

Research Note

Natural Occurrence of Aflatoxins in Peanuts and Peanut Butter from Bulawayo, Zimbabwe

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ABSTRACT

Mycotoxins are toxic secondary metabolites produced by filamentous fungi that may contaminate food and pose a health risk, especially in developing countries, where there is a lack of food security and quality is subsumed by food insufficiency. Aflatoxins are the most toxic known mycotoxins and are a significant risk factor for liver and kidney cancer, teratogenicity, undernutrition, and micronutrient malabsorption in both humans and animals. The main aim of the study was to determine the extent of fungal and aflatoxin contamination in peanuts and peanut butter being sold in both the formal and informal markets in Bulawayo, Zimbabwe. Eighteen peanut samples and 11 peanut butter samples were purchased from retail shops and the informal market. Fungal contamination was determined using standard mycology culture methods, while aflatoxin contamination was determined using high-performance liquid chromatography–fluorescence detection. Four of the six peanut samples tested for fungal contamination were infected with *Aspergillus flavus/parasiticus*, ranging from 3 to 20% of the kernels examined, while 27% (3 of 11) of the peanut butter samples were infected with *A. flavus/parasiticus*. Ninety-one percent (10 of 11) of the peanut butter samples were contaminated with aflatoxins (mean, 75.66 ng/g, and range, 6.1 to 247 ng/g), and aflatoxin B₁ was the most prevalent (mean, 51.0 ng/g, and range, 3.7 to 191 ng/g). Three of the 18 peanut samples were contaminated with aflatoxins (range, 6.6 to 622 ng/g). The commercial peanut butter samples had very high aflatoxin levels, and manufacturers should be sensitized to the detrimental effects of aflatoxins and measures to reduce contamination.

Aflatoxins occur worldwide in many food commodities. They are produced via a complex polyketide pathway by many strains of *Aspergillus flavus* and the closely related *Aspergillus parasiticus* (2, 23). Aflatoxin B₁ (AFB₁) is the most potent natural carcinogen known and has also been classified as a group 1 human carcinogen by the International Agency for Research on Cancer, together with mixtures of AFB₁, aflatoxin G₁ (AFG₁), and aflatoxin M₁ (AFM₁) (13). The potential human and animal health effects of aflatoxicosis can be categorized as acute or chronic. The symptoms of acute aflatoxicosis include hemorrhagic necrosis of the liver, bile duct proliferation, jaundice, gastrointestinal hemorrhage, and lethargy (25, 29). Adult humans have been shown to have a high tolerance for aflatoxins, and in acute cases, it is usually children who die (5, 29).

The main human and animal health impacts of aflatoxins are related to chronic exposure. In the long term, aflatoxins pose a wide range of chronic health risks, including cancer of the liver and kidney, a weakened immune system (and attendant poor infectious disease modulation), a negative effect on micronutrient absorption,

teratogenicity, mutagenicity, and failure to thrive in infants (14, 23, 27, 29). There are also socioeconomic and financial problems associated with aflatoxins (2). These include reduced selling prices for contaminated crops, loss of export markets, and increased animal production losses (3).

Peanuts and peanut butter are considered nutritious, as they contain proteins, oils, fatty acids, carbohydrates, and minerals (24). This, ironically, makes them a rich medium for fungal growth and aflatoxin contamination (1). Routine monitoring of peanuts by the Zimbabwe Government Analyst Laboratory noted seasonal variation in aflatoxin contamination. About 46% of the samples analyzed during the 1995 season and 8% of the samples analyzed during the 1996 season were contaminated with levels greater than 10 µg/kg (12). Studies in the rural villages indicated the presence of AFM₁ in human breast milk at levels up to 0.05 ng/ml, raising concerns about postnatal exposure to aflatoxins (28). A recent study was carried out to monitor aflatoxin carryover during large-scale peanut butter production, and the findings reveal that peanuts from local farmers have high levels of aflatoxin contamination (>80 ng/g in raw peanuts) (26).

Zimbabwe has been plagued with an economic crisis for the past decade that has resulted in the collapse of the agricultural system (18). The available data indicate that

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very little aflatoxin surveillance has been done during this period. Also, the results from the few studies are inconsistent, not comprehensive, and have significant gaps. This preliminary survey was carried out to determine the extent of fungal and aflatoxin contamination of peanuts and peanut butter being sold in both the informal and formal markets of Bulawayo, the second largest city in Zimbabwe.

