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Evaluation of aflatoxin B1 contamination of peanut butter in The Gambia

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Aflatoxins are poisonous, mutagenic, and carcinogenic compounds produced by *Aspergillus* fungi that contaminate various agricultural produce and products including peanut butter. Peanut butter is among the most consumed recipe in The Gambia. Thus, a cross-sectional assessment was conducted to evaluate the levels of aflatoxin contamination in processed peanut butter, sold and consumed locally in the country. In total, 85 peanut butter samples of approximately 2.0 kg each were bought at random within the six administrative regions across the country. All the samples were analyzed for aflatoxin contamination using thin layer chromatography (TLC) technique. Aflatoxin B1 was detected in 8 (9.4%) of the samples and only one (1.20%) of the samples exceeded both the Codex Alimentarius Commission and FAO/WHO Food Standards Program of 15 µg kg^{-1}. Likewise, only 5 of 85 samples representing 5.90% exceeded the European Union maximum limits for total aflatoxin of 4 µg kg^{-1} in peanut and processed products intended for direct human consumption. The remaining samples (77 of 85) representing 90.6% were negative or without any detectable aflatoxins. The analyzed samples therefore indicate that majority of peanut butter especially homemade is safe for human consumption.

Key words: Aflatoxin B1, peanut butter, thin layer chromatography (TLC), The Gambia.

INTRODUCTION

Several agricultural produce are susceptible to fungal attack that produces toxic metabolites referred to as mycotoxins. Aflatoxin is a food or grain-borne toxic secondary metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can infect a wide variety of crops such as corn, groundnut, cottonseed, tree nuts, etc., either in the field or during post-harvest (Horn, 2005; Miningou et al., 2021). Spores if not atoxigenic of *A. flavus* are saprophytic in nature and once they become pathogenic, they are known to produce an array of toxic secondary metabolites including aflatoxins (Nallathambi and Umamaheswari, 2009).

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Infestation by the fungus can occur on these produce even during processing, handling, storage or transportation (Nakai et al., 2008). Both raw and processed products are highly susceptible to mycotoxin contamination including milk and vegetables (Giryn and Szteke, 1995).

Aflatoxins have become a concern in agriculture, trade, animal and human health on a global scale (Bennett and Klich, 2003). Aflatoxin B1 (AFB1) is known as a potent hepatocarcinogen and can play a synergic action with hepatitis B or C viral infections leading to hepatocellular carcinoma (HCC) (Turner et al., 2000; McKean et al., 2006; Groopman et al., 2008; Eaton and Groopman, 2013).

Aflatoxin B1 is strongly linked to immune-system suppression, increased susceptibility to diseases, and growth retardation, notably stunting (Henry et al., 1999; Gong et al., 2002; Turner et al., 2003; Williams et al., 2004). Reports have shown that high exposure to aflatoxin causes infertility, abortions, and delayed onset of egg production in birds (Oladele, 2014). Furthermore, loss of appetite, skin discoloration, and even yellowish pigmentation on skin can be observed in fish. This hazardous toxin can be transformed to aflatoxin M1 when feeds of livestock are contaminated (Carvajal et al., 2003; Mohammadi, 2011). In The Gambia, groundnut is the main cash crop of the country and peanut butter is among the most consumed product in the country. Aflatoxin impact on international trade resulting from price losses and rejected exports is detrimental to the economy of the country. It has been reported by Joseph Ndenn, Iris Consulting (2018) that an average price loss per annum sums to US$1.5 M (2000-2014) and an average annual loss from rejected exports adds US$62,854 (2012-2015).

