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Research Article

Aflatoxin Susceptible Food Consumption Frequency, Prevalence, and Levels in Household Foodstuffs in Southwestern Uganda

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Contamination of household foodstuffs by aflatoxins has been associated with many illnesses, especially hepatocellular cancer and malnutrition. Aflatoxins are toxins produced by fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus,* usually found in food. Literature concerning the S.W. Ugandan foods that are the main aflatoxicosis route and therefore need most aflatoxin preventive measure is scanty. The current study determined the aflatoxin-susceptible food consumption frequency, prevalence, and levels of aflatoxins in selected foodstuffs in households in S.W. Uganda to establish the main food route of aflatoxicosis. Following a food frequency questionnaire, flour samples of common foodstuffs, namely, groundnuts, maize, millet, and sorghum, were randomly picked from seven districts of Southwest Uganda and analyzed for the presence and levels of aflatoxins using competitive ELISA. On average, maize and groundnut were found to be the most frequently consumed foods (seven times a week) by every family. Groundnuts had the highest mean aflatoxin level (96.5 ± 13.37 μg/kg), ranging from 6.2 to 297.3 μg/kg. Over 90% of the groundnut samples had mean aflatoxin levels greater than 10 μg/kg, the East African regulatory limit. Maize flour had a mean aflatoxin level of 34.1 ± 14.1 μg/kg, with one sample registering 336.5 μg/kg. This study found that groundnuts were the main food-route for aflatoxicosis followed by maize flour. In addition, the study re-affirmed the high prevalence and levels of aflatoxins in common food stuff in households in S.W. Uganda reported by previous studies. This study recommends further studies to elucidate its association with the observed recent increase in diseases like hepatocellular cancer and malnutrition in the region.

1. Introduction

Globally, more than 5.5 billion people are continuously exposed to different levels of aflatoxins [1]. Aflatoxins are a group of closely related mycotoxins produced by fungi such as *Aspergillus flavus, A. parasiticus, A. nomius*, *A. niger*, *A. pseudonomius*, and *A. tamarii* [2]. The most common aflatoxigenic species present in Uganda are *Aspergillus flavus* (63%), *A. tamarii* (14%), and *A. niger* (23%) [3]. Aflatoxins have detrimental effects on human health and economics [4]. Ingestion of aflatoxins in contaminated foodstuffs is of major public health concern because these toxins are nephrotoxic, immunotoxic, teratogenic, and mutagenic [5]. Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are found

in milk/milk products and in urine of animals or urine of people that consume feeds or foods, respectively, containing aflatoxin B1 (AFB1) or aflatoxin B2 (AFB2) [6]. The major food sources of aflatoxin exposure are maize and groundnuts [7]. The aflatoxins of health importance include AFB1, AFB2, aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Of these, AFB1 contributes up to 75% of the contamination and is the most toxic [8].

Aflatoxins are most prevalent in tropical and subtropical areas where temperature and humidity are more favorable for the growth of the aflatoxigenic fungi [9]. Aflatoxicosis claims 12–13 per 100,000 liver cases annually in countries like Kenya, Mozambique, China, Phillipines, USA, and Swaziland, which usually consume the susceptible foods [10]. Several episodes have occurred in EAC, e.g., in Kenya, outbreaks of acute aflatoxicosis claimed many lives between 2004 and 2008 [10, 11]. In 2004, an aflatoxicosis outbreak in Kenya resulted in 317 cases and 39% mortality [12]. The first case of death by aflatoxin poisoning in Uganda was recorded in 1970. This was a 15-year-old boy with abdominal pain and swelling who died in two days. Postmortem results showed that he had hepatic necrosis, pulmonary edema, and a dilated flabby heart. Researchers eventually found that the sample of the cassava meal this boy and his surviving siblings had consumed was aflatoxin contaminated at 1700 ppb [13].

The tolerable limits of aflatoxins in foodstuffs have been set as 15 to 20 μg/kg by different regulatory authorities and scientific committees in Canada and USA in an attempt to reduce aflatoxin ingestion [14]. The Codex committee proposed a limit of 0.5 to 15 μg/kg, European commission limits of 2 μg/kg for AFB1 and 4 μg/kg for total aflatoxin, World Food Programme (WFP), 10 μg/kg [15], Food and Drug Administration (FDA) 20 μg/kg [16], and the East African community, 10 μg/kg for total and 5 μg/kg for aflatoxin B1 alone [17].

