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Levels of total aflatoxins in maize and groundnuts across food value chains, gender and Agro-ecological zones of Uganda

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Abstract

Maize and groundnuts are traditional nutrient-rich and high economic value foods grown in Uganda. However, these crops are susceptible to aflatoxin contamination which may result into aflatoxicosis related illnesses. Occurrence of flatoxins in the foods varies across food value chains, gender and agro-ecological zones of Uganda. Therefore, we conducted a cross-sectional study in 80 maize and groundnut foodstuff from Masindi and Soroti agro-ecological districts respectively. We determined levels of total aflatoxins in maize and groundnut samples across food value chains, gender and agro-ecological zones. This was aimed at assessing safety and quality status of the foods in Uganda. Questionnaires were administered to obtain information on food handling practices. 500g of each food sample were obtained and analyzed for total aflatoxin levels using ELISA assay. Data was analyzed using descriptive and analytical statistics. In overall, 45% of maize and 30% of groundnut foods were contaminated with aflatoxins. Mean aflatoxin levels in foods from Eastern and Western agro-ecological zones were 0.052±0.036ppb and 0.045±0.033ppb respectively (p=0.124). Mean aflatoxin levels in groundnuts from both male and female respondents were coincidentally 0.052ppb. Whereas, the mean aflatoxin levels in maize from male and female respondents were 0.056±0.037ppb and 0.039±0.029ppb respectively. Across the food value chain, wholesaler groundnut foods contained the highest mean aflatoxin levels of 0.088ppb (p= 0.27). Growing high polyamine containing crops, routine testing of aflatoxin prone foods and sensitizing food value chain players are important aflatoxin control strategies.

Keywords: Maize; Groundnuts; Aflatoxins; Food value chain; Gender; Agro-ecological zones

1 Introduction

Maize and ground nuts are traditional nutrient-rich foods in Uganda consumed by both humans and animals. However, recent research reports show that some cereals and legumes along the food value chain are contaminated with pathogenic micro-organisms [1, 2]. These microbes are capable of colonizing, contaminating variety of foods and transmitting food borne diseases from one individual to another [3]. Amongst the commonest microbes, are the aflatoxin-producing *Aspergillus flavus* and *Aspergillus parasiticus* fungi that mostly reside in the soil [3]. Aflatoxins are biologically active heterocyclic, oxygen containing mycotoxins that possess the bisdifuran ring system [4, 5]. About 18 different types of aflatoxins have been identified and the most commonly occurring ones are aflatoxin B1, B2, G1, G2, M1 and M2 [6]. The B-aflatoxins, the pentanone derivatives exhibit strong blue fluorescence under ultraviolet light while the G series (six-membered lactones) fluorescence yellow-green on thin-layer chromatography plates, thus the B and G naming [7, 8]. Aflatoxin B2 and G2 are dihydroxy derivatives of aflatoxin B1 and G1 and other aflatoxins are not usually

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reported in the absence of aflatoxin B1 [9]. The M aflatoxins (M1 and M2) are also derivatives of B series that exhibit blue-violet fluorescence and have been reported in milk products of animals fed on aflatoxin contaminated foods hence designation M [10]. Aflatoxin B1, G1 and M1 are major metabolites while aflatoxin B2, G2 and M2 are biotransformation products of the major metabolites [11].

In Uganda, aflatoxin contamination of foods is high and widespread. In one study, 65% of maize from Mubende district and 45% of maize from Kamwenge were found to contain aflatoxin concentration above the Ugandan aflatoxin maximum limit of 10ppb [3]. Ingestion of foods containing moderate to high levels of aflatoxins is characterized by a rapid and obvious onset of toxic responses, which include bleeding, acute liver damage, edema, digestive difficulties, and possibly death, usually within a week of exposure. Chronic aflatoxicosis may also occur in individuals who ingest low doses of aflatoxins in food over prolonged periods and is characterized by immune suppression, low birth weight, impaired child growth and [3] and liver cancer [12]. Different regions and Countries have set up acceptable maximum limits of mycotoxins. According to the European Union food regulatory standards, aflatoxin B_1 and total aflatoxins (B1+B2+G1+G2+M1+M2) in consumable cereal products should not exceed 2ppb and 4ppb respectively [13]. The Codex Alimentarius Commission also set the maximum tolerable level of total aflatoxins in 'ready to eat' tree nuts at 10ppb [14, 15] and peanuts destined for further processing at 15ppb [14, 15]. In comparison with aflatoxin standard limits in Africa, both the East African Region Standards Office [16] and most Countries in Africa [17] have set a tighter aflatoxin maximum edge of 5ppb for aflatoxin B1 and 10ppb for total aflatoxins. Unfortunately, few Ugandan commercial foods do meet this aflatoxin standard limit, hence the products end up being rejected by important international markets. The contaminated foods end up being sold in traditional markets hence increasing health hazards of aflatoxin consumption in the local communities. There are a number of factors believed to fuel aflatoxin contamination of foods in Uganda and these factors vary from biotic, abiotic to food handling practices [18]. In this study, we took the initiative to determine quantities of total aflatoxins in maize and groundnut crops across the food value chains, participants' gender and agro-ecological zones of Uganda. Since maize and groundnuts are among the commonly consumed local crops, it was imperative for us to assess the aflatoxin levels which are a recommendable measurable indicator of food safety and quality.

