Original Article

Liver Cancer Risks Associated with Consumption of Groundnuts and Maize Contaminated with Aflatoxins in Eastern Uganda

Robert Muyinda[1, 2*], Patrick Ogwok[1], Michael Bamuwamye[1]

1- Department of Food Science and Technology, Kyambogo University, Uganda

2- Department of Animal Sciences, Busitema University, Uganda

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ABSTRACT

Background and Objectives: Regular consumption of food contaminated with aflatoxins is associated with the prevalence of liver cancer in humans. Aflatoxin contamination of food occurs because of poor handling practices during drying, storage and processing. The cancer risk for children and adults, who consume contaminated maize and groundnut products with aflatoxins, was assessed.

Materials and Methods: he level of aflatoxin was assessed using enzyme Enzyme-linked Linked immunosorbent Immunosorbent assay Assay. Cancer risk was characterized using margin of exposure and hepatocellular carcinoma risk.

Results: The level of aflatoxins ranged from 2.94– to 3.38 µg/kg in groundnuts and 2.25– to 2.38 µg/kg in maize grains. Groundnut pastes and maize flours included aflatoxin levels of 2.12– to 2.53 µg/kg and 1.51– to 1.54 µg/kg, respectively. The quantities of aflatoxin in groundnuts and maize grains were correspondingly higher than those in the pastes and flours. Levels of aflatoxin were less than the maximum limit of 10 µg/kg, set by the East African Community. The margin of exposure of 9.90–185.76 was less than the safety margin of 10000 for aflatoxin exposure in foods. The hepatocellular carcinoma risk varied between 0.94– and 49.86 cases/100,000 individuals/y with the values for children of being 2– to 3 times higher than the World Health Organization acceptable level of one cancer case/y/100,000 individuals.

Conclusions: Consumption of groundnut and maize products in Eastern Uganda is greatly a concern and should be prioritized as a public health problem.

Keywords: Maize, Groundnuts, Aflatoxin, Cancer risk assessment, Uganda

Highlights

- Regular consumption of foods contaminated with aflatoxins is linked to liver cancer in humans
- Children were further more susceptible to aflatoxin exposure , compared to thethan adults
- The margin of exposure was significantly lesslower than the safe margin of 10000, which increased the likelihood of liver cancer cases in the parishes
- The hepatocellular carcinoma risk ranged between 0.94– and 49.86 cases/100,000 individuals/y with the values for children of being 2– to 3 times higher than the World Health Organization acceptable level of one cancer case/y/100,000 individuals
- Regular monitoring and assessment of aflatoxins in food levels can decrease the risk of exposure and hence liver cancer cases

***Address for correspondence:** Robert Muyinda, Department of Animal Sciences, Busitema University, Uganda
E-mail address: rmuyinda2014@gmail.com

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Introduction

Maize and groundnuts are major staple food crops in Uganda with an estimated annual production of 2,964,017 and 242,243 Metric tons, respectively [1]. Groundnuts are roasted or processed into flour and paste, which are used in sauces and stews as protein sources, while maize is processed into flours [2]. These are consumed in homes, schools, hospitals, prisons and catering institutions and used as ingredients in food products, including baby foods [3;27]. Maize contributes almost 50% of the daily calorie intake while groundnuts are sources of dietary fats and proteins for many Ugandans [4;5]. Contamination of maize and groundnuts with aflatoxins is prevalent in tropical and subtropical regions, especially sub-Saharan African countries, including Uganda [6;7]. The existance of toxigenic *Aspergillus* spp. and over-reliance on traditional postharvest handling practices such as drying on bare ground causes aflatoxin contamination in the food chain [19;30]. Food contamination by aflatoxins is worsened by crop variety, temperature, water stress, drought, moisture content and insect infestation [8]. Aflatoxins, including AFB1,AFB2, AFG1 and AFG2, are greatly concerned for the economy and public health [9].