The Gambia is a tropical country with a sahelian climate; defined by a long dry season (November- May) and a short wet season (June-October). The country has an average monthly temperature range of 18 to 30°C during the dry season and 23 to 33°C during the wet season. The average monthly relative humidity varies from 68 to 70% during the dry and wet season, respectively, and the average rainfall ranges from 800 to 1200 mm (GOTG, 2020). These climatic conditions provide the optimum environments for the growth of the *Aspergillus* fungus. Improper agricultural practice like continuous cropping, late weeding, poor drying and storage of nuts with mechanical damage, coupled with low humidity, drought, insect, crop genotype, and soil condition can increase crop susceptibly to aflatoxin (Leszczynska et al., 2000).

Groundnut and its product, peanut butter are considered nutritious, as they contain proteins, oils, fatty acids, carbohydrates, and minerals (Settaluri, 2012). This, ironically, makes them a rich medium for fungal growth and aflatoxin contamination (Barberis et al., 2012). A 100 g roasted peanut is said to constitute 1.55 g of water, 21.51 g of carbohydrates, 8.0 g of fiber, 9.66 g of lipids (fats), 23.68 g of proteins and a total calories of 2448 kJ (585 kcal) (USDA, 2011). Peanut and its additives could provide such a nutritious diet to satisfy WHO recommended average requirement of 0.66 g of protein per kg of ideal body weight, and a “safe level” of 0.86 g/kg of body weight (Food and Nutrition Board, 2002).

Peanut butter locally referred to as “*De gay*”, is made from roasted groundnut at high temperature (160°C) (Siwela et al., 2011), blanched, deskinned and ground to paste after salt being added as a stabilizer (Peanut Institute, 2012). “*De gay”* is the primary recipe for a popular soup called “*domoda*” across all socioeconomic or demographic status in The Gambia. The stew is consumed nationwide due to its relative affordability and organoleptic properties.

Frequent deaths especially in children are repeatedly reported in many sub-Saharan countries due to malnutrition. Groundnut, a rich source of protein containing essential amino acids, can help in preventing malnutrition (Sanghvi and Murray, 1997).

Schroder et al. (2004) reported that people more adherent to a traditional Mediterranean diet, which includes nuts, had statistically lower body mass index (BMI). And a US food survey data revealed that peanut eaters have lower BMIs than non nut and peanut eaters (Sabate, 2003; Griel et al., 2004).

Peanut butter contains beneficial mono and poly unsaturated fats and is rich in antioxidants, vitamin E and the polyphenol, p-coumaric acid and help to lower blood cholesterol levels, reduce risk of heart disease by 50% (Talcott et al., 2005). The β-Sitosterol (phytosterol) is an anti-cancer compound found in peanut and peanut butter (Lee et al., 2004).

Virtually no scientific data exists on aflatoxin contamination of peanut butter in The Gambia. Considering the wide consumption rate of “*domoda”* and the need to ensure food and public health safety, the study is designed to probe the occurrence and prevalence of aflatoxin in peanut butter nationwide.

MATERIALS AND METHODS

Peanut butter sampling

About 2 kg of 85 samples of peanut butter were incrementally collected using multistage sampling method for markets and homes, while purposive sampling for production areas or processors within the six administrative regions in The Gambia (Figure 1). A total of 20 samples from the Greater Banjul Area (GBA) which include the Kanifing Municipality, 15 samples from West Coast Region (WCR), North Bank Region (NBR) and Upper River Region (URR), while 10 samples from Lower River Region (LRR) and Central River Region (CRR) (Table 1). With respect to sample Collection Points (CP), 36 samples were taken from homes, 30 market samples and 19 samples from processors (Table 1). All the samples were kept in zip bags, and kept in a quart cooler then transported to the Food Chemistry Laboratory at the National Agricultural Research Institute (NARI) for aflatoxin contamination analysis.