Aflatoxins are implicated in many diseases such as cancer, abdominal pain, pulmonary and cerebral edema, fatty liver, kidney and heart diseases, immunosuppression, and convulsions, among others [7]. They also contribute to the occurrence of malnutrition such as marasmus and kwashiorkor in children [7, 18]. AFB1 induces cancer in the liver, kidney, colorectal, breast, ovary, small intestine, and other body parts [6]. It impairs the biological functions of the kidneys, lungs, and brain, including blood coagulation [7].

During metabolism, AFB1 is oxidized to aflatoxin B1-8,9-epoxide by cytochrome P_{450} family enzymes, i.e., CYP1A2 and CYP3A4 [19]. The epoxide then reacts with N7 of the nitrogenous base guanine turning it to thymine through transversion, thereby halting gene transcription and translation. When this occurs in the third position of codon 249, in the tumour suppressor gene p53, it diminishes its tumor suppression activity, letting cancer to start and thrive [20]. AFB1 also causes damage of the mitochondrial membrane, mutation of mitochondrial DNA, and inhibition of the electron transport system, thereby disrupting ATP production, leading to apoptosis [21]. AFM1 and AFG1 inhibits mitochondrial protein synthesis in vitro, whereas AFB1 and AFM1 inhibit cytoplasmic protein synthesis in rats [22]. Aflatoxins also suppress the activity of T and B-lymphocytes and impair macrophage and neutrophil functions as well as the expression of perforins by cytotoxic T-lymphocytes or $CD8^{+}$T-cells [23].

Ingestion of a high amount of aflatoxins leads to acute aflatoxicosis, manifested by liver damage, jaundice, and death, whereas continuous consumption of moderate amounts of aflatoxin leads to chronic aflatoxicosis. Aflatoxicosis increases the risk of hepatocellular carcinoma (HCC) [24], which is ranked as the second leading cause of cancer death in East Asia and sub-Saharan Africa [25, 26], stomach, and colon cancers [10].

In 2010, aflatoxin levels in common market foods in S.W. Uganda ranged from 0 to 55 ppb with a mean aflatoxin level of 15.7 ppb [27]. According to reference [27], there is a high prevalence of HCC in S.W. Uganda. Furthermore, there is a high prevalence of malnutrition [28] despite this region being the national food basket with an abundance of foods. This necessitated the study to determine the aflatoxin-susceptible food consumption frequency, prevalence and levels of aflatoxins in the selected foods, to establish the main food-route of ingestion, in household foodstuffs consumed in the area.

2. Materials and Methods

2.1. Materials. Aflatoxin ELISA kit was purchased from R-Biopharm Company, Darmstadt, Germany. Methanol was purchased from Sigma Aldrich (Steinheim, Germany). All these chemicals were of analytical grade.

2.2. Study Design. This study was carried out in the Southwestern region of Uganda comprising fourteen districts inhabited by communities consuming various types of staple foods. Seven districts were randomly selected following the alphabet, considering every next district. The selected districts included Rubanda, Kabale, Rukiga, Sheema, Mitooma, Bushenyi, and Kasese. From the seven districts, counties, subcounties, parishes, and villages were randomly selected based on the alphabetical appearance of their names, taking every next member.

In any village, a maximum of five households were selected from a different ecological zone/location (e.g., North, South, West, East, or Central). Informed consent was sought from the head or representative of the family of each household. An adjusted food frequency questionnaire (Supplementary 1) was used to establish the type of foods most frequently consumed in each household (Supplementary 2) and with a high risk of aflatoxin contamination, which was considered. Based on the questionnaire results, a total of 106 samples were collected from the households, which reduced to 67 after data cleaning (Supplementary 3). The food samples were placed in tightly sealed polythene bags and kept in ice-cooled plastic containers. The samples were transported to Department of Biochemistry, Mbarara University of Science and Technology laboratory, and stored at −20°C. They were later transferred to Kyambogo University, Department of Chemistry, for aflatoxin quantification.

2.3. Aflatoxin Analysis. Total aflatoxin levels in the food samples was carried out as described by reference [29], using the enzyme-linked immunosorbent assay (ELISA) kit; Ridascreen® Aflatoxin Total (R-Biopharm, Darmstadt, Germany). Briefly, aflatoxins were extracted from 2 g of each ground food sample using 10 mL of 70% methanol (v/v). The sample suspension was well mixed for approximately 10 minutes. The sample suspensions were centrifuged at 3500 rpm for 10 minutes and the supernatant was transferred to another container. An aliquot 50 mL each of the supernatants was analyzed using the total aflatoxin ELISA kit as described in procedures by the manufacturer. The difference among the means was considered at 95% confidence level using the post-hoc methods of Tukey's Multiple Comparison through GraphPad Prism 8.0.1 software.

