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Research Article

Prevalence of Aflatoxin Contamination Across the Food Value Chain for Maize and Groundnuts in Uganda

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Abstract

Background: Maize and groundnuts are some of the most highly contaminated foods on the Ugandan market. Since 2006 there have been some scattered studies on the prevalence of aflatoxin contamination among these grains. However, there were no recent studies of 2022 to highlight the status of aflatoxin contamination.

Methods: Grain samples were collected from two highest producing districts that is Masindi and Soroti districts; and across the value chain, from farmers to wholesalers, retailers, processors and restaurants/homes. The samples were tested using ELISA method and the prevalence calculated.

Results: Aflatoxins were detected in 45% and 30% of maize and groundnuts, respectively. Contamination increases over the value chain from 31% at farm level, to 42% at table level. In general, contamination was highest among processors at 43%, followed by restaurants (42%), wholesalers (40%), farmers (31%) and retailers at 29%.

Keywords: Aflatoxins; Prevalence; Maize; Corn; Groundnuts; Gnuts; Uganda; Value Chain

Introduction

Globally, about 25% of food produced is contaminated with aflatoxins [1]. Aflatoxins are biologically active heterocyclic, oxygen containing mycotoxins that possess bisdifuran ring system [2,3]. Aflatoxins are produced by fungi especially Aspergillus flavus and Aspergillus parasiticus which grow on poorly managed agricultural crops and agro-products mainly ground nuts, rice, millet, sorghum, cassava and maize. About 18 different types of aflatoxins have been identified and the most commonly occurring ones are aflatoxin B1, B2, G1, G2, M1 and M2 [4]. B-aflatoxins (the pentanone derivatives) exhibit strong blue fluorescence under ultraviolet light while G aflatoxins (six-membered lactones) fluorescence yellow-green on thin-layer chromatography plates, thus the B and G naming [5]. Aflatoxin B2 and G2 are dihydroxy derivatives of aflatoxin B1 and G1 respectively [6]. 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 [7]. Aflatoxin B1, G1 and M1 are regarded as major metabolites while aflatoxin B2, G2 and M2 are biotransformation products of the major metabolites [8].

Consumption of aflatoxin contaminated foods results in several health-related conditions including aflatoxicosis, immune suppression, liver cancer, liver cirrhosis and nutrition-related problems such as stunted growth in children.

Maize and ground nuts are staple foods in Uganda and are sources of various nutrients for both animals and humans. Traditionally, these foods are consumed in different forms; such as fresh nuts, roasted, and more recently, as paste and powder (flour). However, these foods are prone to aflatoxin contamination which may happen during pre-harvest or post-harvest activities. Agro-ecological zones have climatic conditions characterized by heavy rains, changing droughts, ambient temperatures, high humidity, poor pre- and post-harvest handling practices by famers and traders also fuel aflatoxin contamination in Uganda [9-11].

In a study carried out in Gambia and Benin, 90% of children had traceable amounts of aflatoxin albumin adducts as opposed to less than 1% of detectable rate in the developed countries [12]. The exposure pattern is a huge public health burden to children especially in Africa. In Uganda, high populations of aflatoxin producing fungi are present in staple foods sold on informal market outlets [13], of which some of them have contamination rates way above the recommended levels set by Uganda National Bureau of Standards (UNBS) and the World Health Organization (WHO) [14,15,18].

In a study by Omara [16], the mean total aflatoxin content of maize samples obtained from Hoima, Mayuge and Ibanda districts were 11.0 μg/ kg, 10.6 μg/kg and 10.1 μg/kg respectively, equivalent to the UNBS maximum limit of 10 ppb. In another related study carried out in various agro-ecological regions of Uganda, up to 65% of maize in Mubende district and 45% in Kamwenge district contained aflatoxin levels exceeding the UNBS maximum limit of 10 ppb [17]. Meanwhile, up to 30% of groundnuts in Iganga district have aflatoxin content above 10 ppb [17].

Prevalence studies have been conducted on maize in Masindi and Groundnuts in Soroti. The PACA 2017 [17] report indicates that the prevalence of aflatoxin contamination of maize in Masindi district is at 25% while contamination of groundnuts in Soroti is at 20%. However, no study, according to our knowledge provides the level of contamination across the value chain from farmers to consumers (farm to fork).

In this study, we therefore specifically determined the prevalence of aflatoxin contamination of maize and ground nuts in Masindi and Soroti districts across the food value chain among farmers, wholesalers, transporters, retailers, processors and restaurants. Information obtained will shed more light on the specific parts of the food chain that require more urgent attention, inorder to reduce the aflatoxin contamination on Uganda grown grains.