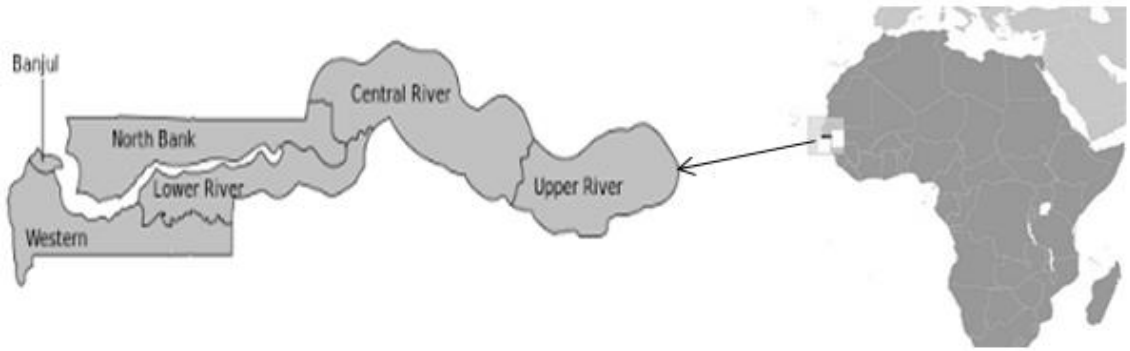

Figure 1. Map of The Gambia with demarcated sampling regions.

Table 1. Samples distribution in homes, markets and processors within regions.

Collection points (CP)/ regions	GBA	WCR	LRR	NBR	CRR	URR	Grand total
Homes	3	3	5	10	5	10	36
Market	10	8	3	3	3	3	30
Processors	7	4	2	2	2	2	19
Total	20	15	10	15	10	15	85

Extraction of aflatoxin

A 100 g representative sub-sample was obtained from each 2 kg sample and blended with 300 ml of distilled H_2O into slurry. A 100 g slurry was blended for 3 min with 250 ml methanol, 100 ml hexane and 2 g of NaCl, filtered using a Whatman filter paper of 32 cm and 50 ml collected. Exactly 150 ml of distilled water was poured in a separating funnel then the 50 ml filtrate and 25 ml of chloroform were respectively added and the separating funnels topped then slightly shaken. When separation settled, elutes were collected in a small beaker containing 2 anti-bumping granules for calm boiling then heat to dryness in a steam bath. The dried beaker was allowed to cool and about 5 to 8 ml chloroform pipetted to it, a dropper was used to rinse its wall to cleanse off any aflatoxin then the chloroform was subsequently filled in a vial containing 2 anti-bumping granules. The vials were evaporated to dryness using an electric vial-rack heater. After dryness and the vials cooled, a 0.25 ml of benzeneacetonitrile (98:2 v/v) solution was pipetted into them and vortexed.

Reading of TLC plates

Samples were spotted using an assipettor-fix for (5-10-40 µl), respectively on the TLC silica gel coated glass plates of 20 cm × 20 cm and thickness layer of 0.25 mm against the standard (1-3-7-10-15-20 µl) labeled using a pencil on top of the plate. The TLC plate is gently lowered in an already loaded tank with diether: methanol: water (94:4.5:1.5 v/v) positioned on a flat surface and covered with the lid. After about 45 to 60 min, when the plate in the tank had absorbed the solvent and at three-quarter length of the plate, the plates were removed and allowed to dry up for about a minute then illuminated below the TLC machine or lamb and viewed under long-wave of UV 366 nm in the dark room. The fluorescence intensities of aflatoxin B1 spots in samples were compared to the respective spots of standard in terms of color and retention factor (R_f). Aflatoxin B2, G1, and G2 spots were compared by the same procedure. Both preparation of the standards and the calculation of aflatoxin B1 concentration were done as stated by Jallow et al. (2019) in part per billions.

Statistical analysis

Data were statistically analyzed using SigmaPlot 12, applying t-test ($P<0.05$) for pairwise comparisons.

RESULTS

The data indicated that aflatoxin contamination in peanut butter is generally low in the country. There was no significant differences ($p>0.05$) among regions (Table 2) not at collection points (Table 3). Aflatoxin was not detected in 77 (90.59%) of the 85 samples analyzed (Table 2). Furthermore, there were no detectable aflatoxin in all the peanut butter samples collected from the GBA and NBR, therefore those regions are regarded as aflatoxin contamination free zones under this study (Figure 2).