Human exposure to aflatoxins through intake of contaminated groundnuts and maize products lead to adverse health outcomes [10]. The ingestion of aflatoxins in foods can breakdown of proteins due to a decrease in protease enzyme activity and decrease the metabolism of vitamins A and C, zinc and other micronutrients [11]. Decreased protein intake due to aflatoxin exposure in foods has led to the development of edema, as well as Kwashiorkor in children [8; 12]. Chronic hepatitis B infection, caused by the hepatitis B virus (HBV) and exposure to aflatoxins, is critical in formation of hepatocellular carcinoma (HCC) in developing countries. Aflatoxin B1 interacts synergistically with HBV, leading to the development of HCC [13]. The prevalence of hepatitis B in eastern Uganda varies 2.1–4.4%, which increases risk of HCC [14;22]. In Uganda, cancer is estimated at 34,008 cases; of which, 3,700 cases are liver cancer [6;15]. However, liver cancer cases in eastern Uganda due to aflatoxin synergism with hepatitis B is unknown. Therefore, the current study assessed aflatoxin levels and characterized the liver cancer risks as an input for improvement.

Materials and Methods

Study area

The study area comprised 32 parishes from four major maize and groundnut producing districts in eastern Uganda. The parishes were contrasted by altitude, average temperature and rainfall. Eastern Uganda is located at the altitude of 1,085–1,143 m above sea level with an average temperature of 26 °C and annual rainfall greater than 1,000 mm (Table 1). Farmers in these areas practice subsistence farming, relying on the rain for the cultivation of crops. Two sub-counties were selected with the assistance of extension workers from the district agriculture office. In every sub-county, four parishes were randomly selected for food sample collection.

Table 1. Attributes and geographical locations of the study area

District	Altitude (meters above sea-level)	Annual Rainfall (mm)	Annual average Temp. (ºC)
Namutumba	1,135	2050	24.0
Iganga	1,138	1,436	23.5
Soroti	1,130	1194	26.0
Serere	1,085	1,250	26.0

Sampling of foods for aflatoxin analysis

Samples of maize grains, maize flours, groundnuts and groundnut pastes were collected from each district following the sampling procedure of the International Organization for Standardization (ISO) method 24333:2009(E). A total of 198 groundnut paste, 279 maize grain, 270 maize flour and 261 groundnuts samples were collected from the study parishes (Figure 1). Each food type was composed of 96 samples of 500 g, making a triplet per parish. All samples were transferred to the Analytical Biosciences Laboratory, Makerere University, on the similar day and stored at -20 °C to prevent further contaminations.

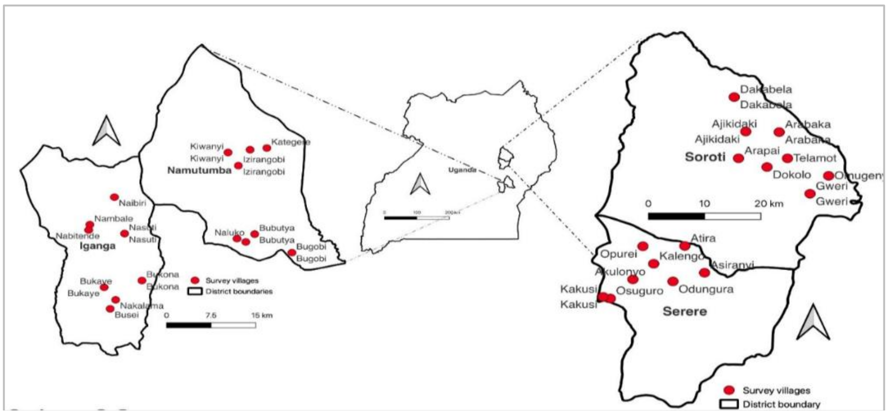

Figure 1. Map of Uganda, showing distribution of sampling sites in the study areas

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Sample Preparation

The 500 g food samples were separately ground for 30 s using laboratory stainless-steel blender, passed through a 20-mm sieve and thoroughly mixed. Aflatoxin was extracted by weighing 20 g of the ground sample in a clean conical flask of 250 ml with a glass lid and 100 ml of 70% methanol (methanol:distilled water 70:30) added. The conical flask was tightly sealed, then stirred thoroughly for 30 min at room temperature (RT) using a AS1-C-19 Orbicult shaker (Dutscher, France). The samples were extracted in a ratio of 1:5 (w:v) of sample to extraction solution. The samples were settled and then filtered using Whatman no. 1 filter papers (Whatman, USA). The filtrate was collected for aflatoxin analysis using enzyme-linked immunosorbent assay (ELISA) kits.

