Aflatoxins Contamination of Locally Processed Peanut Butter sold in Retail Markets in Arusha, Tanzania

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Abstract

Aflatoxins contamination of agricultural produce affects both the quality and safety of food and feedstuff. While peanuts are important crops of economic and nutritional significance in tropical regions, they are highly susceptible to aflatoxins contamination. This study reports on the assessment of aflatoxins contamination of 50 samples of peanut butter, processed locally by 10 different firms and sold in local markets, supermarkets and retail shops in Arusha City. Immunoafinity columns were used for sample clean-up followed by analysis using High Performance Liquid Chromatography (HPLC). Total aflatoxins were determined in 48% of the samples with concentration ranging from1.00 to 1,981.37 µg/kg, out of which 44% of the samples exceeded 15 µg/kg, the East African maximum tolerable limit for total aflatoxins in foods. The aflatoxins B1 (AFB1) ranged from 25.98 to 300.39 µg/kg, aflatoxins B2 (AFB2) from 1.01 to 34.20 µg/kg, aflatoxins G1 (AFG1) from 52.51 to 1,832.17 µg/kg and aflatoxins G2 (AFG2) from 1.00 to 27.03 µg/kg. The mean total aflatoxins from all the firms ranged between 14.1 to 908.6 µg/kg. The use of poor quality raw materials and unhygienic processing practices in local peanut butter production chains might have contributed to significant aflatoxins contamination of these products. Creation of awareness of aflatoxins contamination and enforcement of legislations throughout the chain may contribute to its reduction to protect consumers' health.

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Introduction

Food safety is a major public health concern in sub-Saharan Africa (Mutegi et al., 2013; WHO, 2006) and worldwide. Contamination of agricultural produce mainly cereals, oily seeds and nuts such as peanuts with mycotoxins producing fungal compromises the safety of food and poses a serious health risk to consumers (Gong et al., 2002; Lewis et al., 2005). Since the realization of mycotoxins contamination in foods, extensive researches has been conducted on the nature of mycotoxins and modes of action, effects to human health as well as methods for their determination (Shephard, 2008).

Mycotoxins are natural fungal metabolites that have pathogenic roles (Ostadrahimi et al., 2014) and have been estimated to contaminate 25% of the food produce worldwide (Kabak, 2010). Their contamination in foods differs depending on the climate such that hot and humid conditions favors their growth and toxin production (Cotty & Jaime-Garcia, 2007). Fungi producing mycotoxins belong to the genera *Aspegillus*, *Penicillium* and *Fusarium* while the mycotoxins of public health concern are aflatoxins, zearalenone, ochratoxin A, fumonisins and trichothecenes, with aflatoxins being reported as the main potent (Bankole et al., 2006). Aflatoxins are one of the mostly potent food borne mycotoxins in Africa (Ndung'u et al., 2013; Who, 2006). They are natural fungal toxins produced by *Aspergillus parasiticus* and *Aspergillus flavus* that contaminate agricultural products before and after harvest (Elshafie et al., 2011; Walke et al., 2014). The most common types are aflatoxins B1, B2, G1 and G2. *Aspergillus flavus* is the main source of B aflatoxins, while *Aspergillus parasiticus* produces both B and G types (Mutegi et al., 2012).

Worldwide, the major health risk associated with consumption of foods contaminated with aflatoxins is liver cancer (hepatocellular carcinoma) which leads to approximately 550,000 to 600,000 new cases annually (Liu and Wu, 2010). In Eastern African region the outbreaks of aflatoxicosis have been reported in countries like Somalia in 1997-98 and Kenya in 1982, 2001, 2004, 2005 (Who, 2006). Other health risks associated with aflatoxins includes immune suppression and child growth retardation (Gong et al., 2002; Kimanya et al., 2008; Turner et al., 2003; Unnevehr & Grace, 2013; Williams et al., 2004). As such, aflatoxins have been reported to cause malnutrition problems such as kwashiorkor and vitamin A deficiency due to nutritional interference, such that binding of aflatoxin to the DNA leads into the decrease in protein

synthesis, while aflatoxin concentration interfere vitamin A metabolism in the body. Aflatoxins is known to lower the immunity and thus creates room for virus replication (Kamika & Takoy, 2011; Kimanya, 2015; Shirima et al., 2015; Williams *et al*., 2004).

