanut samples	AFB <sub>1</sub> (µg/kg)	Peanut-products samples	AFB <sub>1</sub> (µg/kg)
1	32.0	6	22.0
2	24.0	7	30.5
3	87.5	8	31.8
4	38.0	9	69.8
5	45.0	10	72.5
		11	71.3
		12	42.8
		13	23.2
		14	84.6
Mean 8.6% (5/58)	45.3	20.9% (9/43)	49.8

Of the peanut samples analyzed, only 5 were contaminated, with AFB<sub>1</sub> levels of 24.0 to 87.5 µg/kg. For the peanut products analyzed, 9

samples were found to contain AFB<sub>1</sub> contamination, at levels of 22.0 to 84.6 µg/kg. Even if the number of contaminated samples is

low, the AFB1 levels detected in this study are a risk for consumer health and for the Brazilian economy.

Studies conducted in Brazil, especially in the southeast region, analyzing the occurrence of aflatoxins in peanuts and their products typically report higher AFB1 levels than those observed in the present study. For example, Nakai et al. (2008) studied the occurrence of aflatoxins in stored peanut samples from the State of São Paulo, Brazil, and found mean levels of AFB1 from 7.0 to 116.0 µg/kg.

In a review of mycotoxin research in Brazil from 1991 through 2000, Rodriguez-Amaya and Sabino (2002) reported aflatoxin contamination in peanuts and peanut products from 1.0 to 12.9 µg/kg Sabino et al. (1999), researching the occurrence of aflatoxins in peanuts and peanut products consumed in the State of São Paulo, Brazil, concluded that aflatoxin contamination in peanuts is decreasing but remains a problem because the maximum level of contamination detected was 536.0 µg/kg.

However, few studies of aflatoxin contamination have been conducted in the southernmost region of Brazil. Mallmann et al. (2003) analyzed 664 samples of peanuts and peanut products available in the State of Rio Grande do Sul over a two-year period (from March 2000 through April 2002) and found aflatoxins in 31.3% of the samples tested, with mean contamination levels of 92.1 µg/kg and a maximum level of 5476.0 µg/kg.

Worldwide, levels of aflatoxin contamination are variable but can sometimes exceed the contamination levels found in this study. Craufurd et al. (2006), in a study of aflatoxin contamination in peanuts from Niger, found values ranging from 34.0 to 208.0 µg/kg. Wang and Liu (2007) analyzed aflatoxin contamination in different types of foods from China and found the highest level of contamination (28.39 µg/kg) in peanuts. Finally, Sulaiman et al. (2007) studied the occurrence of aflatoxins in raw, shelled peanut samples from three districts in Perak, Malaysia and found a range of AFB1 contamination from 0.85 to 547.51 µg/kg.

In the present study, peanut samples were found to contain lower levels of AFB1 contamination than peanut products. This finding is important because peanut products are more widely consumed than peanuts, and it suggests that poorer-quality peanuts are used for processing. Similar data have been reported in previous studies (Sabino et al. 1999;

Freitas and Brigido 1998) and also suggest that the peanuts used for processing are of lower quality. Moreover, the data presented here reaffirm the inefficiency of government agencies that are responsible for monitoring these products and removing contaminated products from trade.

## CONCLUSIONS

The present study detected high levels of AFB1 contamination in peanuts and peanut products marketed in the south of Brazil. The maximum AFB1 level detected was 87.5 µg/kg, and all contaminated samples are in violation of current Brazilian regulations and exceed the tolerance level of 20.0 µg/kg. The toxic effects of aflatoxins represent a severe health risk for consumers and are also a risk factor for the economy because contaminated products do not satisfy export requirements. Further studies should be conducted to establish the occurrence and levels of AFB1 contamination in peanuts and peanut products in the south of Brazil and to examine the agricultural practices applied by farmers to minimize field, harvest and post-harvest contamination.

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