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Maize meal (posho) served at selected boarding schools in western Uganda is highly contaminated with aflatoxins

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Abstract

The study aimed to evaluate food handling practices and assess the maize-bean supply chain in selected boarding schools of western Uganda. Additionally, it sought to determine the extent of aflatoxins exposure in the maize-bean meal consumed by students within the age range of 5 to 24. A cross-sectional study was conducted in two districts of Bushenyi and Sheema to gather 95 samples (31 beans, 32 maize flour, and 32 cooked maize flour-*posho*). In addition, 262 individual interviews were conducted. The findings revealed that only 9.38% of the school, store food as per World Health Organisation standards. The majority of schools (57.9%) purchase maize flour and beans from any stockist with no contractual obligations (random supplier), and all the 32 schools base on visual parameters especially white colour and smell to rule out aflatoxin contamination. Maize flour stored on the ground registered significantly (*P=0.0018*) higher levels (54.3±10.1 µg/kg) of total aflatoxins than that stored on a raised platform (14.7±2.62 µg/kg). Likewise, the supplier significantly influenced total aflatoxins levels (*P=0.0064*), with higher levels (44.6±8.02 µg/kg) detected in maize flour supplied by a random supplier than a pre-qualified one (14.7±2.62 µg/kg). Given the low levels (1.3 to 2.8 µg/kg), which are far less than the Maximum Tolerated Limit (MTL) of 10 µg/kg, aflatoxins don't pose a significant problem in beans. Aflatoxins exposure in younger children (5–12) was higher (61.5 ng/kg bw/day) than in older ones (13–24; 41.5 ng/kg bw/day). Similarly, younger children had a higher risk of developing hepatocellular carcinoma (1.84) than older ones (1.24). The findings underscore an urgent need to formulate guidelines for procurement, storage, and food handling in schools, alongside intensifying inspection programmes for food handling. Additionally, there is need to expand the research scope by incorporating a greater number of schools from varied geographical areas within Uganda, while rigorously monitoring the entirety of the food supply chain.

Key words: Boarding schools, aflatoxins, risk, posho, beans, storage.

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1.0 Introduction

Aflatoxins are toxic secondary metabolites produced by fungi especially *Aspergillus flavus* and *Aspergillus parasiticus* (PACA, 2017). These fungi infect crops mainly peanuts, maize, sorghum, millet and root crops like cassava both in the field and post-harvest (Meijer *et al.*, 2021). Continuous exposure to aflatoxins is highly linked to Hepatocellular carcinoma (HCC) which accounts for 75–85% of global liver cancer cases (Mcglynn *et al.*, 2020). A recent study revealed that contamination of African staple foods by aflatoxins is a major issue for human health and that overall exposure is high, leading to a substantial increase in long-term disease burden (Meijer *et al.*, 2021). In Uganda, there has been a notable surge in reported cases of HCC at the country's largest healthcare facility-Mulago referral hospital, prompting the government and researchers to advocate for extensive investigation of food contamination (Bukirwa *et al.*, 2021).

Aflatoxin contamination not only poses health risks but also results in financial standstills and hampers export activities. A recent report by the Agricultural and Food Authority (AFA) of Kenya revealed excessively high aflatoxin levels in Ugandan maize. Consequently, Kenya imposed a ban on maize imports from Uganda, leading to an estimated loss of USD 121 million (The Independent, 2021). In East African Community (EAC) member states, maize exceeding the total aflatoxin limit of 10 parts per billion (ppb) is forbidden for trade (Mufumbi, 2021). Consequently, traders export high-quality maize meeting aflatoxin limits, leaving substandard grain for local consumption (Edgar Mugizi et al., 2021). It is expected that a significant portion of this lower-quality maize, failing to meet export standards, is circulated within the domestic market, and is potentially consumed by students in schools (Nicholaus et al., 2021).

The main sources of nutrition for most children in boarding schools in low-income nations like those in East Africa are maize and legumes, particularly maize and beans (Reardon *et al.*, 2021). An estimated 420 million children receive rotating school meals each day (World Food Program (WFP), 2023). The WFP and UNICEF have persistently called for a balanced diet for school children, but despite this advice, implementation is lax, leaving maize flour and beans the "daily bread" for children in boarding schools. Maize and corn are used interchangeably depending on geographical location, in this study maize has been adopted to mean corn. Usually, in schools, maize flour is cooked by combining hot water (90-100^{0}C) with flour and stirring until a thick, sticky paste known as posho is obtained. This posho is normally served alongside beans for lunch and dinner, while maize porridge is consumed at breakfast (Tukahirwa, 2021). However, recently it has been discovered that maize and bean meal are significantly contaminated with aflatoxins in Tanzania and Kenya, posing a serious hazard to human life (Nicholaus *et al.*, 2021; Wangia *et al.*, 2019). Nevertheless, there is limited information regarding the food supply chain, and handling practices. Schools normally buy a lot of maize, store, and use it to feed students for the rest of the school term (WFP, 2023). These bulking practices and improper storage are likely to foster the development of *Aspergillus* species, which produce aflatoxins and pose a concern to schools with potentially non-existing detection capabilities.