2.4. Ethical Approval. Ethical approval for this study was received from Gulu University Research and Ethics Committee (GUREC) and numbered GUREC-110-18.

3. Results

3.1. Food Types and Household Food Consumption Frequency. The common types of foodstuffs and their weekly consumption frequency in households per district are presented in Table 1. Results showed that flours of maize and groundnut were the most commonly consumed foods in daily meals. Maize flour was consumed as breakfast porridge or lunch/supper bread in each district. Maize flour was mostly consumed (8 times/week) in Rukiga district and least consumed (4 times/week) in Bushenyi district.

Groundnuts came second (once a day) in all districts except Rubanda (6 times/week) and Sheema (5 times/week). Third was millet flour (5 times/week), which was consumed mostly in Kasese district, and only 2 times/week in Rubanda district. Lastly, sorghum was mostly consumed in Rubanda district (3 times/week) as a local porridge called *Obushera*, a local brew called *Omuramba,* or as lunch/supper bread called *Obuhemba*.

3.2. Aflatoxin Levels in Selected Food Types from S.W. Uganda. Aflatoxin levels in selected foods sampled from households in S.W. Uganda are presented in Table 2. Results showed that all groundnut samples in the region had detectable levels. Ninety-one percent of the groundnut samples had aflatoxin levels of 10 μg/kg or above. The mean aflatoxin level in groundnut flour was 96.5 ± 37.4 μg/kg ranging from 6.2 μg/kg to 297.3 μg/kg (Table 2). This level was significantly higher than levels in all other foods ($p < 0.05$) except maize ($p = 0.08$).

The mean total aflatoxin level in maize flour was 34.1 ± 14.1 μg/kg, which was three-fold the East Africa regulatory limit of 10 μg/kg. Approximately, 74.2% of maize samples were contaminated with aflatoxins, with one sample containing 336.5 μg/kg. More than half (58.1%) of the maize samples tested had aflatoxin levels higher than 10 μg/kg (the East Africa regulatory limit).

In the millet flour samples, the mean aflatoxin level was 11.7 ± 4.2 μg/kg. The sample with the highest contamination level was at 46.5 μg/kg (Table 2). Compared to other food types, millet had the least detectable levels of aflatoxins. Only 43% of the millet samples contained aflatoxin levels above the East African regulatory limit of 10 μg/kg.

The mean aflatoxin level in Sorghum was 12 ± 2.8 μg/kg. This value is in a comparable range to that of millet samples (Table 2). The sorghum sample with the highest level of total aflatoxin was 25.5 μg/kg. Approximately, 66.7% of all sorghum samples was contaminated with aflatoxins, and 58.3% had aflatoxin levels above the East African regulatory limit of 10 μg/kg.

3.3. Aflatoxin Levels in Groundnut Flour Samples from Selected Districts of S.W. Uganda. The mean aflatoxin levels in groundnut flour from selected districts of S.W. Uganda are presented in Figure 1. Like, for maize samples, the mean aflatoxin levels in groundnut flour were exceptionally high in samples from Kasese (211.2 ± 82.7), followed by Kabale (150 ± 128 μg/kg). The lowest level was in those from Sheema district (6.2 μg/kg).

3.4. Aflatoxin Levels in Maize Flour from Selected Districts of S.W. Uganda. The different levels of aflatoxin in maize flour from selected districts of S. W. Uganda are presented in Figure 2. The most contaminated samples were from Kasese district which had approximately nine-times the East African regulatory limit of 10 μg/kg. Sheema district had the lowest mean aflatoxin levels (7.31 ± 7.31 μg/kg). About 71% of the seven districts had mean total aflatoxin levels equal to or above the East African regulatory limit of 10 μg/kg; viz *Kasese* (88.1 ± 50.3 μg/kg), *Bushenyi* (24.9 ± 13.8 μg/kg), *Rukiga* (19.6 ± 6.4 μg/kg), *Mitooma* (14.5 ± 7.1 μg/kg), and *Rubanda* (10.0 ± 4.0 μg/kg) districts.