MATERIALS AND METHODS

Sample collection. Eighteen peanut and 11 peanut butter samples were bought at random from both retail shops and vendors at informal markets in the Bulawayo metropolitan area (Southern Zimbabwe) between October and November 2011. The bulk peanut lots were sourced from Gokwe (a small town about 450 km from Bulawayo) and transported by buses and other modes of public transport to Bulawayo. The commercial peanut butter samples were manufactured by several companies and were purchased from retail shops. The homemade peanut butter was purchased from vendors at the informal market.

Isolation and identification of fungi. All of the peanut butter samples and six randomly selected peanut samples were analyzed for fungal contamination according to the method of Pitt et al. (22). The peanut samples were surface sterilized with 3.5% sodium hypochlorite for 1 min and rinsed twice with sterile water before 100 kernels (5 kernels per plate) were plated on the selective *Aspergillus flavus* and *parasiticus* agar (AFPA) medium to obtain the percentage of infected kernels. The plates were incubated at 30°C for 72 h, and infected kernels were deemed positive for *A. flavus/parasiticus* according to the bright yellow/orange pigmentation of the underside of colonies. The spread plate method was used for peanut butter samples. One gram of sample was mixed with 9 ml of sterile distilled water, followed by serial dilution. One milliliter from each dilution was transferred onto the petri dish, and the cooled AFPA medium was poured on top and gently mixed. The mixture was allowed to set before being incubated at 30°C for 3 days. The isolated fungi were enumerated from plates showing well-separated colonies and identified using the colonies' reverse colors (22). The number of *A. flavus/parasiticus* colonies per gram of food was calculated and expressed as CFU per gram.

Aflatoxin extraction and clean up. The aflatoxin extraction method was adapted from a previously described method (9). In brief, 10 g of sample was mixed with 1 g of sodium chloride and extracted with 25 ml of 80% aqueous methanol. The mixture was shaken using a Stuart Orbital Shaker (Bibby Scientific Ltd., Stone, UK) at 250 rpm for 10 minutes and then centrifuged at 4,000 rpm (3,000 × g) and 5°C for 5 minutes using an RC-3B refrigerated centrifuge (Sorvall, Bohemia, NY). The supernatant was filtered into a conical flask using Whatman 4 filter paper, and a 10-ml aliquot from the filtrate was diluted with 40 ml of distilled water. Then, 10 ml of the diluted sample was loaded onto an AflaTest immunoaffinity column (Vicom, Watertown, MA) at a flow rate of 1 or 2 drops per second. The immunoaffinity column was then washed with 15 ml of distilled water, and aflatoxin analytes eluted with 3 ml of methanol into an amber vial. The extract was dried at 60°C under a stream of nitrogen gas. The residue was reconstituted in 200 µl of methanol and stored at 4°C until analysis.

Chromatography. The reconstituted samples and aflatoxin standards (Sigma-Aldrich, St. Louis, MO) were analyzed on an 1100 series Agilent high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) equipped

with a quaternary pump and a Phenomenex Ultracarb 3-µm particle size Octadecylsilane (ODS) (20) column (100 by 4.60 mm with a 3-mm internal diameter; Phenomenex, Torrance, CA) at 35°C. Ten microliters of sample was autoinjected using an autosampler connected to a fluorescence detector with wavelengths of 365 nm for excitation and 435 nm for emission. An online postcolumn derivatization was performed using a PHRED (photochemical reactor for enhanced detection) UV lamp (Aura Industries, Inc., New York, NY). The mobile phase of 0.01 M KH₂PO₄-acetonitrile-methanol-acetic acid (690:150:75:20, vol/vol/vol/vol) was pumped at a flow rate of 1.5 ml/min. Data were collected and analyzed with the Agilent ChemStation software, and quantification was achieved by comparing the areas under the curve with those of authentic aflatoxin standards.

Method validation. The analytical method was validated for specificity, linearity, accuracy, and precision. Specificity validation was performed by injecting the aflatoxin standard three times, each time before injecting extracted samples, and comparing retention times. Peanut and peanut butter samples purchased from a retail shop in Cape Town (South Africa) were spiked in triplicate with aflatoxin standards at 5, 10, and 20 ng/g. Linearity, accuracy, and precision were assessed by performing triplicate intraday injections at each of the spiking levels.