The problem of aflatoxins is more pronounced in children who are at high risk of dietary aflatoxins exposure compared to old people. This vulnerability can be attributed to their weaker immune systems, increased food demand, and unregulated dietary habits (Wangia et al., 2019). In Uganda, limited research has been conducted to investigate the school food supply chain, storage and handling practices as well as levels of total aflatoxins exposure. Although Asiki et al., (2014) partially addressed this issue by assessing aflatoxins exposure in Ugandan rural communities using blood samples from

100 adults and 96 under the age of three, the study did not delve into the food value supply chain, handling and storage or specifically target students who typically consume a monotonous diet of maize and beans on a daily basis. Hence, the objective of this study was to assess the maize and bean supply chain in schools, examine storage and handling practices, and determine the levels of total aflatoxins among students aged 5-24 years in two selected districts. The study's specific objectives were to: (1) understand the food supply chain of maize and beans handling practices, (2) Assess the knowledge on aflatoxins among food handlers and determine levels of aflatoxins in maize and bean meals served to students, and (3) determine aflatoxins exposure among students through their daily dietary intake.

2.0 Materials and Methods

2.1 Survey to assess the current maize and bean food supply chain and handling practices.

2.1.1 Study area, design and sampling.

The study was granted ethical clearance by the Ethical Review Committee of Mbarara University Science and Technology under reference MUST-2023-766. In two districts, Bushenyi and Sheema, a cross-sectional study was conducted to gather food samples for lab testing. Individual interviews were conducted to collect data on demographics, the supply chain for maize, and beans, the quantities provided by suppliers, the storage and general food handling practices. The two target districts are located in western Uganda, 59 to 100 kilometres south of the equator and 330 kilometres from Kampala, the nation's capital. Residents of the districts are urban and semi-urban. Both districts experience high levels of malnutrition (Kikafunda et al., 2014) and thus were selected for the study.

The RaoSoft online sample calculator (http://www.raosoft.com/samplesize.html) was used to determine the sample size. 1.96 was chosen as the standard deviation with a 95% confidence level. The response rate was set at 85%, and the margin of error at 5%. The design effect was 1.0. According to information obtained through district offices from the Ministry of Education, Bushenyi and Sheema together have 459 schools (208 in Sheema and 251 in Bushenyi). The following exclusion criteria was followed to draw a sample: A school shall have a primary and secondary division, excluding nursery and tertiary institutions, a boarding portion with students aged 5 to 24, have a mixed gender and store food for at least a month as opposed to purchasing and consuming without storing. When this criterion was applied, the number of schools in the overall sample shrank to 78. 34 schools were chosen as the sample size by entering the specifications into the Raosoft calculator.

2.1.2 Data collection process

Primary data were gathered using pre-tested and validated semi-structured questionnaires with the consent of the school and the children. Two different questionnaires were given out: one to food handlers, primarily the cook, shop manager, and procurement person/director, and the other to school children between the ages of 5 and 24. In the latter, data on demographic traits, frequently encountered health issues and a few anthropometric parameters, primarily weight, were gathered. A computerised Soehnle weighing scale that was precisely calibrated was used to weigh schoolchildren in accordance with a methodology established by the World Health Organisation. The experienced enumerators weighed each child three times to assure precision and accuracy.

From a pool of 78 schools, 34 public and private schools were chosen at random. Schools located more than 3 kilometres from the town centre were chosen to rule out the possibility of consuming maize and beans that were purchased at the market or in the town. After receiving permission from the school administration, research assistants were split into two groups. One group interviewed food handlers (cooks, shop managers, procurement personnel/directors), while the other group interviewed schoolchildren. Following a thorough description of the study's objectives, schoolchildren and food handlers voluntarily agreed to participate in the study with approval from school authorities. Following approval, a simple random sample procedure was used to select eight students from each school, yielding a total of 272 responses. Despite expecting 272 respondents, only 226 completed the meal frequency and dietary recall questionnaire. Participants in an interview have the legal right to terminate it ethically. As a result, some children easily withdrew from interviews when the time for providing meals coincided with the interview. Because most schools lacked established processes, it was impossible to get the requisite sample size for school food handlers. For instance, at certain schools, the cook also served as the storekeeper and at the same time as a procurement officer. Hence, 38 questionnaires were administered for food handlers.

2.1.3 The assessment of the status of food stores.

The status of food stores was assessed in accordance with the guidelines provided by the World Health Organisation, (1996). These guidelines recommend the use of visual inspection and a scoring system to assess essential factors that contribute to an appropriate food store for storing food products. Critical factors taken into consideration include adequate ventilation, the presence of pallets, moisture-proofing measures, strategic positioning, and prevention of rodent infestation.

2.2 Samples collection and total aflatoxins analysis.

2.2.1 Sample collection

Each school provided three separate samples for laboratory analysis: dry beans in storage, maize flour in storage, and cooked maize flour (referred to as posho). Each sample was taken in triplicate. A total of 98 samples, each weighing 500g, were collected (35 samples of beans, 33 samples of flour, and 30 samples of posho), sealed in zip lock bags, and kept in a cooler box for 5-8 hours. Samples were moved to the biology lab at Mbarara University of Science and Technology, where they were kept at 4°C for 2 days until laboratory analysis at the International Institute of Tropical Agriculture (IITA).