Materials and Methods

Study design and population set up

Masindi and Soroti districts are located in the Western and Eastern agro-ecological zones of Uganda respectively. Masindi was chosen because it is the second highest maize producing district in Western Uganda and is aflatoxin prone [17]. The maize variety commonly grown in Masindi is *Zea mayis* L. Soroti is the highest groundnut producing district in Uganda and is also aflatoxin prone [17]. The Groundnuts variety commonly grown in Soroti is *Arachis hypogaea* L.

The study employed proportionate sampling after establishing the sampling frame. In January 2022, a total of 80 food samples were obtained from chain dealers in Masindi and Soroti districts. Producers comprised of food samples picked from homes or households of farmers or weekly markets. Transporters from pickup trucks, wholesalers were sourced from wholesale shops, produce granaries/storage bans or exporter suppliers. Retailers were from retail shops, supermarkets or local markets while processors are those with value addition facilities, mainly transformation to paste, flour or pellets. Cooked food was sourced from homesteads, restaurants, hotels or roadside street food. The food was in form of boiled paste (posho, porridge), sauce, roasted seeds or boiled seeds.

The samples were randomly selected among willing participants who provided written consent to participate in the study.

Sample collection and analyses

A structured questionnaire was administered to participants of the study to obtain knowledge, attitudes and practices associated with food contamination by aflatoxins. 500grams of each food specimen (maize n = 40 and groundnut n = 40) were bought and packaged in sterile zip lock bags, sealed, labeled and placed in ice cool boxes at temperature range between 2°C to 6°C. Thereafter, samples were transported to the Uganda Industrial Research Institute Laboratory and analyzed using ELISA method. ELISA is a quantitative test that returns numbers representing the level of contamination, meaning that the higher the number, the more contaminated the sample. We used the Elabscience AF (Total Aflatoxin) ELISA Kit [19].

The ELISA method

The ELISA assay involved the following sample pre-treatment and analysis steps.

Sample pre-treatment

We homogenized the samples (maize andgroundnuts) with a homogenizer and mixed them thoroughly. Weighed 2grams of homogenate sample into the 50 mlscentrifuge tube and added 5 mls of 70% Methanol. Vortexed the mixture for 5 min, centrifuged at 4000 r/min for 10 min at roomtemperature, transferred 0.5 mls of supernatant to another centrifuge tube, added 0.5 mls of deionized water, mixed fully.

Sample analysis

All reagents and samples were restored to room temperature (25°C) before use. All reagents were mixed thoroughly by gently swirling before pipetting. Multiple wells withsample and standard numbers in the ELISA plate were labeled. We then added 50 μL of standard and sample per well, added 50 μL ofHRP Conjugate to each well, then added 50 μL of antibody working solution. We covered the plate with plate sealer, oscillatedthe mixture for 5 seconds and incubated for 30 min at 25°C in shading light. Immediatelyadded 300μL of wash buffer to each well andwashed. We repeated the wash procedure fivetimes, at an interval of 30 seconds, then inverted the plate and pat it against thickclean absorbent paper. 50 μL of substrate reagent A was added to each well, and another 50 μL of substrate reagent B. Gently oscillated for 5 seconds to thoroughly mix thepreparation and incubated at 25°C for 15 minin shading light. We added 50 μL of stopsolution to each well, oscillated gently to mixthoroughly. Thereafter, determined the optical density (OD) of each well at 450 nmusing microplate reader.

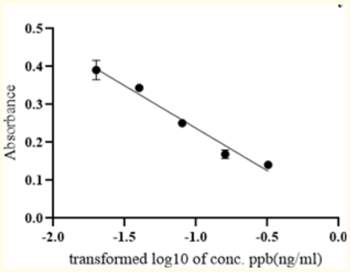

Figure 1: The Standard curve obtained.

We created a standard curve displayed in Figure 1, by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot.

A sample was declared positive once aflatoxins were detected and declared negative if no aflatoxins were detected. The test was sensitive to aflatoxin concentrates of as low as 0.01 ng/ml.

Statistical analysis

Data analysis was performed using both descriptive and analytical statistics. Prevalence of aflatoxin contamination of food was calculated using the formula below Where P refers to prevalence of Aflatoxin contamination, N the Number of food types contaminated with aflatoxins and T as the Total number of contaminated and uncontaminated food samples.

$$P = \frac{\mathrm{N}}{\mathrm{T}} * 100$$

Tests of association were based on chi-squaretests at 5% level of significance, which compared the proportions of the outcome of aflatoxin contamination across the foodsamples (maize and groundnuts) and the foodvalue chain.