Out of the 15 samples from WCR, only two (2) samples (13.33%) were contaminated with aflatoxin by 2.91 and 38.76 ppb of aflatoxin. While from the 10 samples of LRR, only one (1) sample (10%) was found to have been contaminated with aflatoxin with 2.91 ppb of aflatoxin. CRR and URR both registered 20% of aflatoxin contamination of the 10 and 15 samples analyzed, respectively. The two (2) contaminated samples from

Table 2. Aflatoxin B1 concentration of peanut butter samples from the six regions of The Gambia.

Region	No. of samples	AF-positive samples, *n* (%)	Range of contamination (ppb)	Mean of contamination (ppb)	SD (±)	CV (%)	LSD
GBA	20	0 (0)	ND	ND	0.00		
WCR	15	2 (13.33)	ND - 38.76	2.78	9.98		
LRR	10	1 (10)	ND - 2.91	0.29	0.92		
NBR	15	0 (0)	ND	ND	0.00	23.60	2.82
CRR	10	2 (20)	ND - 12.60	2.23	4.74		
URR	15	3 (20)	ND - 7.26	0.84	1.92		
Total	85	8 (9.41)					

ND= Not detected; ppb= parts per billion, SD = standard deviation, CV= coefficient of variation, Lsd=less significant difference.

Table 3. Aflatoxin B1 concentration of peanut butter samples from the collection points (CP).

CP	No. of sample	No. of positive samples, n (%)	Range of contamination (ppb)	Mean	SD	CV (%)	LSD
Homes	36	2 (5.56)	ND - 2.91	0.61	0.67		
Markets	30	4 (13.33)	ND - 38.76	1.71	7.14	29.40	3.87
Processors	19	2 (10.53)	ND - 12.60	1.17	3.54		

CRR recorded 12.60 and 9.65 ppb, while 2.91, 6.74 and 7.26 ppb from URR. Out of the total samples (85) analyzed, only eight (8) samples (9.4%) were found to have contaminated aflatoxin B1. Only 1.20% (1 of 85) of samples in WCR at the Collection Point “Homes” Table 3 with 38.76 ppb exceeded both the Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program of 15 µg/kg (ppb) intended for further processing (Codex Alimentarius Commission, 2001). Furthermore, only 5.90% (5 of 85) samples exceeded the EU maximum limit for total aflatoxins of 4 µg/kg (ppb) for groundnut, nuts, and processed products intended for direct human consumption or use as an ingredient in foodstuffs (EU, 2010). Only 1.20% (1 of 85) of samples exceeded all international acceptable limits with 38.76 ppb (Table 2).

A 42.35% were collected from homes, 35.29% from markets and 22.35 from processors (Table 3), two (2) came out positive for both homes and processors 5.56 and 10.53% and 4 samples (13.33%) from the markets (Figure 3).

DISCUSSION

These finding reports the level of aflatoxin contamination in peanut butter samples processed and consumed in The Gambia. The negative results of the peanut butter in the GBA which is mostly supplied from NBR could be as a result of the frequent and quick completion of peanut butter consignments sale due to the high demand of the densely population of the area (about 20% of the country’s population). In the same light, a 5 km ocean separates GBA from NBR, a region known as the most peanut grown hub. Consequently, the farmers in this agro-ecological zone (sudano-sahelian) are more experienced in good agronomical practices that mitigate aflatoxin in groundnut and practice proper post-harvest like drying, sorting and screening, thus the negative results. And unlike GBA, most consumed peanut butter is homemade in NBR.