2.2.2 Laboratory analysis of total aflatoxins.

In accordance with the manufacturer's instructions, total aflatoxins were extracted and quantified in three replicates using the Reveal Q+ immunoassay aflatoxins detection kit (© Neogen corporation, 2021). The protocol was initially verified and optimized. Each sample was specifically ground into smaller particles and about 10 g ±0.1 of each sample was weighed using an Ohaus Navigator NV123 digital balance. 50 ml of 65% ethanol was added to each sample after they had been weighed. With a Waring blender, the mixture was mixed for one minute (MX 1200XTS, 24000rpm). The mixture was allowed to settle and filtered using Whatman filter paper 185mm (Sigma-Aldrich). 100µl of the sample filtrate was added to 400 µl of aflatoxins sample diluent and homogenized in the red sample dilution cap. Reveal Q^{+} aflatoxins strip was inserted into the raptor cartridge and transferred into any of the three ports within the raptor machine. 100 µl of diluted sample extract was placed into the raptor cartridge while ensuring that the strips are in contact with the liquid for a period of 6 minutes. Results were read within a period of 30 seconds using the Raptor Neogen machine. It should be mentioned that samples with values above 100 ppb were diluted and retested before being taken into consideration. Each sample was measured three times and an average considered during analysis.

2.3 Risk assessment for aflatoxins in maize flour consumed by students.

The age group of students was divided into two categories (5-12 and 13-24 years) to calculate the risk of aflatoxins exposure, and the following parameters were evaluated: the estimated aflatoxins daily intake (EDI), the margin of exposure (MOE), the hazard index (HI), and the population at risk for hepatocellular carcinoma (HCC). EDI was calculated based on the concentration of total aflatoxins and calculated daily intake of posho as indicated in equation 1.

$$\text{The estimated aflatoxins daily intake } \textbf{(EDI)} = \frac{\text{Daily intake} \times \text{Mean aflatoxins levels}}{\text{Average body weight}} \quad (1)$$

The European Food Safety Authority (EFSA) and WHO recognizes two methods in risk characterization for genotoxic and carcinogenic substances, these are MOE and HCC. The benchmark lower dose is set at 400 ng/kg bw/day by EFSA, equation 2.

$$\text{Margin of Exposure } \textbf{(MOE)} = \frac{\text{Bechmark lower dose set at 400 ng/kg bw/day}}{\text{Estimated Aflatoxins daily intake}} \quad (2)$$

Hazard index was calculated as indicated in equation 3, where TD50 is the dosage (ng/kg bw/day) required to induce tumors in half of the test population that would have remained tumor free at zero doses as described by (Ismail *et al.*, 2016).

$$\text{Hazard Index } \textbf{(HI)} = \sum_{n=0}^{n} \left(\frac{\text{EDI/TD50}}{50000} \right) \quad (3)$$

HCC is a measure of the proportion at risk for hepatocellular carcinoma within a population of 100,000 people/year. It is calculated from average potency which utilizes data from Hepatitis B antigen (+/-). Average potency= 0.3 × positive prevalence rate for proportion of Hepatitis B antigen surface (0.01 negative prevalence rate for proportion of Hepatitis B antigen surface). The positive prevalence of Hepatitis B in Uganda is at 7% (Kitandwe *et al.*, 2021)

$$\text{Hepatocellular Carcinoma } \textbf{(HCC)} = \text{Average potency} \times \text{EDI} \quad (4)$$

2.4 Data analysis

Stata version 17 (Stata 2017) was used to analyse quantitative data from laboratory analysis, food frequency, and dietary recall. Data were tested for normality using the Shapiro-Wilk and heteroskedasticity using the Breusch-Pagan tests prior to analysis. Since the data passed these two checks without needing to be transformed, the analysis was purely parametric. The association between aflatoxins levels, school-related practices, and other categorical variables was investigated using the chi-square test. The means of continuous variables were compared using independent student t-tests. All data were analysed a 95% confidence level. The risk assessment analysis was carried out in accordance with Ismael et al (2016). ArcGIS 10.8.1 (Esri), was used to develop a risk distribution map for aflatoxins. Qualitative data from school cooks, store managers, procurement officers, and school bursars were analysed following the thematic content analysis (O. Nyumba et al., 2018).

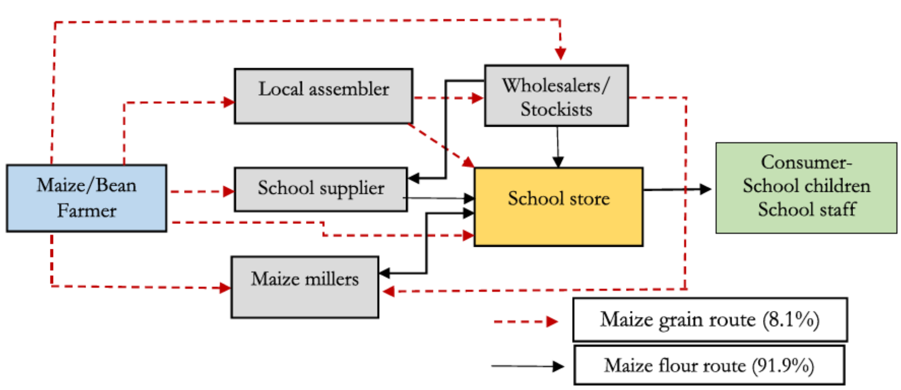

Figure1. School maize and bean value chain

3.0 RESULTS

3.1 Characteristics of respondents.

3.1.1 Food handlers

A total of 34 schools—17 schools in each district—were involved. Twenty of the 34 schools were primary and fourteen were secondary. The management of 17 schools was public (government controlled), whereas 17 were privately managed. Cooks (12), head teachers (10), procurement officers/store managers (10), and school directors (06), in that order, were the most frequently interviewed food handlers (Table S1). Private schools have distinct food handling management and operating procedures than public schools. For instance, the study found out that school directors in privately owned schools have taken on the function of procurement officers, which they acknowledge to reduce expenses, for this reason no single private school had a procurement officer. In public schools, procurement officers existed and concurrently performed the duties of a store manager. Men respondents made up the greatest share of respondents (60.5%). The respondents had an average age of 40 and at least 7 years of experience in the role they held on the day of the interview. A school could have up to 1418 students, with 604 being the average (Table S1).

