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A survey of the incidence and level of aflatoxin contamination in a range of locally and imported processed foods on Malawian retail market

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ABSTRACT

Samples of locally (Malawian) processed and imported maize- and groundnut-based food products (peanut butters, roasted groundnuts, peanut based therapeutic foods, instant baby cereals, maize puffs and de-hulled maize flour) were collected from popular markets of Lilongwe City, Malawi. The samples were analysed in order to determine the frequency and extent of aflatoxin contamination, using immuno-affinity column and reversed-phase liquid chromatography with post-column photochemical derivatization and fluorescence detection. No aflatoxins were detected in all samples of imported baby cereal and locally processed de-hulled maize flour. However, all locally processed maize based baby foods had aflatoxins above EU maximum tolerable level of 0.1 μg/kg. In 75% of locally processed maize puffs, aflatoxins were detected at levels of up to 2 μg/kg. Peanut based therapeutic foods had aflatoxin level between 1.6 and 2.9 μg/kg, exceeding the EU tolerable maximum level (0.1 μg/kg) set for food for health purposes. Locally processed peanut butters had aflatoxins levels in the range of 34.2–115.6 μg/kg, which was significantly higher than their imported counterparts (<0.2–4.3 μg/kg). Samples of locally processed skinned and de-skinned roasted groundnuts had aflatoxins in range of 0.5–2.5 μg/kg and 0.6–36.9 μg/kg, respectively. These results highlight the need for rigorous monitoring of aflatoxins in commercially available processed products in order to reduce likely health risks associated with dietary aflatoxin intake.

1. Introduction

Several maize- and groundnut-based ready-to-eat food products are commercially available, some of which are promoted as infant/baby and therapeutic foods. However, maize and groundnuts are prone to pre-harvest and post-harvest contamination with aflatoxins. Aflatoxin contamination has been reported in samples of maize and groundnuts from Malawi (Matumba, Monjerezi, Chirwa, Lakudzala, & Mumba, 2009; Monyo et al., 2012) and across Africa (Bankole, Schollenberger, & Drochner, 2006; Sibanda, Marovatsanga, & Pestka, 1997; Shephard, 2003, 2008). There is potential that the contaminated raw materials pass on aflatoxins to the final product (Bullerman & Bianchini, 2007).

Aflatoxins have been shown in many studies to be immunosuppressive, teratogenic, mutagenic, carcinogenic, genotoxic and hepatotoxic (Fung & Clark, 2004; Hendrickse, 1997; IARC, 1993; Peraica, Radic, Lucic, & Pavlovic, 1999; Preisler, Caspary, Hoppe, Hagen, & Stopper, 2000; Wangikar, Dwivedi, Sinha, Sharma, & Telang, 2005; WHO, 1998) to humans and animals, depending on the duration and level of exposure. Maize- and groundnut-based ready-to-eat food products may constitute aflatoxin exposure risk, particularly because they are consumed by infants/babies, malnourished children and people living with HIV/AIDS (Manary, Ndkeha, Ashorn, Maleta, & Briend, 2004; Ndekha, Manary, Ashorn, & Briend, 2005; Sandige, Ndekha, Briend, Ashorn, & Manary, 2004). It has been postulated that a synergy exists between HIV and AFB1 in AIDS development (Jiang et al., 2008; Jolly et al., 2013). In addition, aflatoxins cause decreased transport of soluble nutrients (Fink-Gremmels, 2008), disrupt protein, carbohydrate and lipid metabolism (Cheeke & Shull, 1985), alter growth

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factor expression and impair child growth (Gong, Turner, Hall, & Wild, 2008; Khlangwiset, Shephard, & Wu, 2011).

There is however, limited knowledge about the frequency and levels of aflatoxins in processed products in Malawi. In this context, this study reports, for the first time, on the occurrence of aflatoxins in industrial processed food products marketed in Malawi. The data presented in this study may be useful in facilitating improved food regulation and dietary risk management in Malawi.

2. Materials and methods

2.1. Food samples

A total of 125 samples of local and imported food products were purchased from the market in Lilongwe City, Malawi, in December 2012. The local products were: 14 cans of peanut butters; 15 packs of de-skinned roasted groundnuts; 9 packs of un-skinned roasted groundnuts; 6 cans of peanut based therapeutic foods, 36 packs of instant baby cereals, 12 packs of maize puffs; 15 packs of de-hulled maize flour samples. Imported products included 7 packs of instant baby cereals and 11 cans of peanut butters.

2.2. Aflatoxin analysis by HPLC-FLD method

2.2.1. Chemical and reagents

Acetonitrile, methanol and HPLC-grade water were supplied by Merck (Darmstadt, Germany). 5.0 μg/mL total aflatoxins (aflatoxin B1 (AFB1)/aflatoxin B2 (AFB2)/aflatoxin G1 (AFG1)/aflatoxin G2 (AFG2) (4/1/4/1, v/v/v/v)) were purchased from Trilogy Analytical Laboratory (Lot # 120316-090, Washington, MO, USA). After reconstitution in 10 mL acetonitrile, the standard solution was kept securely at −15 °C, wrapped in aluminium foil to avoid photo-degradation and held for 6 months. Working aflatoxins standard solutions were made by diluting the stock solution in methanol/water (50/50, v/v).