Similar results were reported by Azer and Cooper (1991) who analyzed 73 samples of peanut and peanut butter and found the contamination in only one (1) sample equal to 61 µg/kg. Again in Turkey, where only one peanut butter sample from a total of 85 peanut and peanut product samples had 2.0 ppb (Ozay, 1989). A 91% (10 of 11) peanut butter aflatoxin contamination with a mean 75.66 ng/g was found in Zimbabwe (Mupunga, 2014) and 27% (3 of 11) exceeds the EU maximum limits of 4 ppb. In Sudan, a range of 26.6 to 853 µg/kg in peanut butter was reported with 90% exceeding the EU maximum limit (Elzupir et al., 2011). This two African countries are known to be prone to drought and erratic rainfall. A survey conducted in Taiwan showed aflatoxins were detected in 10 out of 21 peanut butter samples, but the highest level of AFB1 was only 2.59 µg/kg (Ying-Chun et al., 2013). Also, higher total aflatoxin level was reported in Nepal with 42.5% (43 of 101) of aflatoxin contamination (Koirala et al., 2005). This might be due to high rainfall, hot and humid conditions that are prevalent in the Asian subcontinent.

Though consumption of aflatoxin susceptive crops most specifically peanut and it products has been identified as a strong risk factor for hepatocellular carcinoma (HCC). A

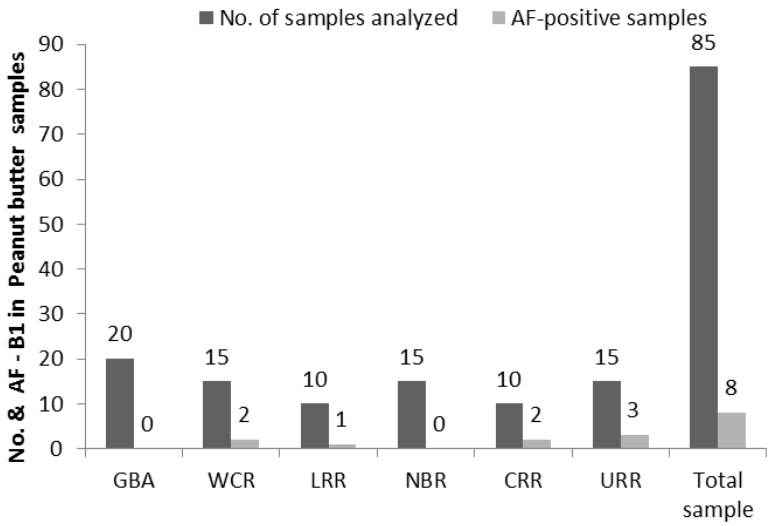

Figure 2. Aflatoxin contaminated samples at region.

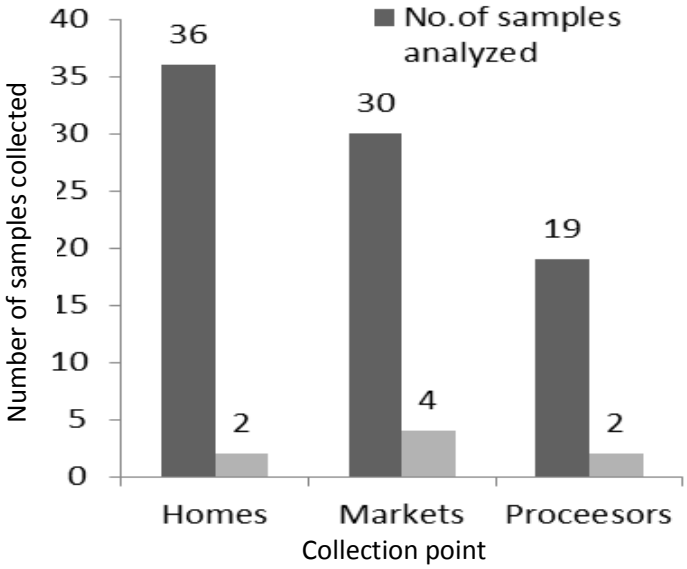

Figure 3. Aflatoxin contaminated samples at collection points.

report by PACA (2018d) revealed 285 liver cancer cases in 4 years in The Gambia.