3.1.2 School children

The study involved a total of 226 students, with a 14-year-old average age. All the students included had spent at least one academic year in that particular school, which is judged sufficient to address inquiries about the school food schedule, and food handling practices. The number of people living in each family of the children examined ranged from 6 on average to a high of 13. Typically, students paid between 10,000 ($2.7) and 930,000 UGX ($251) per term for school fees, with 418,800 UGX ($113) being the average. Findings, however, showed that privately managed schools pay a little higher than public ones. On average, a child in public school paid 407,000 UGX, while for private schools it was 421,900 UGX. (Table S1).

3.2 The food supply chain of maize and beans used at schools.

The study investigated the pathways through which beans and maize reach school stores as depicted in Figure 1. It was discovered that schools receive maize in two different forms: (i) maize flour, and (ii) maize grain. Maize grain is later processed into flour at a piecemeal rate. Due to the time-consuming process of converting maize grain to flour and the risk of maize grain spoilage during storage, 91.9% of schools opt to purchase maize flour instead of maize grain. The findings in Table S1 indicate that only 41.1% of schools have established pre-qualified suppliers, while the majority of schools (58.9%) prefer to buy maize flour and beans from any supplier without contractual obligations (random supplier). Schools acquire these food items differently depending on the supply route. For the flour route, the primary sources of maize flour are pre-qualified suppliers and stockist/wholesalers located near commercial centers. A small percentage (8.11%) of schools choose to stock maize grain and process it into flour themselves, such schools buy grain from a nearby assembler, wholesaler, or pre-qualified source. In some cases, parents provide maize grain, maize flour, and/or beans as a substitute for school fees.

Table 1. Association between storage status and school catagory[1]

Storage surface	School category	
	Private	Public
Bare ground	6 (85.7%)	1 (14.3%)
Pallets/raised platforms	5 (33.3%)	10 (66.7%)

[1] Pearson Chi^2 = 5.24, Prob = 0.0221.

Table 2. Practices conducted after receiving produce

Variable	Freq.	Percentage
Pre-dying and store	3	8.60
Weigh and store	30	79.6
Add rat poison, store	3	8.60
Sorting and store	2	3.23

3.3 The current handling practices and quality aspects of maize and beans in schools.

3.3.1 The public vs private schools

The quality of the food served in schools can be predicted by how it is handled. Findings in Table 1 revealed that there was no clear distinction in food handling between public and private schools, except for a noteworthy ($P<0.05$) correlation between storage conditions and school type. Private schools were found to be more likely (85.6%) to store food on the ground compared to public schools (Table 1). Further results in Table 2 indicated that both private and public schools prioritize quantity over quality when it comes to the supplied food. The majority of schools (79.6%) weigh and store the food (maize flour and beans) upon receiving it, while only a small percentage of schools (11.8%) consider quality factors such as pre-drying and sorting before storage (Table 2). Interestingly, all schools whether private or public base on subjective visual inspection to determine if maize flour or beans are of high quality. For high-quality maize flour, including that free of aflatoxins, all schools rely on white colour, good smell, and sealed packaging as indicators. Similarly, in two schools, the management waits for student complaints and strikes as an indicator of subpar meal processed from maize flour (posho) and (or) beans.

The study highlighted the issue of food adulteration. According to the findings, 75% of the interviewed food handlers reported rejecting posho and (or) beans due to poor quality (Table 3). On average schools reject between 25-5000 kg of maize flour per term, leading to a financial loss ranging from 87,500 UGX (23 USD) to 17,500,000 UGX (4,729 USD) (Table 4). The main reason for rejection was the mouldy smell (65.4%). It was surprising to note that two schools reported incidents of adulteration where suppliers intentionally mixed cement, a binding agent used in construction, with maize flour to increase its weight and gain more profit (Table 3). However, it's worth noting that adulteration was not solely attributed to suppliers. In peculiar cases, four school food handlers admitted to inserting 'tablet-like' rat poison in food storage bags to combat rodents that normally consume and contaminate the produce. Of these four schools, three confessed to adding crude kerosene 'paraffin' to tame the sexual libido among students.

Furthermore, the conditions of the stores were investigated according to World Health Organisation (WHO) minimal standards. Upon personal observation, there were no existing guidelines in place for proper food storage. Both private and public schools employed different storage methods based on convenience rather than adherence to any criteria. Out of the 34 school stores evaluated, only 9.38% met the WHO standards for a suitable storage facility, indicating that the majority of them (90.6%) stored food in substandard conditions as depicted in Figure 2.

3.3.2 Food handling -the student perspective.

The study findings in Table 4 revealed that the majority of students (46.2%) consume their meals in the dining hall. However, based on WHO guidelines, 89% of the food serving surfaces in the dining hall were in unsanitary conditions (Figure 2-C). Additionally, 16.4% of the students admitted to consuming their meals outside, particularly under a tree. In terms of food preparation, students indicated that only 35.7% of the cooks wear uniforms/aprons while preparing and serving meals (Table 4) and Figure 2-E. Furthermore, results in Table 4 revealed that 35% of students have experienced health issues after consuming posho and beans that were potentially contaminated. The most reported symptoms were occasional stomach ache (48.2%), nausea and vomiting (16.1%), frequent headache (12.5%), frequent cough (10.7%) and diarrhoea (7.14).