High levels of aflatoxins have been reported in peanuts and cereal grains such as corn (Kamika & Takoy, 2011; Kimanya et al., 2008). Peanuts are important crops of economic and nutritional significance in tropical regions (Guo et al., 2009) and are consumed either as roasted, boiled or processed into peanut butter and oil (Afolabi et al., 2015; Chang et al., 2013). In some communities, peanuts are used in food preparation as an ingredient or in placement of oil. Of a major concern, peanuts are milled in combination with cereals to obtain flour used to prepare porridge type of complementary food. Occasionally, peanuts are consumed raw as snacks during harvesting, dehulling, packaging, meetings or in between meals; and especially consumption of raw peanut is believed to enhance sexual stamina especially in men. Nevertheless peanuts are prone to fungal and mycotoxins contamination (Mutegi et al., 2013), posing a great challenge on food quality and safety with a subsequent health risk to consumers (Mutegi *et al*., 2013; Ostadrahimi et al., 2014). The risk of aflatoxins contamination in peanuts increases after harvest which is associated with poor handling practices along the chain (Kaaya et al., 2006).

The quality of a processed food product such as peanut butter begins from farm production throughout processing stages including shelling, storage and product manufacturing. The choice of raw materials is very crucial during peanut butter processing, as it contributes to the quality and safety of the final product. Not only the full matured and well dried peanut of uniform size, but also free from fungal contamination are recommended for peanut butter processing (ITDG, 2002). Therefore, this min-surveillance aimed at determining aflatoxins contamination of locally processed peanut butter sold in retail markets in Arusha, Tanzania.

Materials and methods

Study site, design and sampling

The study was conducted in Arusha city, the headquarter of the Arusha region located on the northern Tanzania. It lies between the latitude 2° and 6° south and longitude 35° and 38° East

(Office, 1998). The site was chosen due to presence of local peanut butter processors and the availability of different brands of locally processed peanut butter. A survey was conducted to retail market to identify common peanut butter brands. A random sampling technique was used to collect samples from retail market outlets. According to the survey, 10 common brands were identified and from each, 5 samples were collected to make a total of 50 samples.

Sample preparation and Extraction of Aflatoxins

Aflatoxins were extracted from peanut butter samples by the method described by (Stroka et al., 2000), with external derivatisation as reported by (Tarter et al., 1984). A portion of 10 g of homogenized peanut butter sample was weighed and 1g of NaCl was added. 40 ml of extraction solution (80% methanol and 20% water) and 20 ml of hexane were added and blended for two minutes and immediately filtered through Whatman filter No. 1. Then, 10 ml of filtrate was mixed with 70 ml of Phosphate Buffer Saline (PBS). Immunoaffinity columns were then activated with 10 ml of PBS and 80 ml of sample extract was passed through the column fitted to a vacuum manifold. Then, 15 ml of double distilled deionized water was used to wash the column and the column was dried further by slightly application of pressure. Aflatoxins from the column were eluted with 3 ml of elution solvent (99% Methanol and 1% acetic acid) into a test tube with a maximum flow rate of 1ml/min.

The eluent was evaporated to dryness with nitrogen gas, followed by derivatisation with 200 µl of n-hexane and 50 µl of trifluoroacetic acid, then the mixture was left for 20 minutes. After complete derivertisation, the mixture was evaporated to dryness with nitrogen and the residues were re-dissolved with 200 µl of a mobile phase (methanol: water: acetonitrile: acetic acid at a ratio of 23: 57: 20: 0.1 v/v respectively), 50 µl of the mixture was then injected into HPLC for aflatoxins quantification.

Analysis of Aflatoxins by HPLC

A Shimadzu HPLC system equipped with RF-20A fluorescence detection system and an auto sampler SIL 20AHT was connected to C18 (250×4.6 mm, 5 µm) column with the oven temperature maintained at 20ºC. The mixture of methanol: acetonitrile: water at a ratio of 23: 20: 57 v/v was used as mobile phase at a flow rate of 0.3 ml/min and a running time of 25 minutes.

The fluorescence detection system was set at wavelengths of 360 nm excitation and 440 nm emissions. Difference in occurrence of aflatoxins between firms were analysed by Kruskal-Wallis rank sum test.

Results

The average percentage recoveries were 105.7%, 91.5%, 101.8% and 94.5% for aflatoxins B1, G1, B2 and G2 respectively. These average recoveries are within the percent required range of 70% to 110 % **(**Graya et al 2014). The relative standard deviation (RSD) for aflatoxins B1, G1, B2 and G2 were 6, 7, 10 and 18 respectively. The limits of detection for aflatoxins B1, G1, B2 and G2 were 0.219, 0.021, 0.219 and 0.211µg/kg respectively.