RESULTS AND DISCUSSION

Fungal contamination. Peanuts are among the food-stuffs most susceptible to contamination by aflatoxigenic fungi in both pre- and postharvest stages (17). Four of the six peanut samples analyzed in this study were contaminated with *A. flavus/parasiticus*, ranging from 3 to 20% of the kernels examined. In comparison, Mphande et al. (17) reported *Aspergillus* species from 94% (113 of 120) of peanut samples from Gaborone, Botswana, and the *A. flavus/parasiticus* were detected on 66% of the samples. In Thailand, 100% of the peanut samples showed some fungal infection and 84% of all kernels examined were infected (21). *A. flavus* isolates were the most dominant, followed by *Aspergillus niger*, being found in 95 and 86% of all the samples examined, respectively.

Three of the peanut butter samples in this study showed *A. flavus/parasiticus* growth, with the most contaminated sample yielding 1×10^2 CFU/g of peanut butter. This is below the maximum tolerance limit (10^4 CFU/g) recommended by the International Commission on Microbiological Specifications for Foods (6). Commercial peanut butter is made by dry roasting shelled peanuts at 160°C and blanching, deskinning, and grinding them (26). Salt, sugar, and dehydrogenated fat (as stabilizer) are also added (20, 26). Homemade peanut butter is also made by roasting peanuts, blanching, and grinding, although the roasting temperature is rarely monitored. The roasting is believed to destroy most of the vegetative fungi present.

Results of method validation. Recoveries of AFB₁, AFB₂, AFG₁, and AFG₂ at 5, 10, and 20 ng/g are shown in Table 1 below.

The mean recoveries (relative standard deviation [RSD]) for total aflatoxins were 65 (14), 61 (2.5), and 85% (8%) for peanuts at 5, 10, and 20 ng/g respectively. For peanut butter, the mean recoveries (RSD) for total

TABLE 1. Summary of recovery validation of various aflatoxin analogues for peanuts and peanut butter using HPLC

Aflatoxin analogue	Mean % recovery (RSD [%]) for sample spiked with indicated amt of aflatoxin standards					
	Peanuts			Peanut butter		
	5 ng/g	10 ng/g	20 ng/g	5 ng/g	10 ng/g	20 ng/g
AFB ₁	73 (7.5)	84 (1.3)	93 (18.5)	107 (4.2)	81 (0.9)	86 (2.1)
AFB ₂	73 (4.4)	73 (0.7)	85 (18.9)	82 (5.0)	79 (0.6)	74 (7.9)
AFG ₁	55 (23)	50 (4.7)	77 (18.3)	98 (3.4)	65 (5.3)	72 (20.4)
AFG ₂	59 (5.7)	38 (3.3)	84 (8.9)	94 (10.4)	76 (0.9)	68 (14.9)

aflatoxins were 95 (11), 75 (1.9), and 75% (10%) at 5, 10, and 20 ng/g, respectively. These recoveries are similar to aflatoxin recoveries reported in spiked cassava samples (10) and in spiked maize samples (15). Specificity validation was demonstrated using spiked samples. The reproducibility of the HPLC method for aflatoxins was determined using the working standards. The coefficients of variation for the working standards were 2, 1, 1, and 0% for AFB₁, AFB₂, AFG₁, and AFG₂, respectively. After validation was performed, the samples were tested for aflatoxin contamination.

Aflatoxin contamination. Three (17%) of the peanut samples were contaminated, with total aflatoxins ranging from 6.6 ng/g to as high as 622.1 ng/g. The latter sample was contaminated with AFB₁, AFB₂, and AFG₁. All of the contaminated samples exceeded the maximum total aflatoxin level set by the European Union (EU), and two samples exceeded the limit set by Zimbabwean legislation. The EU limit for total aflatoxins is 4 µg/kg in peanuts and peanut products meant for direct human consumption (8), and the Zimbabwe regulatory limit is 15 µg/kg in all foods (4). AFB₁ was detected in all three samples and had the highest concentrations, ranging from 6.3 to 528 ng/g, which was above the regulatory limit of 5 µg/kg of AFB₁ permitted in Zimbabwean food.