Results

Out of 80 respondents who participated in thestudy, 70% (n = 56) of them were femaleswhile 30% (n = 24) were males. Theproportion of females was substantially higher among participants who provided groundnut samples than among those who provided maize samples (82.5%, n = 33 versus57.5%, n = 23). Amongst males, theproportion of participants who providedmaize samples (42.5%, n = 17) were higher than those who provided groundnut samples (17.5%, n = 7). The food value chain was composed of n = 21 (26.3%) retailers, n = 19 (23.6%) restaurants, n = 14 (17.5%) processors, n = 13 (16.3%) farmers, n = 10 (12.5%) wholesalers and n = 3 (3.8%) transporters.

Prevalence of aflatoxin contamination of maize and groundnuts samples

All the 80 maize and ground nut samples were tested for aflatoxins using ELISA method with diagnostic sensitivity of 0.01 ng/ml. Thirty (30) samples were found contaminated with aflatoxins representing theoverall prevalence of 37.5% wherecontamination was higher in maize (45%, n = 18) than in groundnuts (30%, n = 12). Figure 1 illustrates the overall level of contaminationof both grains.

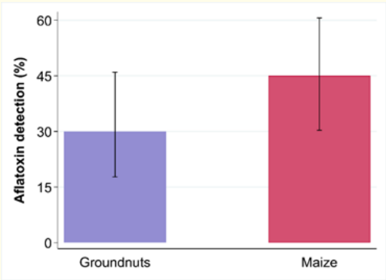

Figure 2: Prevalence of aflatoxin by the type of grain.

Among those contaminated, the overall meanaflatoxin levels was 0.057 ng/ml (Standard deviation = 0.034) broken down as 0.055 as the mean contamination for maize and 0.062for groundnuts. However, the number of samples collected could not provide sufficient statistical power to detect the aflatoxin contamination difference between maize and ground nuts (p-value = 0.124).

Table 1 provides details of the prevalence of the contaminated samples.

Agro-Ecological Zone (District)	Sample (N)	Positive samples n (%)	Mean Aflatoxin Levels (SD)
Western (Masindi)	Maize(40)	18 (45)	0.055 (0.033)
Eastern (Soroti)	Groundnuts (40)	12 (30)	0.062 (0.036)
Western and Eastern (Masindi and Soroti)	Maize and groundnuts (80)	30 (37.5)	0.057 (0.034)

Table 1: Occurrence of aflatoxin in maize and groundnut from Western and Eastern agro-ecological regions in Uganda.

Prevalence of aflatoxin contamination across the food value chain

Prevalence of aflatoxin contamination across the value chain is provided in figure 3. It is observed that for maize, contamination seems to be constant across the value chain with high contamination prevalence of almost half of the samples contaminated at each of the stages. The story is different for groundnuts, which exhibits an increase across the value chain. Figure 3 illustrates the results which suggest that aflatoxin contamination increases over the value chain of groundnuts from 20% at farm level, reaching 45% in restaurants.

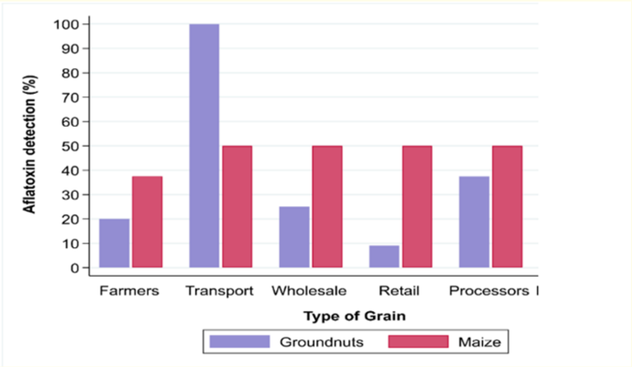

Figure 3: Prevalence of aflatoxin contamination across the value chain by groundnuts and maize.

The detailed prevalence of aflatoxin contamination is across the value chain is provided in Table 2 which shows that for groundnuts, 1 of 5 (20%) of samples at farm level are contaminated, 1 of 1 (100%) in transit (transporters), 1 of 4 (25%) among wholesalers, 1 of 11 (9.1%) among retailers, 3 of 8 (37.5%) among processors and 5 of 11 (45.5%) among restaurants. The one sample obtained during transit is too small to generalize contamination among transporters of groundnuts. So, the highest contamination is registered among processors and restaurants.