3.5. Aflatoxin Levels in Millet Flour from Selected Districts of S.W. Uganda. Results in Figure 3 show the mean aflatoxin levels in millet flour from selected districts of S. W. Uganda. The aflatoxin levels in millet flour were the highest in Rubanda district (33.3 μg/kg) and were undetectable in Rukiga and Sheema. The mean total aflatoxin level in millet samples from Kasese was 26.1 ± 13.7 μg/kg, Bushenyi 6.0 ± 4.8 μg/kg, Mitooma 11.7 μg/kg, and Kabale 5.2 ± 5.1 μg/kg.

3.6. Aflatoxin Levels in Sorghum Flour from Selected Districts of S.W. Uganda. Figure 4 shows aflatoxin levels in sorghum flour from selected districts of S. W. Uganda. The mean aflatoxin levels in sorghum flour samples were the highest in *Bushenyi* district (18.3 ± 7.2 μg/kg) and the lowest in *Kabale* and *Kasese* districts where levels were not detectable (ND). In *Rubanda* district, the aflatoxin level was 13 ± 4 μg/kg and 17.4 and 13.5 μg/kg in *Rukiga* and *Mitooma* districts, respectively. Therefore, the mean total aflatoxin levels in sorghum flour were above the East African regulatory limit of 10 μg/kg in 67% of the districts where samples were taken.

Table 1: Types of food and mean household consumption frequency per week, per district.

Food flour	*Consumption frequency, per week, per district*							
	Rubanda	Kabale	Rukiga	Sheema	Bushenyi	Mitooma	Kasese	Mean
Maize	6.83	6.89	8	6.5	3.89	6.67	6.67	6.49 ± 0.47
Groundnut	5.5	7.3	7	5	7.1	6.67	6.73	6.47 ± 0.33
Millet	1.67	1.78	2.5	4	3.28	3.3	5.26	3.1 ± 0.4
Sorghum	2.83	2.2	2	0	1.17	0.67	2.07	1.56 ± 0.37

Table 2: Total aflatoxin levels in selected food types in S.W. Uganda.

Food type	*Total aflatoxin levels (μg/kg)*			Detection levels (%)	Samples above 10 μg/kg (regulatory *limit)* (%)
	Mean ± SE	Minimum	Maximum		
Maize flour	34.07 ± 14.05	ND	336.5	74.2	58.1
Groundnut flour	96.46 ± 37.37	6.2	297.3	100	91
Millet flour	11.69 ± 4.24	ND	46.51	50	43
Sorghum flour	12.02 ± 2.75	ND	25.54	66.7	58.3

ND = not detectable. Maize flour $n = 31$, groundnut flour $n = 11$, millet flour $n = 14$, sorghum flour $n = 11$.

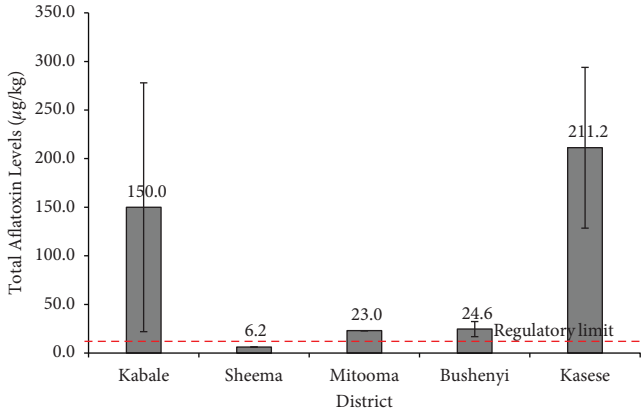

Figure 1: Total aflatoxin levels in groundnut flour samples (the regulatory limit for E. Africa is in broken lines).

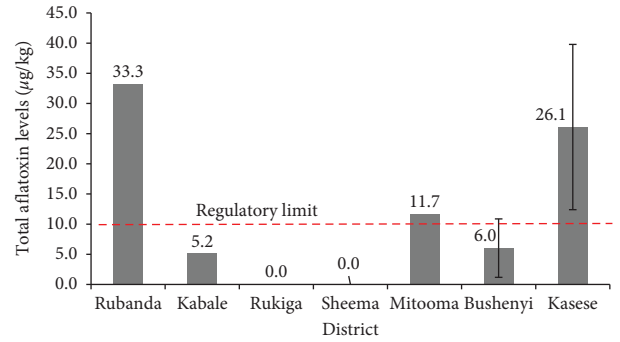

Figure 3: Total aflatoxin levels in millet flour samples in selected districts of S.W. Uganda. (The regulatory limit for E Africa is in broken lines).