2 Material and methods

2.1 Study design and population set up

The study was a cross-sectional design and employed proportionate sampling after establishing the sampling frame. In January 2022, food chain dealers from the Western agro-ecological zone (Masindi district) and the Eastern agro-ecological zone (Soroti district) were recruited into the project comprising of transporters, processors, retailers, wholesalers, restaurant operators and producers. The Food and Agriculture Organization of the United Nations categorized Africa into five agro-ecological zones according to rainfall and the length of the growing season [20]. These include; Humid zone with up to 329 days growing period, Moist sub humid zone with up to 269 growing days, Dry sub humid zone with up to 179 growing days, Semiarid zone with up to 119 growing days and the Arid zone with less than 60 growing days [20]. According to Wortmann and Eledu, (1999) [21], Uganda's agro-ecological zones were defined by considering 25 variables which include; climatic variables (mean annual rainfall and temperature), soil variables, two population variables (population density and male: female ratio), four land use types (farmland, woodland, grassland and wetland) and ten food crop types (banana, maize, cassava, sweet potatoes, Irish potatoes, finger millet, beans, groundnuts, sorghum and rice). Western Savannah grassland is one of the agro-ecological zones located in Mid-Western part of Uganda. It covers 2423km^2 with temperatures above 20^{o}C and receives more than 1200mm of rainfall per year. In the Western agro-ecological zone, we selected Masindi district as the representative study site because it has diverse cropping system and is well known for growing maize [3]. The Eastern agro-ecological zone covers 1684km^2, with temperatures above 20^{o}C and receives more than 1000mm unimodal rainfall. The zone has a moderately low population density of 58 persons per km^2 and a very low male: female ratio [21]. From the Eastern agro-ecological zone, we chose Soroti district for groundnut sampling since it is well known for growing groundnut crops.

2.1.1 Sample collection and analysis

Structured questionnaires were administered to heads or representatives of the maize and groundnut value chain study units. Questionnaires were used obtain information from the respondents about maize and groundnut handling habits and practices that are responsible for aflatoxin contamination of the foods. 500grams of each food specimen (maize n= 40 and groundnut n=40) were bought from maize and groundnut value chain dealers in Masindi and Soroti agro-ecological districts respectively. The samples were packaged in sterile zip lock bags, sealed, labeled and placed in ice cool boxes at temperature range between 2^{o}C to 6^{o}C. Thereafter, samples were transported to the Uganda Industrial Research Institute and analyzed for aflatoxins using ELISA method as described by Florence et al., (2023) [19].

2.1.2 Statistical analysis

Data analysis was performed using both descriptive statistics and analytical statistics based on STATA statistical package. Tests of association were based on chi-square tests at 5% level of significance, which compared the proportions of the outcome of aflatoxin contamination across the factors for both maize and groundnuts.

3 Results

Eighty (80) maize and groundnut samples were collected from male and female value chain dealers in two agro-ecological zones. The food types collected consisted of raw flour/paste (47.4%), raw shelled/kobbed grains (26.3%), raw unshelled/dekobbed grains (21%) and boiled/roasted foodstuff (5.3%). The study assessed total aflatoxin levels in the foods across various respondents as presented below;

3.1 Agro-ecological zones

From the Western agro-ecological zone, Masindi district provided n=40 maize foods of which 45% (18 out of 40) of the samples were contaminated with aflatoxins. Meanwhile, Soroti district in the Eastern agro-ecological zone provided n=40 groundnut foods where by 30% (12 out of 40) of the samples were found to contain aflatoxins *(Figure 1)*. Mean aflatoxin contents in the samples from the Eastern and Western agro-ecological zones were 0.052±0.036ppb and 0.045±0.033ppb respectively. Mean room (operating) temperatures for Masindi and Soroti agro-ecological districts were 33.2^{0}C and 32.9^{0}C respectively. The difference in aflatoxin contamination between the two agro-ecological zones was not statistically significant (p-value=0.124).