Food value category	Groundnuts		Maize		
	Number of samples N1	Prevalence n1 (%)	Number of samples (N2)	Prevalence n2 (%)	Total (T)
Farmers	5	1 (20.0)	8	3 (37.5)	13
Transporters	1	1 (100.0)	2	1 (50.0)	3
Wholesalers	4	1 (25.0)	6	3 (50.0)	10
Retailers	11	1 (9.1)	10	5 (50.0)	21
Processors	8	3 (37.5)	6	3 (50.0)	14
Restaurants	11	5 (45.5)	8	3 (37.5)	19
				Total	80

Table 2: Aflatoxin contamination across the maize and groundnut value chains.

The specific prevalence of aflatoxin contamination of maize samples ranged from 37.5% (3 of 8 samples) amongst farmers, 50%, (1 of 2) among transporters, 50%, (3 of 6) wholesalers, 50% (5 of 10) retailers, 50%, (3 of 6) processors and 37.5% (3 of 8) restaurants. There was no statistical difference in aflatoxin contamination amongst the maize food value chain categories (p = 0.788).

In general, contamination was highest among processors at 43%, followed by restaurants (42%), wholesalers (40%), farmers (31%) and retailers at 29%.

Assorted Prevalence of aflatoxin contamination within the food value chains

Our results revealed some interesting trends such as:

- 25% of grains picked from home (the farmers directly) was contaminated.
- 37.5% of food in the warehouses was contaminated.
- Contamination seems to drop at retail level for groundnuts (14.3%) but does not for maize (50%).
- 35% of grains in the local market were contaminated as opposed to 0% from supermarkets and local shops

- 60% of groundnut paste was contaminated as opposed to 0% of groundnut flour
- 75% of groundnut boiled sauce was contaminated as opposed to 40% of roasted seeds
- Boiled seeds are 13% healthier than roasted ones.
- 75% of the sauce in restaurants was contaminated as opposed to 35% in homes

Discussion

This study has revealed that overall, maize is more contaminated than groundnuts which tallys with the previous studies by [20] for the Sahel region of Africa - Burkina Faso, Mali, and Niger and by PACA [16] for Uganda. Secondly, the overall contamination of maize from Masindi district, has increased from 25% in 2017 to 45% in 2022, while that in groundnuts has increased from 20% in 2017 to 30% in 2022 [17,21].

Notable is that all the samples we obtained registered aflatoxin levels below the threshold limit of 10ng/ml as set by the Uganda National Bureau of Standards. However, the detected aflatoxins can easily multiple quickly to reach the unhealthy limits in a short time. Also, the samples were picked from production districts and the levels of contamination is likely to change for samples sourced from the big cities (non production districts) in Uganda. This means that more effort is needed to bring the prevalence levels down.

It is interesting to note that contamination is lowest among retailers. This is due to the fact that 92% of retailers in this study reported that they sort out their grains by removing the damaged ones and also their stock spends little time since they buy less due to space constraints, and sell it of quickly.

The results of this study reveal that contamination at wholesale levels is 19% higher than that at retail level. These results differ from those by Kaaya [15], which reported higher contamination levels among retailers. The possible reasons to explain the shift is that in 2022, retailers are more likely than wholesalers, to sort out the grains.

Contamination is highest among processors because they don't sort out the seeds, but crush them all into paste or floor. Most of the rejected seeds end up at the processors who simply convert them into another form that can not be visibly inspected for signs of contamination, unlike seeds. Restaurants also exhibited high levels of contamination because they are more likely to buy paste/floor than seeds, while homes of producing districts are more likely to crush the seeds themselves and thereby sorting out what to crush.

This study has revealed that processed seeds are more likely to be contaminated compared to the seeds. This tallys with previous work by Muzoora [21]. However, in 2017 the proportion of contaminated groundnuts from Soroti was 12.7% as opposed to 30% in 2022.

It is surprising to note that contamination at farm/household level has reduced from 60% in 2006 to 31% in 2022 [15]. This may indicate effective sensitization of farmers (and retailers) in best practices against contamination. There is need to increase sensitization drives to all actors across the value chain, especially the processors and restaurant workers.

Conclusion

This study delved into details of contamination of Ugandan maize and groundnuts along the value chain in 2022. The findings reveal that maize remains more contaminated than groundnuts and contamination levels remain almost constant across the value chain, unlike for groundnuts which increases across the value chain. Secondly, if the results show contamination of as much as 30% at the producing district level, then how much more will manifest in the non-producing or commercial (urban) districts? The increase in prevalence rates across the years in these two districts can only indicate the same trend for all the districts in Uganda. Therefore, there is urgent need to curb the aflatoxin contamination rates across the whole value chain, especially among processors and restaurants.

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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 person was also obtained from Soroti and Masindi study district headquarters

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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