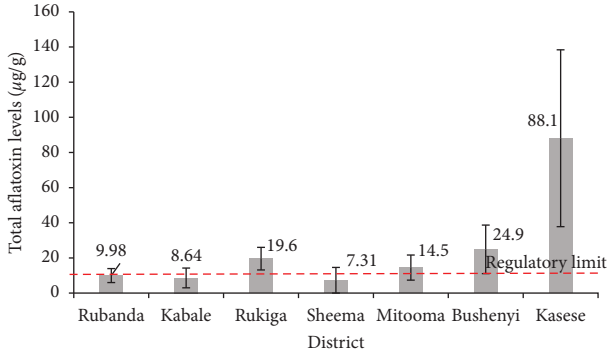

Figure 2: Total aflatoxin levels (mean ± SEM) in maize flour samples in selected districts of S.W. Uganda (the regulatory limit for E. Africa is in broken lines).

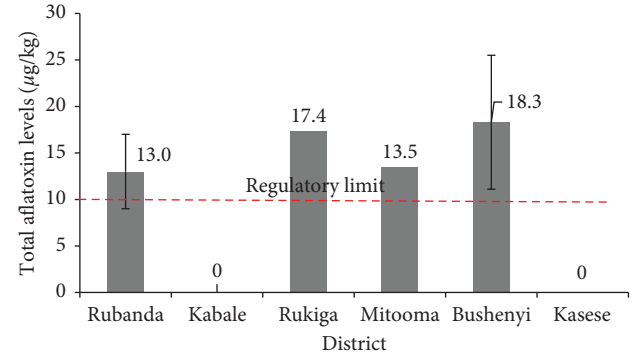

Figure 4: Total aflatoxin levels in sorghum samples in selected districts of S.W. Uganda (the regulatory limit for E. Africa is in broken lines).

3.7. Aflatoxin Levels in Foods from Rural versus Urban Areas. Figure 5 shows a comparison of aflatoxin levels in foods from rural versus urban areas. Aflatoxin levels were higher in all foods from urban areas than those from rural areas except for groundnut flour samples. The mean aflatoxin levels in maize flour samples from urban areas (60.9 ± 30.0 μg/kg) were fivefold greater than those from rural areas (12.0 ± 2.5 μg/kg). Similarly, the mean aflatoxin level of

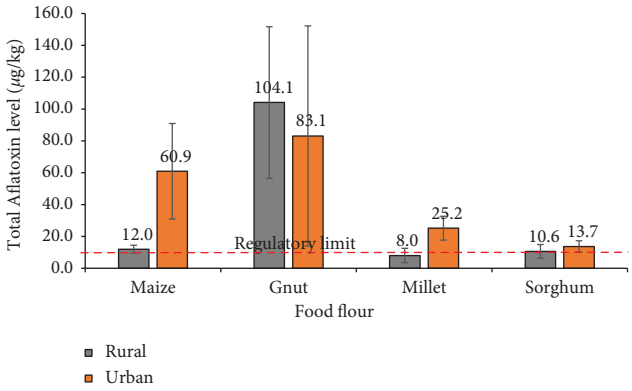

FIGURE 5: Aflatoxin levels (mean ± SEM) in foods from rural versus urban areas (the regulatory limit for E. Africa is in broken lines).

millet samples from urban areas (25.2 ± 4.5 μg/kg) is about 3 times that from rural areas (8.0 ± 4.5 μg/kg). In addition, the mean aflatoxin level of sorghum samples from urban areas (13.7 ± 3.6 μg/kg) is higher than that from the rural area (10.6 ± 4.3 μg/kg). Groundnuts are the only food samples with a lower mean aflatoxin level 83.1 ± 69.1 μg/kg in urban areas than (104.1 ± 47.6 μg/kg) for samples from rural areas.

4. Discussion

From the food consumption frequency results (Table 2), groundnut and maize flours were most frequently consumed. In addition, groundnut flour was the most contaminated among the selected foods (Table 2). This implies that since Bushenyi district had a low consumption frequency of maize flour but consumes groundnuts most frequently (Table 1); groundnuts could be the most important food-route for aflatoxicosis in Bushenyi that may be responsible for the reported 46% malnutrition rate [30].

As presented in Figure 1, the mean aflatoxin levels in groundnut flour in were exceptionally high in samples from Kasese, followed by those from Kabale and the lowest in those from Sheema district. This could be because Kasese is on the equator, with both high atmospheric temperature and humidity that support the thriving of aflatoxigenic fungi [9]. On the other hand, Sheema populations grow them but Kabale buys them. This implies that they take long to reach Kabale district. The longer they take in the supply chain, the higher the aflatoxin accumulation [31]. It would therefore be safer for Kabale populations to plant them or buy ones in very dry pods since they would be less contaminated [32].