Ninety-one percent (10 of 11) of the peanut butter samples were contaminated, with total aflatoxins ranging from 6.1 to 247 ng/g (mean, 75.66 ng/g), as shown by the

TABLE 2. Aflatoxin contamination levels in peanut butter

Sample (type ^a)	Mean amt (ng/g) of:				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total aflatoxins
PB 1 (i)	32.5	6.2	9.3	ND ^b	48
PB 2 (i)	10.1	ND	ND	ND	10.1
PB 3 (i)	7.7	ND	ND	ND	7.7
PB 4 (c)	191	25.7	30.3	ND	247
PB 5 (c)	42.9	6.3	47.1	8.8	105.1
PB 6 (c)	38.2	ND	ND	ND	38.2
PB 7 (c)	186	24.9	30.3	ND	241.2
PB 8 (i)	6.1	ND	ND	ND	6.1
PB 9 (c)	19	ND	10.4	ND	29.4
PB 10 (c)	23.8	ND	ND	ND	23.8
PB 11 (c)	ND	ND	ND	ND	ND

^a i, informal market sample; c, commercial sample.

^b ND, not detected.

results in Table 2. The mean aflatoxin concentration was about 5 times higher than the limit of 15 µg/kg set by the Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program (4), for total aflatoxins in food and 19 times higher than the limit allowed by EU regulations (8). The EU has stricter regulations, with a maximum allowable limit for total aflatoxins of 4 µg/kg for groundnuts, nuts, and processed products intended for direct human consumption or use as an ingredient in foodstuffs (8). AFB₁ was detected in all of the samples that were contaminated and accounted for the highest toxin levels (mean, 55.73 ng/g, and range, 6.1 to 191 ng/g).

All of the contaminated peanut butter samples exceeded the maximum AFB₁ level set by both the EU and Zimbabwean legislation. The EU limit for AFB₁ is 2 µg/kg in peanut and peanut products for direct human consumption (8), and the Zimbabwe regulatory limit is 5 µg/kg in all foods. In Sudan, much higher total aflatoxin levels (range, 26.6 to 853 µg/kg) in peanut butter were reported, with 90% of the samples exceeding the EU maximum limit by a factor of >20 (7). Only 27% (3 of 11) of samples in the current study exceeded the EU limit by a factor of >20. The differences might be due to the hot and humid storage systems for peanuts prevalent in Sudan (7, 19). In addition, the Sudanese peanuts were crushed and stored in plastic bags at ambient temperatures before processing (7), which might have created conditions conducive to high-level production of aflatoxins. Higher total aflatoxin concentrations in peanut butter were also reported in Nepal (16), with 42.5% (43 of 101) of the samples contaminated and 19.8% (20 of 101) of the samples having a total aflatoxin concentration of >30 ng/g. However, the Indian subcontinent is considered a hotspot for aflatoxins due to the hot and humid conditions that are prevalent in that part of the world (3). The sample size in this study was small, but this can be considered representative of market size in the region, and hence, the findings are important in showing the possible phytosanitary problems currently pertaining in Zimbabwe.

In conclusion, this study has shown the presence of both fungal and aflatoxin contamination in peanuts and peanut butter from Bulawayo. Peanut butter is widely promoted as food for infants and school children. It is used in soft porridge (commonly called soft *pap* or *bota*), sandwiches, and vegetable relish (26). It has excellent nutritional benefits, including proteins and micronutrients, especially phosphorous, potassium, zinc, folate, vitamin E,

and some phytochemicals (20). High levels of aflatoxin and fungal contamination result in decreased quality and nutritional value of the peanuts and peanut butter (11). Furthermore, the available information suggests that children are most vulnerable to the detrimental effects of aflatoxins (5, 29), and hence, the detection of aflatoxins in peanut butter is a worrying development.

The results from this preliminary study show that peanuts and peanut butter from Bulawayo in particular and Zimbabwe in general are contaminated with very high levels of aflatoxins. The fact that there was no difference between aflatoxin contamination in commercial peanut butter samples and homemade peanut butter samples is an important revelation from the study. This shows that quality control among manufacturers as required by the law either is not being done or is compromised. The regulatory authorities that are charged with enforcing phytosanitary regulations should ensure that they are complied with by manufacturers. Enforcement of regulations in resource-poor settings is usually difficult to do (25). In most developing countries, the food consumed is usually from subsistence production, consumption of formally traded food items is minimal, and laboratories to test the food are absent or have little capacity (25, 29). At the country level, the least contaminated, best quality food is usually exported, leaving the highly contaminated food to be consumed by a population already at risk (29).

Further research on commercial peanut butter should be carried out with a much larger sample size to confirm these results. Dietary exposure studies in children around Zimbabwe should also be carried out to ascertain the extent of the aflatoxin food contamination problem.

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