3.2 Gender of respondents

Of the respondents who provided maize samples, 42.5% (n=17) were males while 57.5% (n=23) were females. For the respondents who provided groundnut samples, 17.5% (n=7) were males whereas 82.5% (n=33) were females. In *Table 1,* female respondents (10 out of 33; 30%) had more aflatoxin contaminated groundnuts than male counterparts (2 out of 7; 28.6%). On the other hand, male respondents (8 out of 17; 47.1%) registered higher aflatoxin contamination of maize foods than females (10 out of 23; 43.5%). Of the 20 female respondents who had aflatoxin contaminated maize and groundnuts, 10% (n=2) stored their foods inappropriately in open and torn sacks, 15% (n=3) dried their foods on bare ground, 20% (n=4) had their foods in unshelled/dekobbed form while 55% (n=11) both dried their foods on bare ground and stored them in torn sacks. Out of the 10 male respondents who provided aflatoxin contaminated maize and groundnut foods, 50% (n=5) had unsorted maize containing particulate matter while 50% (n=5) had undercooked foods. Mean aflatoxin levels in groundnuts from both male and female respondents was coincidentally 0.052ppb while maize foods from male respondents contained higher mean aflatoxin levels (0.056±0.037ppb) than those from female respondents (0.039±0.029ppb). In both food types, there was no statistical difference in aflatoxin contamination between males and females (males; p-value: 0.93, female; p-value: 0.82)

3.3 Value chain cadres

The maize value chain was composed of n=10 (25%) retailers, n=8 (20%) restaurants, n=8 (20%) farmers, n=6 (15%) processors, n=6 (15%) wholesalers and n=2 (5%) transporters. The groundnut value chain consisted of n=11 (27.5%) retailers, n=11 (27.5%) restaurants, n=8 (20%) processors, n=5 (12.5%) farmers, n=4 (10%) wholesalers and n=1 (2.5%) transporter. In the maize value category, 50% of the maize foods bought from retailers, wholesalers, transporters and processors were contaminated with aflatoxins. 50% (1 out of 2) of the maize transporters never covered their foods from dust during transit, 83.3% (5 out of 6) of the maize wholesalers and 100% (10 out of 10) of the maize retailers did not routinely monitor the growth conditions for aflatoxin producing moulds. Meanwhile, 100% and 45.5% of groundnuts bought from transporters and restaurants respectively were contaminated *(Table 1)*. The groundnut transporter also did not cover food during transit while 66.7% (6 out of 9) of the groundnut restaurant owners never adequately cooked or roasted their foods. The highest mean aflatoxin levels were observed in groundnut samples from wholesalers (0.088ppb) followed by maize foods from farmers (0.062 ±0.018ppb). However, aflatoxin contamination across the value chain categories for maize (p-value; 0.98) and for ground nuts (p-value; 0.27) were not statistically significant.

Table 1 Occurrence of total aflatoxins in maize and groundnut samples across food value chains, participants' gender and two agro-ecological zones

	Groundnuts		**Maize**	
Respondents	**Proportion of aflatoxin positive samples n/N (%)**	**Mean±S.D levels of aflatoxins in samples (ppb)**	**Proportion of aflatoxin positive samples n/N (%)**	**Mean±S.D levels of aflatoxins in samples(ppb)**
Agro-ecological zone				
Western (Masindi)			18/40 (45)	0.045 ±0.033
Eastern (Soroti)	12/40 (30)	0.052 ±0.036		
Gender of the respondent				
Males	2/7 (28.6)	0.052 ±0.040	8/17 (47.1)	0.056 ±0.037
Females	10/33 (30.3)	0.052 ±0.038	10/23 (43.5)	0.039 ±0.029
Value chain category				
Farmers	1/5 (20)	0.055	3/8 (37.5)	0.062 ±0.018
Transporters	1/1 (100)	0.031	1/2 (50)	0.034
Wholesalers	1/4 (25)	0.088	3/6 (50)	0.053 ±0.050
Retailers	1/11 (9.1)	0.016	5/10 (50)	0.037 ±0.038
Processors	3/8 (37.5)	0.07 ±0.029	3/6 (50)	0.059 ±0.035
Restaurants	5/11	0.054 ±0.047	3/8	0.034 ±0.033