Aflatoxins contamination of peanut butter

Results of aflatoxins contamination of peanut butter are presented in table 1. About 48% of the samples were contaminated with total aflatoxins at a range of 1.00-1,981.37 µg/kg. Aflatoxins B1 was detected in 40% of the samples with levels ranging from 25.98 to 300.39 µg/kg (mean, 54.95 µg/kg), B2 was detected in 32% of the samples with levels ranging from 1.01 to 34.20 µg/kg (mean 5.19 µg/k), G1 was detected in 28% of the samples with levels ranging from 52.51 to 1,832.17 µg/kg (mean, 324.09 µg/kg) and G2 was detected in 30% of samples with levels ranging from 1.00 to 27.03µg/kg (mean, 3.63 µg/kg). Of the samples, 44% had total aflatoxins levels that exceed the East African standard for maximum limit of total aflatoxins which is 15 µg/kg (EAC, 2013) set by the East African Community. The categorical frequency of the occurrence of the total aflatoxins is shown in Figure 1. Mean levels of total aflatoxins in samples from all firms ranged from 14.07 to 908.58 µg/kg. The difference in levels of total aflatoxins between firms was found statistically insignificant amongst 9 firms, p value 0.3607 ($p > 0.05$).

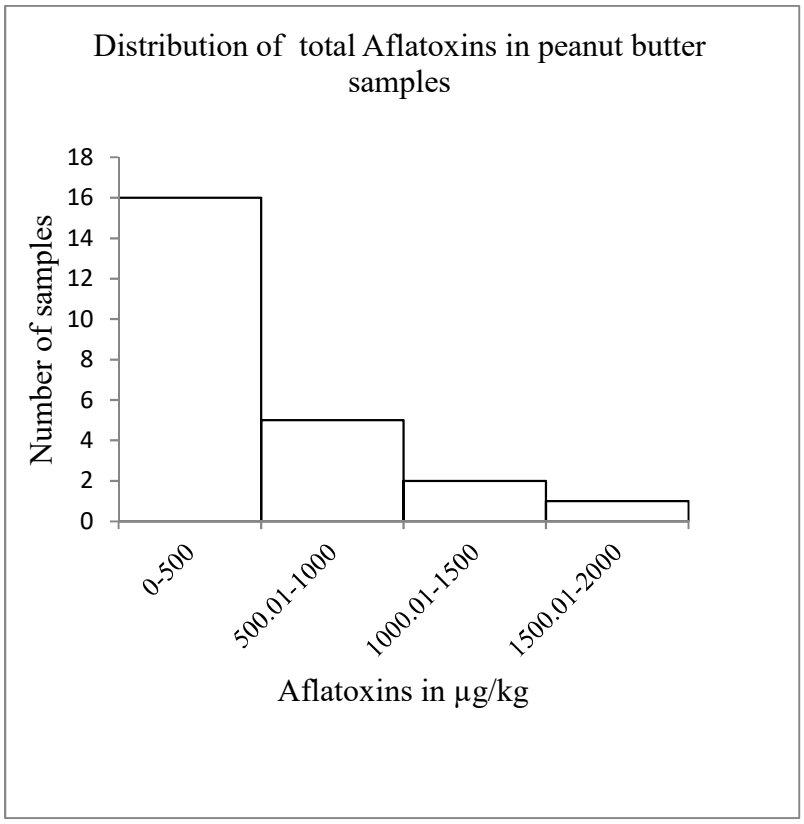

Figure 1. Categorical frequency of occurrence of aflatoxins in peanut butter.

Discussion

Both human and animals are predisposed to mycotoxins through consumption of diets naturally contaminated with these toxins at different points of the food chain. Studies have revealed association between long term dietary consumption of aflatoxins and high incidence of liver cancer in Africa (Wagacha & Muthomi, 2008). Groundnuts are among the dietary food staples that cause most of the mycotoxin poisoning problems in Africa. The ingestion of aflatoxins contaminated food has been reported to cause 250,000 hepatocellular carcinoma deaths annually and has led to recently fatal aflatoxins outbreak occurred in 2004, 2005 and 2006 in Africa (Lewis et al., 2005; Wagacha & Muthomi, 2008).

Table 1. Occurrence of aflatoxins among firms

Firms Code	Mean µg/kg ± SE
A	569.3 ± 543.3
C	312.2 ± 171.5
D	908.6 ± 393.9
E	14.1 ± 11.9
F	449.9 ± 423.9
G	68.1 ± 42.1
H	589.8 ± 322.8
I	26.9 ± 14.9
J	111.6 ± 84.6

The difference between the levels of total aflatoxins was found statistically insignificant amongst 9 firms, $p>0.05$.

The present study quantified total aflatoxins in peanut butters sold in retail markets in Arusha City, Tanzania. The findings have revealed high levels of total aflatoxins contamination of up to 1981.37 µg/kg. Of the samples 48% were contaminated with aflatoxins, in which 44% had levels above the East African standard for maximum allowable limit of total aflatoxins in food 15 µg/kg (EAC, 2013). On contrary, a study in Haiti and Kenya by Filbert & Brown, (2012), reported aflatoxin contamination of all peanut butter samples with levels of up to 799.8 µg/kg, which is more than two times lower the level of contamination found in this study. In Turkey, all samples of peanut butter were contaminated with total aflatoxins of up to 75.74 µg/kg (Yentur et al., 2006). Peanut butter samples from Sudan showed 100% aflatoxins contamination (Elshafie et al., 2011). The variation in aflatoxins contamination found in this study as compared to those reported in other studies could be attributed by differences in geographical location, agriculture practices and climatic conditions (Williams et al., 2004) among other factors.