3.3.1 Consumption patterns of posho and beans served in schools in Uganda.

Investigated were the dietary indices for beans and posho. In all schools, posho and beans are consumed twice daily, whereas maize porridge is taken once at breakfast (Table 5). Depending on the number of students enrolled, schools typically purchase between 246 and 52,500 kg of maize flour and 16 to 50,000 kg of beans for a 90-day academic term. Each student consumes an average of 8.84 kg of beans and 16 kg of posho over one academic year. Posho and beans are consumed every day by each student at the school in amounts of 0.17 kg and 0.14 kg, respectively (Table 5).

Table 3. Drivers for the choice of *posho* and beans purchase by schools

Variable	Freq.	Percentage
How do you determine that maize flour/beans are of high quality?		
Student complaints	2	7.14
Good smell	10	35.7
Sealed packaged	1	3.57
White colour	15	53.6
Have you ever rejected posho*/beans due to poor quality?*		
No	9	25.7
Yes	26	74.3
What were the reasons for rejecting such posho*/beans?*		
Maize mixed with cement	2	7.69
Mixed with sand	4	15.4
Mouldy smell	17	65.4
Student' strike	2	7.69
Weevils	1	3.85

Table 4. Food handling practices and associated health complications

Variable	Freq.	Percentage
From which facility do you eat your meal?		
Classes	40	17.7
Dining	104	46.0
Dormitory	45	19.9
Under the tree	37	16.4
Do cooks wear uniform/apron while serving food?		
Yes	145	64.3
No	81	35.7
Have you ever experienced complications after eating poor quality posho*/beans?*		
Yes	79	35.0
No	147	65.0
What are those complications ever experienced?		
Occasional stomach-ache	38	48.2
Nausea and vomiting	13	16.1
Frequent headache	10	12.5
Frequent cough	8	10.7
Irregular diarrhoea	6	7.14
Fever	4	5.36

Figure 2. (A) to (D) represents appalling food storage and handling practices

Table 5. Consumption frequency for *posho* and beans

Variable	Mean proportion	Std. dev.	Min	Max
Consumption characteristics (n = 33)				
Maize flour purchased in a term (kg)	11,230	12,820	246	52,500
Maize flour consumed/child/term (kg)	16.0	15.5	0.1	61.76
Maize flour consumed/child/day (kg)	0.17	0.17	0.1	0.69
Beans purchased in a term (kg)	6,942	12,312	16	50,000
Beans consumed/child/term (kg)	8.84	24.49	0.1	142.86
Beans consumed/child/day (kg)	0.14	0.32	0.01	1.59
Frequency of consumption (n = 225)				
Posho	1.88	0.39	0	3
Beans	1.89	0.41	0	3
Maize porridge	1.34	0.70	0	3
Rejected maize flour due to poor quality (n = 24)				
Maize flour (kg)	853.83	1289.7	25	5,000

3.3.2 Knowledge on aflatoxins and total aflatoxins levels in maize and beans used at schools.
The results presented in Table 6 indicate that 59.4% of food handlers have heard about aflatoxins. They are aware that poor storage (37.3%) and long-term storage (29.3%) contribute to increased levels of aflatoxins. It is noteworthy that 36.8% of food handlers are aware of the long-term carcinogenic potential of aflatoxins, and some (31.6%) are familiar with acute symptoms of food poisoning and vomiting. In addition, the study explored the perceptions of food handlers regarding the extent of aflatoxin contamination in foods consumed by schools surrounding the area. The findings reveal that 62.5% of food handlers believe that less than 30% of schools consume aflatoxin-contaminated food. Interestingly, one food handler holds the belief that the foods, particularly posho and beans, consumed in schools are always free of any aflatoxin contamination.

Table 6. Aflatoxin awareness among food handlers

Variable	Freq.	Percentage
Have you ever heard of aflatoxins?		
No	13	40.6
Yes	19	59.4
What do you think causes aflatoxins?		
Poor storage	7	37.3
Overstaying in store	6	29.0
Poor conditions after harvest especially drying	5	26.4
Premature harvest of crops	1	7.26
What do you think are the consequences?		
Brings sickness	3	15.8
Cancerous	7	36.8
Cause food poisoning, vomiting	6	31.6
Don't know	3	15.8
According to you, what is the proportion of schools consuming aflatoxins in foods?		
0	1	6.25
1-10%	5	31.2
11-20%	3	18.8
21-30%	1	6.25
31-40%	2	12.5
41-50%	3	18.7
71-80%	1	6.25

Table 7. Aflatoxin levels an dry beans, maize flour and *posho*

Aflatoxin levels (µg/kg)	Obs.	Mean	Min	Max	% samples > 10 µg/kg
Dry beans	34	2.15 ± 0.1	1.4	2.8	0
Maize flour	34	31.1 ± 5.4	2.7	122	64.7
Posho	34	13.1 ± 1.5	3.4	36	58.8

The aflatoxins levels for frequently consumed foods in boarding schools (dry beans, maize flour, and posho) are presented in Table 7. No matter what kind of food was tested, there were traces of total aflatoxins in every sample. Total aflatoxins levels in dried beans ranged from 1.3 to 2.8, but because they were much lower than the Maximum Tolerated Level (MTL) of 10 µg/kg, they are thought to be safe. Total aflatoxins concentrations in posho varied from 3.4 to 36 µg/kg, and more than 58% of samples had concentrations above the MTL. In comparison to posho, maize flour had more (64.7%) samples with total aflatoxins levels above 10 µg/kg; these levels varied from 2.7 to 122.2 µg/kg. Total aflatoxins levels were generally decreased during the posho-making process. According to the calculated data, there was a 32.8% decrease in total aflatoxins levels following cooking. The mean total aflatoxins levels were 2.15±0.06 µg/kg in dry beans, 31.1±5.43 µg/kg in maize flour and 13.1±1.49 µg/kg in posho (Figure S1).