Although, no established correlation was made to relate the cancer cases to the consumption of peanut and or its products or as a result of aflatoxin contamination. Other natural, environmental and habitual factors like, genetic makeup, exposure to hazardous chemical, smoking, alcohol, and underlying medical history may also be a factor to consider for the cancer cases. It is estimated that the risk of developing aflatoxin-induced liver cancer in The Gambia is 8.3 cases per 100,000 people. This is because of the HBV prevalence (15%) of the population (Gambia Aflatoxin Control, 2015). In The Gambia, HBV, HCV and aflatoxin exposure are known aetiologies of HCC with HBV accounting for a majority of cases. In a study, Bah et al. (2001) and Kirk et al. (2004) showed 10% cases of cancer were as a result of smoking**.** A Similar report indicates 57% of liver cancer cases in The Gambia are attributable to chronic hepatitis B infection (Kirk et al., 2006).

The low aflatoxin sequence of the CPs (Table 3) could be correlated to the shelf life of the product which might be as a result of the oil in it during production. Secondly, in the presence of phytoalexins, an inhibiting protein in seed for fungal colonization (Jallow et al., 2018) and time of sampling. Homemade peanut butter as expected,

recorded the lowest contamination level. This is certainly because more precautionary measures like sorting the black and moldy and all foreign materials are done with precisions and much care is also given in its storage, since is purposely for home and family consumption. The product is transformed to soap if it loses one of its organoleptic features. Processors serve as the first and transitional points of the product, fewer and lower contamination are as a result of less time spent at this point before entering its finally stage. The market recorded the highest aflatoxin level which could be due to contrary practices to homemade and possible adulterations of the peanut butter to increase its volume for profit.

When proper food safety practices are maintained, this study confidently shows that the peanut butter consumed in The Gambia is safe, and therefore encourages the continuation of the consumption of this delicious and cultural stew "*domoda"* in order to gain the maximum health benefits. As stated by Kelly and Sabaté (2006), 37% lower from cardiovascular disease (CVD) and stroke for regular consumers of the product than those who do not at all. Furthermore, a water soluble vitamin (B9) known as folate or folic acid found in peanuts could help in human growth and aid the normal functioning of nerves and brain and may also help protect against cancers of the lung, colon, and cervix (Fishman et al., 2000).

Again these findings are substantiated with the attestation of a popular US TV talkshow called The Drs (2013), where a dietitian described this Gambian stew as a factor that may lower cancer risk. As a result the country is having one of the lowest cancer cases in the world. Epidemiological studies have confirmed that consuming peanut and its products, and snack food, at least four to five times per week may contribute to protect against, type two diabetes and gallbladder disease (Hu et al., 1998; Jiang et al., 2002; Tsai et al., 2004), weight management (Jennette, 2005), no positive correlation to increase body mass index (BMI) (Hu and Stampfer, 1999). The high protein and high unsaturated fat nature of peanuts may also contribute to the lack of weight gain associated with peanut consumption (Johnston, 2005).

Conclusion

The overall results demonstrate that 90% (77 of 85 samples) were not contaminated with aflatoxin. From the contaminated samples, eight (9.41%) sample exceeded the EU limits of 2 µg/kg (ppb) for aflatoxin B1 for direct consumption and only one sample exceeds the Codex Alimentarius maximum limits of 15 ppb. This study reassures the safety of peanut butter consumed in The Gambia, and therefore encourages the frequent consumption of this peanut butter stew in order to gain the maximum health benefits it had in it. And also recommends a national policy and regulatory document with budgetary allocations for aflatoxin management activities should be setup for the nation to redeem herself from the huge aflatoxin economic impact she is facing. A regular awareness creation and analytical surveillance programs be conducted by food control agencies and stakeholders is highly recommended to monitor the incidences of aflatoxin contamination in the country.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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