2.2.2. Extraction and clean-up

Modified Aflatest® immuno-affinity procedures for extraction and clean-up of aflatoxins in cereals and nuts were used (VICAM, 1999). For maize-based samples, sub-samples (30 g) of finely ground products (to pass sieve #20) were added to 3 g of NaCl and extracted with 60 mL of methanol/water (80:20, v/v) and blended at high speed for 2 min. The extract (10 mL) was diluted four folds with HPLC grade water and filtered twice (firstly through a coarse fluted filter, and secondly through a glass filter) before passing a 20 mL (2 g sample equivalent) of the diluent through Aflatest® affinity column (VICAM, Watertown, MA, USA). For all groundnut-based products, sub-samples (15 g) were added to 3 g of NaCl and extracted with 75 mL of methanol/water (70:30, v/v), blended at high speed for 2 min, the filtrate diluted two folds with water and re-filtered through a glass-fibre filter. A 30 mL (2 g sample equivalent) of the diluent was passed through Aflatest® affinity column as described earlier. For both maize- and groundnut-based foods, the columns were then washed with 23 mL of water/methanol (85/15, v/v) to remove maize intrinsic compounds and finally the aflatoxins were selectively eluted with 1 mL of 100% methanol followed by 1 mL of 100% HPLC water. The total volume of the eluent (2 mL) was mixed using a vortex mixer for 30 s after which the sub-sample was ready for HPLC analysis. In case the total aflatoxins exceeded 25 μg/kg, a sample was re-analysed ensuring that only 0.1–0.2 g sample equivalent was passed through the affinity column.

2.2.3. Aflatoxins determination using HPLC-FLD

Determination of aflatoxins was done using Agilent 1200 Series HPLC System (Agilent, Waldbronn, Germany) consisting of G1322A degasser, G129A autosampler, G1330B thermostat, CY1311A quaternary pump, G1316A temperature controller and G1321A fluorescence detector (FLD). Chromatographic separation was achieved using ZORBAX Eclipse® XDB-C18 column (150 mm × 4.6 mm I.D., 5 μm particle size), protected by C18 security guard cartridge (4 × 3 mm i.d.) (both supplied by Agilent Technologies). An isocratic mobile phase consisting of water/methanol/acetonitrile (55/35/10, v/v/v) was used at a flow rate of 1 mL per min. The column oven temperature was maintained at 30 °C and the injection volume was 40 μL for both standards and samples. Post-column derivatization (PCD) was achieved using a photochemical reactor (LCTech UVE, Dorfen, Germany). Fluorescence excitation and emission wavelengths were set at 365 and 440 nm, respectively. Retention times of AFG2, AFG1, AFB2 and AFB1 were 5.5, 6.4, 7.6 and 9.0 min respectively. Data acquisition and processing was achieved using chromatographic software (ChemStation®). Aflatoxin determination in samples was based on a five point external standard calibration curve, using a mixture of aflatoxin standards (AFB1 and AFG1, each ranging from 0.5 to 15 ng/mL, and AFB2 and AFG2, ranging from 0.125 to 3.755 ng/mL). Calibration curves, with strong regression ($R^2 \geq 0.995$) were classified as valid.

Quality control in the aflatoxin analysis was achieved using naturally contaminated reference materials (Product #: TR-A100, Batch #: A-C-268, R-Biopharm AG, Darmstadt, Germany). Five samples of each product type, spiked with 12.5 μg/kg total aflatoxins, were used to assess recovery and recoveries between 70 and 110% were classed as valid. The results were corrected by mean recovery rates obtained from the recovery experiments (Table 1). Limits of detection (LODs) and quantification (LOQs) were determined at a signal-to-noise (S/N) ratio of 3/1 and 10/1, respectively, for each food category separately. For data evaluation, half the values of LOD or LOQ of the respective category were assigned to values below the LOD and between the LOD and LOQ, respectively.

Since aflatoxin concentration in the samples was not normally distributed, data were log transformed before statistical analysis. The statistical analysis was performed on SPSS version 16 (SPSS inc., Chicago, IL, USA). P values < 0.05 were considered statistically significant.

Table 1
Recovery percentages of the aflatoxins and limit of quantifications (LOQs) for tested products.

Aflatoxin	Extruded maize-soybeans mixture			Maize flour			Maize puffs			Peanut butter		
	Recovery %		LOQ[a] (μg/kg)	Recovery %		LOQ[a] (μg/kg)	Recovery %		LOQ[a] (μg/kg)	Recovery %		LOQ[a] (μg/kg)
	x[b]	RSD		x[b]	RSD		x[b]	RSD		x[b]	RSD	
AFB1	86	3	0.5	92	5	0.7	78	4	1.0	96	2	0.5
AFB2	83	4	0.2	88	4	0.3	71	3	0.3	93	3	0.2
AFG1	85	5	0.6	83	4	0.7	75	5	0.7	84	4	0.6
AFG2	82	4	0.3	74	3	0.3	70	4	0.5	76	3	0.3

[a] Limit of quantifications (LOQs) determined at a signal-to-noise (S/N) 10/1.
[b] Mean Recovery rates were determined from five (5) analyses of spiked blank food (each product type) with AFB1 and AFG1, each at 5 μg/kg and AFB2 and AFG2, each at 1.25 μg/kg.