3.3.3 Relationship between total aflatoxins levels, methods of storage, and type of supplier.
A student-independent t-test was carried out to determine if there was a statistically significant difference in total aflatoxins levels based on the method of storage and type of supplier. The results presented in Table 8 revealed a significant difference (P=0.0018) in total aflatoxins depending on the method of storage. Schools that stored maize flour on bare ground had significantly higher levels of aflatoxins (54.3±10.1 µg/kg) in their flour compared to those that store it on raised surfaces/pallets

(14.7±2.62 μg/kg). Similarly, the supplier of maize flour in schools played a significant (P=0.0064) role in aflatoxins contamination. Maize flour supplied by any random supplier had significantly higher levels of total aflatoxins (44.6±8.02 μg/kg) compared to flour supplied by a pre-qualified or contracted supplier (14.9±5.46 μg/kg).

Table 8. Significance difference in aflatoxin levels among methods of storage (bare ground and pallets) and who supplies (prequalified vs random)

Aflatoxin in flour	Mean aflatoxin levels (μg/kg)	Std. dev	n	T	*P*-value
Bare ground	54.3 ± 10.1	37.9	14	3.52	0.0018
Raised surface	14.7 ± 2.6	9.08	12		
Pre-qualified supplier	14.9 ± 5.5	21.2	15	−2.93	0.0064
Random supplier	44.6 ± 8.0	34.0	18		

Table 9. Risk assessment indicators for posho and beans

Age group	Av. body weight (kgs)	EDI (ng/kg bw/day)	Av. Potency (ng/kg bw/day)	MOE	HCC	HI
5-12	36.1	61.5	0.03	6.51	1.84	6.14
13-24	53.5	41.5	0.03	9.64	1.24	4.15

[1] Mean aflatoxin level for *posho* – μg/kg, maize daily intake – 0.17 kg, TD_{50} = 0.2 ng kg bw.

[2] EDI = estimated aflatoxins daily intake; MOE = margin of exposure; HCC = population at risk for hepatocellular carcinoma; HI = hazard index.

3.4.1 Risk characterization of aflatoxins in maize and posho.

The estimated daily intake, margin of exposure, hazard index, and hepatocellular carcinoma were computed to better understand the risk associated with consuming total aflatoxins-contaminated posho. Students between the ages of 5 and 12 consumed more (61.5 ng/kg bw/day) than their counterparts between the ages of 13 and 24 (41.5 ng/kg bw/day). For younger children, the margin of exposure was 6.51; for older ones, it was 9.64. Younger school children had a significantly higher (1.84) chance of being exposed to hepatocellular carcinoma than older ones (1.24). Similarly, younger children had a greater hazard index than older ones (Table 9). Figure 3 shows the risk distribution map for total aflatoxins concentrations in maize flour with levels above the Maximum Tolerated Level-MTL (>10 μg/kg). Given greater values above MTL in maize flour in Bushenyi than Sheema district, schools in Bushenyi are more at risk of aflatoxins contamination.

4. Discussion

This study evaluated the food supply chain of maize and beans, and food handling practices, assessed the knowledge on aflatoxins among food handlers and determined aflatoxins exposure among students through their daily dietary intake.

4.1 The maize and bean food supply chain and handling practices currently used in selected boarding schools.

Results show that the entire food supply chain is informal, and there are no guidelines in place to regulate food purchases, which gives room for all kinds of arrangements, as in private owned schools where the function of procurement officers has been neglected. In odd situations, the school's director serves as the store's manager, the procurement officer, and the supplier all at once. Furthermore, aflatoxins levels were significantly *(P=0.0064)* associated with the supplier, most schools don't care who supplies the maize flour and beans as long as they have food in stock. The importance of pre-qualified suppliers and specialised procurement officers in the food supply chain has been highlighted. According to John et al., (2022), suppliers tend to focus more on volume than quality when there are no contracts binding them to specific obligations, which leaves room for food adulteration and quality compromise. The practice of hiring specialised individuals to ensure food procurement and contract suppliers to meet high-quality standards has been overlooked in Ugandan schools, particularly private ones. This is often attributed to the perception that hiring procurement officers is expensive and requires them to be continuously paid during holidays when most schools are out of business. For this reason, school directors in private schools have assumed this position which compromises quality because they are loaded with other responsibilities.