The quality of raw materials, storage conditions, amount and frequency of consumption are important determining factors for aflatoxins exposure (Yentur et al., 2006). The high levels of contamination observed in this study may be attributed by the use of low grade raw materials, improper processing that allows cross contamination, storage and preservation practices (Ndung'u et al., 2013). According to Mutegi et al, 2013, storage and packaging materials for peanuts contribute to the higher level of aflatoxins due to poor ventilation resulting in the humidity increase that favours fungal growth. A research done by Hell et al., (2000), has depicted the evidence that, storage methods and storage time can facilitate the proliferation of fungal and aflatoxins contamination in maize. In addition, climatic conditions of relative humidity, temperature and moisture which are important factors for the growth of *Aspegillus flavus* and *Aspegillus parasiticus* (Wagacha & Muthomi, 2008), contributes largely to the high levels of aflatoxins contamination of produce and processed products. Omer et al, (1998), reported the correlation between humid local storage conditions and high levels of aflatoxins concentrations in peanut products.

The similarities in aflatoxin levels between firms in this study suggest similarity in the quality of raw materials used, preparation practices and processing of peanut butter. The use of poor quality and mouldy peanuts during processing under poor hygienic conditions may be the cause for high aflatoxin levels in the final product (Elshafie et al., 2011). The processors of peanut butter products may not consider the quality of the raw material or perform the aflatoxins management practices to control contamination during processing (Filbert & Brown, 2012). Lack of quality control and protective measures in food chain systems and the negligence of good hygiene practices during food handling and preservations might be among the contributing factors (Wagacha & Muthomi, 2008). Improved quality control practices by peanut butter manufactures may significantly reduce the levels of aflatoxins contamination (Oliveira et al., 2009). Laboratory segregated and prepared peanut butter samples had significantly reduced aflatoxin to an acceptable level for human consumption compared to those purchased from retail stores (Elshafie et al., 2011).

Product contamination at the stages of production and processing affect the shelf life, quality and safety of packed foods. Stages in peanut butter processing involves, cleaning of groundnuts to remove unwanted materials; dry roasting of nuts for 10 to 30 minutes; cooling to stop cooking

process; skinning and sorting for testa and undesirable nuts removal. Grinding of roasted nuts to smooth consistency paste and then additional ingredients (salt, sugar, vegetable oil and stabilizer) are added prior butter filling in clean containers (ITDG, 2002). Roasting, testa removal and blanching can reduce aflatoxins to an acceptable level (Afolabi et al., 2015; Siwela et al., 2011). Peanut butter is being used widely as an ingredient in foods for children such as porridge, and according to (Siwela et al., 2011) young are more vulnerable to aflatoxin effects due to their small body size and immature immune system. Therefore, considering the high aflatoxins risks of exposure to humans, especially children, care should be taken during peanut butter preparation intended for human consumption.

The presence of contaminated peanut butter for human consumption, calls for the aflatoxins management strategies, including adoption of quality control measures during processing to ensure food quality and safety. Various strategies are being enforced to control aflatoxins during crop production and food preparation including good agronomic practices, early crop harvesting, proper drying of crops for moisture control, physical separation such as sorting, and use of improved storage structures that prevents moisture inlet, insects and rodents from entering the crops. Biological control and resistant varieties have shown to reduce aflatoxins contamination (Bankole & Adebanjo, 2003). Monitoring of processing flow, inspection of lots prior market release and the use of principles of Hazard Analysis and Critical Control Points during processing have been reported to improve food safety (Calhoun, 2013).

High levels of aflatoxins contamination of peanut butter revealed in this study is alarming and might pose a health threat to consumers. As well, this can leads to the significant loss of economy due to the loss of product`s export value (Yentur et al., 2006). Therefore, this alarming contamination calls upon the regulatory bodies and food value chain actors to enforce control measures in order to reduce the magnitude of the problem with an understanding of its related problems on human health and the economy. Continuous aflatoxin national surveillance and creation of awareness intervention has been advocated by (Who, 2006) to sensitize stakeholders to take into consideration the mycotoxins prevention strategies along the food chain. In addition, diversification of food intake such as the use of fruit jams, margarine apart from peanut butter alone is also recommended as continuous consumption of peanut butter may pose a risk of aflatoxins exposure to consumers.

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