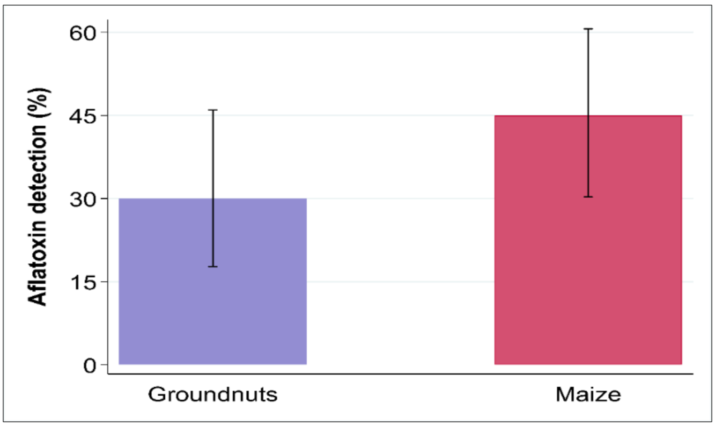

Figure 1 Percentage of aflatoxins in maize and groundnut samples

4 Discussion

In Uganda, maize is the most consumed cereal and a main export earner of worth US $ 27,277,000 while groundnuts are the second most important legumes [3]. However, these foods are prone to mycotoxin contamination due to a number of factors that propagate growth of mycotoxin producing fungal species [18]. In our study, we found out that both agro-ecological zones contained some aflatoxin contaminated foods. One of the most unique abiotic factors observed in the two agro-ecological districts is moderate temperature (30^{0}C to 37^{0}C) that favors growth of mesophilic aspergillus species. One of the probable mesophiles is aflatoxin producing aspergillus fumigatus which is capable of growing rapidly up to 37^{0}C and tolerating temperatures above 50^{0}C due to thermotolerance factors [26, 27]. However, size of the study food samples was small to give sufficient statistical power to detect the difference in aflatoxin contamination between maize and groundnuts from the agro-ecological zones. On a good note, detectable levels of aflatoxins in all the food types were lower than the recommended maximum total aflatoxin limit of 10ppb [16, 17]. Samples were collected during the month of January which is a dry season for both Masindi and Soroti agro-ecological districts. During the dry spell, water content in the atmosphere is always low and the resulting dehydration state does not favor production and accumulation of large amounts of aflatoxins. Therefore, all foods were deemed safe for human consumption and eligible for sale across international markets. In comparison with a similar study by PACA, (2017) [3], 97% of the mean maize grains sampled from the Western agro-ecological districts of Masindi, Kamwenge and Mubende and 40% of the mean groundnut samples from Soroti, Iganga and Tororo districts in the Eastern agro-ecological zone were contaminated with aflatoxins. Sadly, almost all the foods contained unacceptable levels of total aflatoxins and this put consumers at increased risk of developing illnesses associated with aflatoxicosis. [12]. This study was carried out in 2017 where by majority of the agricultural value chain actors were not aware of aflatoxins and their negative effects on agriculture. Hence, this could explain the high proportions of aflatoxin positive foods registered by the above study. In another research by Timothy Omara, (2019) [22], highest concentration of total aflatoxins were recorded amongst maize samples got from Hoima district located in the Western agro-ecological region compared to other agro-ecological districts. Because of the high nutritional and economic value, maize is routinely and widely grown hence creating a higher possibility of the crop being colonized by ecologically favored aflatoxin producing fungi [23, 24]. Furthermore, maize and groundnuts have various quantities of biotic factors like polyamines which play important roles in aflatoxin resistance and drought tolerance [28]. Polyamines are found in all forms of plant life but their relative amounts depend on tissue types, developmental stages, and coexistence of other abiotic factors. Since polyamines are universal and pivotal in crop survival and aflatoxin resistance, efforts should be put in exploring maize and groundnut crop breeds with high polyamine levels.