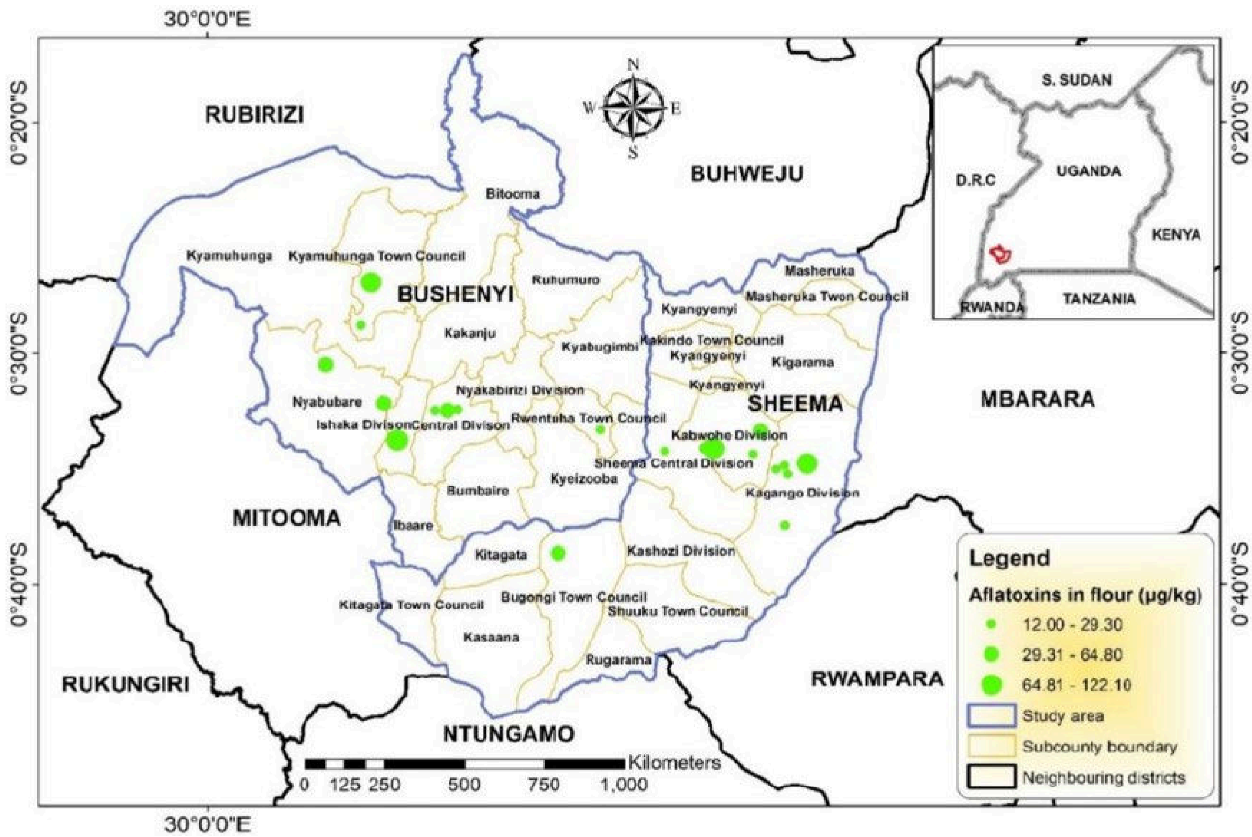

Figure 3. Risk distribution map for aflatoxins in Bushenyi and Sheema districts

Four schools reported placing rat poison near food products in stores, particularly beans. Similarly, two schools reported mixing cement with maize flour, and two schools discovered sand combined with maize flour. Studies (Grace, 2017; Imathiu, 2017; Ngabirano and Birungi, 2022) have demonstrated a similar tendency of adulteration in various items, particularly street foods, where disinfectants such as formalin, formaldehyde, and rat poison are used to prevent food from spoiling and keep pests away. Although adulterants have occasionally been discovered in foods, for the first time, this study reports cement as another adulterant that is intentionally added to increase weight and obtain more profits. Such practices are fatal. For instance, in India, 22 children died after eating a meal that was contaminated with rat poison, which was placed purposefully next to food to deter and kill vermin (Grace, 2017). Given the significant health risk involved, adulteration procedures should be severely limited through a robust school inspection program.

Findings also showed that the state of the food storage facilities (stores) was a mess; Only 9.38% representing 3 of the 32 school stores assessed, met the standards of a store as per FAO guidelines, leaving the biggest percentage (90.6%) storing food in an unsanitary and subpar conditions. Every school adopted its unique design for the store and method of storing food. Personal observations show that many schools considered any enclosed place to be a store as long as it had enough room to store the purchased produce. As a result, 31.82% of schools kept food on bare ground, which was significantly associated with higher aflatoxins levels than schools that kept maize flour on raised surfaces or pallets. Produce stored on the bare ground encourages the growth of *Aspergillus* species that produce aflatoxins by increasing ambient humidity (Kaaya and Eboku, 2010). The findings support those of numerous authors who also reported that storing produce on the bare ground caused high levels of aflatoxins (Kyalo *et al.*, 2022; Namubiru *et al.*, 2022; Sahar *et al.*, 2022)

4.2 Knowledge on aflatoxins and levels of total aflatoxins in maize, maize flour and beans.
According to the results, schools use white colour, good smell, and sealed packaging as indicators to determine if posho or beans meet quality standards. These are subjective visual assessments that are deluded and may produce false negatives or false positives. A sample may appear very white but still have high amounts of aflatoxins, or conversely, it may appear dingy but contain low levels of aflatoxins (Doolotkeldieva, 2010; Korley Kortei *et al.*, 2021). It is crucial that in addition to the visual assessments, educational institutions purchase affordable ELISA kits for aflatoxins detection that may be used to detect aflatoxins in foods especially maize flour. The average cost of a Reveal Q^+ kit for 25 extractions is 250 USD (© Neogen corporation, 2021). In this study, on average each child pays 113 USD per term, thus it would require the school fees of only 2 students to buy the kit. Moreover, the government can make subsidies or partner with existing NGOs to make the kits more affordable. While we acknowledge the need for further research on the feasibility of implementing ELISA testing in schools, our study serves as a starting point to highlight the importance of aflatoxin testing kits in schools.