According to Figure 2, the most contaminated maize samples were from Kasese district. This suggests that maize flour could be the main food route for aflatoxicosis in Kasese populations. The finding of the current study agrees with that of the authors of reference [29], who reported a 74% prevalence of aflatoxin in maize flour consumed in Kampala, with levels ranging from 1.8 to 268 μg/kg. This was probably because Kasese district lies on the equator, and therefore has frequent rains, high temperatures, and humidity that favor the flourishing of *Aspergillus Spp.* This could be synergized by poor methods of food handling and storage [27]. Therefore, there is need to improve awareness on harvesting and storage practices among Kasese populations.

Millet contamination was highest in Kasese district (Figure 3) where it was consumed five times a week. This suggests that millet contributes to aflatoxicity mostly in Kasese district. The millet aflatoxin prevalence in the current study is slightly lower than that of the authors of reference [27], who reported 75% in millet samples from S.W. Uganda and a mean level of 14.0 ± 1.22 μg/kg.

Sorghum samples that were most contaminated were those from Bushenyi and Rukiga districts (Figure 4). This implies that sorghum contributes to aflatoxin intoxication in these districts. The finding of the current study is comparable with that of reference [27], which reported a mean aflatoxin level of 15.2 ± 0.2 μg/kg in sorghum from the same region. The highest aflatoxin level in sorghum in Bushenyi (Figure 4) could be because it is rarely grown there and its availability depends on purchases from the markets or shops, allowing for the delay and accumulation of the toxin [31].

The current study showed that it was more than five times riskier to consume maize flour in urban than that from rural settings (Figure 5). In addition, it was three times riskier to consume millet from urban than that from rural settings (Figure 5). Furthermore, this study shows that it is riskier to consume sorghum from urban than those from rural settings (Figure 5). Therefore, the study revealed that urban populations consume more contaminated cereal foods than rural populations. This agrees with the findings of reference [33], which reported that populations that lived closest to trading centers were at a higher risk of aflatoxin exposure than those living in rural settings.

On the other hand, groundnut flour samples from rural areas were surprisingly more contaminated than those from urban centers (Figure 5). This could be because most groundnut flour in rural areas is bought from urban traders who sort out good seeds for sale and grind the residual broken left over, even if visibly contaminated, shielding the evidence of contamination from buyers [34]. The wholesale buyers take the flour to the village for sale to the retail consumers. This happens especially in districts, which do not commonly grow/produce groundnuts. The wholesalers who take this flour to rural shops take long selling the groundnut flour unprotected from any weather changes, thus increasing aflatoxin accumulation since milling provides a conducive environment for *Aspergillus* spp. to thrive [34]. This finding agrees with that of reference [35], which reported that maximum urinary AFM1 was approximately four-fold greater among rural populations compared to urban ones, the chief contributor being groundnut consumption. However, it disagrees with the findings of reference [34], which reported 67.1% aflatoxin prevalence in urban groundnut samples compared to 47.6% in the rural ones.

5. Conclusion

Groundnut and maize flours were the most frequently consumed and contaminated with the highest prevalence

and levels of aflatoxins among the selected foodstuffs and therefore the likely main food-route for aflatoxicosis in S.W. Uganda. Except for groundnut flour, urban samples were more contaminated than rural ones. Community awareness and postharvest food handling/storage and processing technologies need further evaluation. Readily accessible methods of aflatoxin detection in foods for farmers, traders, and consumers, are desirable. This study recommends further studies to elucidate its association with the observed recent increase in diseases like hepatocellular cancer and malnutrition in the region.

Data Availability

The food frequency questionnaire and results are attached (Supplementary 1 and 2 respectively). The Aflatoxin levels data used to support the findings of this study are included within the article too (Supplementary 3).

Conflicts of Interest

All authors declare no conflicts of interest regarding the publication of this article.

Authors' Contributions

Authors 1, 2, and 3 wrote the proposal and manuscript. Authors 1 and 3 performed the experiment. Authors 4, 5, and 6 analyzed results. All authors read and approved the final manuscript before submission.

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Supplementary Materials

Supplementary 1: Food frequency questionnaire. Supplementary 2: Food consumption frequency raw data. Supplementary 3: Aflatoxin levels in household foods (raw data). (*Supplementary Materials*)

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