The maize and groundnut value chain composed of retailers, restaurants, processors, farmers, wholesalers and transporters in the hierarchical order *(Table 1)*. The study recruited more retailers probably because of their field abundance over the other value chain cadres at that particular time. 50% of the maize foods bought from transporters, wholesalers, retailers and processors were contaminated with aflatoxins while all the ground nuts bought from transporters and nearly half of them bought from restaurants were also contaminated with aflatoxins. The number of study respondents was small to give statistical power about difference in aflatoxin contamination across the food value chains. Majority of the contaminated ground nut and maize foods were roasted samples and were bought from home-based restaurants with evidence of low sanitation levels. According to O' Neil et. al, (2001) [25], melting temperature of aflatoxins ranges from 237^0C to 299^0C. It is uncertain that roasting yields heat ≥ 237 ^{0}C that is capable of degrading aflatoxins. This could explain the higher occurrence of aflatoxins in the roasted ground nuts and maize foods. In addition, transporters were observed to carry their foods in damaged sacks and the trucks they used in transporting goods were dusty. Dust provides a medium for harboring spores of aspergillus species. Under favorable conditions, spores become viable and end up producing aflatoxins that cause food contamination. The study also observed that most wholesalers had warehouses which stored the foods up to a month. However, most of them did not routinely inspect the foods for mold growth, insects and other foreign materials. Even worse, all wholesalers under the maize value chain did not participate in the monitoring of storage temperature, humidity and moisture. This could explain why half of the maize foods were contaminated with aflatoxins. Meanwhile, wholesalers dealing in groundnuts had the highest mean total aflatoxin levels despite carrying out routine monitoring and inspection of their foods. We did not have a justifiable explanation for this phenomenon since the sample size was small. Most retailers had market stalls and those dealing in maize barely carried out routine food inspection. More to that, maize bought from retailers had overstayed in storage stalls before being sold out to consumers. This implies that poor storage and monitoring practices amongst retailers were responsible for the higher contamination of maize foods compared to groundnuts. Farmers dealing in both maize and groundnuts had a common practice of drying food on bare ground. As groundnut crop farmers dried groundnuts in pods which are protective coats against aspergillus infiltration, maize crop farmers instead dried their maize in de-shelved aflatoxin susceptible form. We can relate this to the high mean total aflatoxin levels in maize foods from farmers. As an intervention strategy, there is need to carry out sensitization and re-training of food value players on safe food handling practices and storage methods.

According to Guerra & Abad, (2019) [29], Gender plays an important role in disease epidemiology and aflatoxicosis is not exceptional. Guerra & Abad classified hypotheses that explain gender bias in disease burdens into behavioral and physiological. Physiological hypothesis explains biological differences between sexes while behavior hypothesis elaborates on the gender specific food handling practices responsible for aflatoxin contamination. In this study, female respondents across the food value chain provided more maize and food samples than the male counterparts. However, foods supplied by men respondents contained higher mean aflatoxin levels than women. We noted that most male respondents neither sorted out debris from their merchandise nor undercooked the food which they sold. Presence of debris provides a breeding ground for aspergillus species while undercooking propagates aflatoxins accumulation. Despite having lower mean aflatoxin levels in the foods, female respondents practiced poor crop handling methods characterized by inappropriate storage of foodstuffs in open torn sacks, drying foods on bare ground and other practices prone to aflatoxin contamination. In addition, the study sample size could not give us enough statistical power to differentiate aflatoxin contamination between male and female respondents. Based on the above findings, food is prone to aflatoxin contamination. There is need to carry out routine assessment of aflatoxin levels in foodstuff across the various crop value chains. Secondly, value chain players need to sensitized and routinely trained on good food handling practices so as to ensure food safety and quality in Uganda.

The study had funding limitations. Shortage of funds limited our capacity to collect large number of representative food samples.

5 Conclusion

In our study, we found out that total aflatoxin levels in maize and groundnuts across food value chains, gender and agro-ecological zones were within the Uganda's acceptable maximum total aflatoxin limit. The study was limited by shortage of funds and so sample size was not large enough. Since biotic factors such as polyamines are pivotal at increasing crop resistance to aflatoxins, they can be explored as a probable strategy of producing aflatoxin resistant food crops. Routine testing of aflatoxin prone foods, sensitization and re-training of food value chain players are important.

Recommendations

The study recommends establishment of routine food testing services for common food borne diseases. This strategy can also include development of quick, accurate and reliable aflatoxin testing kits. Secondly, food value chain players need to be sensitized and re-trained routinely on good food handling practices.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

The study was approved by the Mbale Regional Referral Hospital Research and Ethics Committee. Written consent was also obtained from Soroti and Masindi study district headquarters prior to field data collection.

Statement of informed consent

Informed consent was obtained from all respondents who participated in the study. All their information was kept confidential.

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