Further results indicated that low levels of aflatoxins contamination in beans (1.4–2.8 μg/kg), and all samples were much below the Maximum Tolerated Limits (MTL) of 10 μg/kg for East African Community (Mufumbi, 2021). A study by Ms et al (2021) indicated low levels (<2 μg/kg) of aflatoxins in common beans. Beans naturally contain phenolic compounds with antimutagenic effects that protect against aflatoxins (Cardador-Martínez *et al.*, 2010), perhaps this could be one of the reasons for low levels of aflatoxins. However, more experimental trials outside the purview of this study could be set up to uncover additional explanations for this finding. Total aflatoxins levels were reduced by

32.9% in the cooked sample (posho), suggesting that cooking could reduce aflatoxins contamination but not completely eliminate it. Aflatoxins are thermal stable, however the levels can be significantly decreased by cooking, depending on the cooking time and temperature. In an experiment to determine the effect of cooking on aflatoxins Hwang & Lee, (2006) discovered that heating at 150^0C and 200^0C lowered aflatoxins levels by 50 and 90%, respectively. However, in schools, the first step in preparing a posho meal (cooked maize flour) is boiling water until it reaches the boiling point (90–100°C) after which it is then processed for around 10-15 minutes. Thus, the cooking methods used in schools are incapable of reaching temperatures as high as 150°C to reduce aflatoxins levels by half. Even if temperatures as high as 150°C were achieved, this would lead to different reactions, altering the nature of the posho significantly.

4.3 Risk of exposure to total aflatoxins in students through daily intake of posho

In general, younger children (5–12) consumed more aflatoxins daily (61.5 ng/kg bw/day) and were marginally more exposed (6.51), with a higher risk of developing hepatocellular carcinoma (1.84). Results agree with Asiki et al (2014) who detected higher levels of aflatoxins-albumin adduct in the blood of younger children than older ones in Uganda. Aflatoxins contamination in cereals and legumes cannot be overlooked (Atongbiik Achaglinkame *et al.*, 2017) noting that dietary exposure to aflatoxins may result in severe toxic and carcinogenic outcomes in humans and animals and human exposure occurs throughout an individual's lifetime (Wangia et al, 2019). Children who are exposed to infections early and for a long time are more likely to develop chronic illnesses as they age. Prolonged exposure implies a buildup of aflatoxins over time. Indeed, in the study short-term health complications were reported by children because of consuming contaminated posho and beans which included occasional stomachache, nausea and vomiting, frequent headaches, cough irregular diarrhea and fever like aflatoxins poisoning observations in Eastern and Central Provinces in Kenya (Lewis *et al.*, 2005).

5. Conclusion

The objective of this study was to assess the maize-bean food supply chain, handling practices, and the levels of total aflatoxins in maize, posho and beans, while also evaluating the associated risk of aflatoxin exposure among school students aged between of 5 and 24. The findings indicate that only a small percentage of schools have established pre-qualified suppliers, with the majority opting to buy from random suppliers, yet it is evident in the study that maize flour and beans sourced from pre-qualified suppliers significantly had low aflatoxin levels compared to purchasing from random suppliers. Therefore, emphasizing the need for pre-qualified suppliers and implementing a rigorous supplier pre-qualification process is crucial. Furthermore, both the public and private schools prioritize quantity over quality when purchasing food and rely on visual aids to check the quality of maize flour and beans, however, such parameters cannot detect aflatoxins, hence a need to include affordable ELISA kits in guidelines for food handling in schools. The absence of proper guidelines for food storage, the failure for most stores to meet minimal standards set by the World Health Organization and unsanitary conditions in dining halls all call for urgent drafting and implementation of guidelines on food handling in schools. Compliance with such guidelines can be monitored using the already-existing, under-utilized government structures, particularly the District Education Officer and District Inspector of Schools offices. Similarly, in this study, posho was found to contain high levels of aflatoxins beyond MTL, yet it is directly consumed by students two times a day and the risk characterization of aflatoxins in maize and posho indicated that school children, especially younger ones, are at a higher risk of exposure and hepatocellular carcinoma. The findings underscore the critical importance of enhancing food handling practices, storage conditions, and supplier selection, as well as fostering increased awareness among all stakeholders involved in the food chain, from farm to fork. By addressing these key areas, it is possible to mitigate the risks associated with aflatoxins, thereby ensuring the provision of safe and nutritious meals for students in schools across Uganda. As a follow-up to this study, it is recommended to expand the research scope by incorporating a greater number of schools from varied geographical areas within Uganda, while rigorously monitoring the entirety of the food supply chain.This expanded investigation would provide a more comprehensive understanding of the sources of contamination, which were beyond the scope of the current study.

Supplementtary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.24885081
Table S1. Summary characteristics of respondents.
Figure S1. Mean aflatoxin levels within sampled food types.

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Conflict of interest

The authors do not declare any conflict of interest for this research.

Author Declaration

The author contribution for this study is summarized as follows:
Conceptualization and proposal writing: EO, JBN, WM and RA; developing and pretesting data collection tools: EO, JBN, WM and RA, method validation: EO, GM and AM, data collection: EO, JBK and RA, laboratory sample analysis: EO, GM, data analysis and writing the first draft, EO, JBN, WM, reviewing the first draft and rearranging manuscript: AM and GM, supervision: AM and GM, funding acquisition: EO. All mentioned authors have read and agreed to this submitted manuscript.

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