Detection of aflatoxins

Total aflatoxin content was detected using ELISA commercial kits (Elabscience Biotechnology, USA). All reagents and kits were set to 25 °C before use. The kit consisted of a solid-phase competitive enzyme immunoassay and polystyrene microwells coated with an antibody with a high affinity for aflatoxins. One microwell was used for each sample or standard (0, 0.02, 0.04, 0.08, 0.16 and 0.32 ppb). Using single-channel pipettor, 50 µl of the standard or sample were added to the microtiter plate, followed by adding 50 µl of HRP conjugate and 50 µl of an antibody working solution to initiate the reaction. The contents were carefully mixed by pipetting the solutions for three times. The microwell plate was sealed and gently oscillated for 5 s. This was incubated at 25 ℃ for 30 min. After incubation, the seal was carefully removed and the contents of the wells were discarded. The wells were washed three times with washing solution to remove unbound toxins. Fifty microliters of the substrate (chromogen) were added to each well and mixed gently by shaking the plate manually. The microplate was incubated at RT for 15 min in dark. The reaction was terminated by adding 50 µl of stop (acid) solution to each well until the color changed to yellow. The absorbance was measured photometrically at 450 nm within 30 min after the addition of the stop solution using a UT-6550 ELISA microplate reader (MRC, Israel).

Validation of the enzyme-linked immunosorbent assay kits

Samples of groundnuts, groundnut pastes, maize grains or maize flours with values less than the limit of detection (LOD) for aflatoxin were spiked with aflatoxin standard of 0.02 µg/l following a method described by Nguegwouo et al. (2023) [16]. The food sample was homogenized with the standard for 10 min to ensure toxin dispersion. The LOD and limit of quantification (LOQ), coefficient of variation (CV) and recovery rate (%) of the ELISA kits were assessed using Equations 1, 2, 3 and 4, respectively (Table 2).

$$\text{LOD} = 3 \times \text{standard deviation} \quad (1)$$

$$\text{LOQ} = 10 \times \text{standard deviation} \quad (2)$$

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Arithmetic mean}}\, x\, 100 \quad (3)$$

$$\text{Recovery (\%)} = \frac{\text{Arithmetic mean}}{\text{Concetration}}\, x\, 100 \quad (4)$$

Aflatoxin exposure assessment

Human exposure to aflatoxin was assessed using probable daily intake (PDI). The PDI was assessed using the mean aflatoxin concentration (µg/kg) in the foods, the daily food intake (g/d) and the average body weight (BWa) (kg). The consumption rates for maize grains, maize flours and groundnuts/pastes used in the calculation respectively included 177, 400 and 93.2 g/person/d for adults [1;17]. Similarly, a consumption rate of 100 g for maize grains and groundnuts/pastes and 200 g/person/d for maize flours was used to calculate the PDI for children. Average weights of 60 kg for adults and 20.5 kg for children of 3–6 years old were suggested [18]. The PDI was computed using Eq. 5.

$$\text{PDI} = \text{C} \times \frac{\text{S}}{\text{BWa}} \quad (5)$$

Where, PDI was the probable daily intake, C was the consumption rate, S was the aflatoxin contamination and BWa was the average body weight.

Table 2. Validation parameters of the food samples using aflatoxin standards of 0.02 µg/l

Food matrix	Theoretical conc. (µg/kg)	Arithmetic mean conc. (µg/kg)	SD	LOD (µg/kg)	LOQ (µg/kg)	CV (%)	Recovery rate (%)
Groundnuts	0.02	0.018	0.001	0.002	0.006	3.557	90.09
Groundnut paste	0.02	0.019	0.001	0.002	0.006	3.293	92.80
Maize grain	0.02	0.018	0.001	0.003	0.009	5.106	91.69
Maize flour	0.02	0.020	0.002	0.006	0.019	9.502	99.72

Risk characterization

Risk characterization is an estimate of the likelihood of adverse health effects in human populations as a consequence of the exposure to a hazard [19]. This was assessed using qualitative and quantitative risk approaches. Qualitative risk assessment was carried out using the margin of exposure (MOE) while the quantitative approach assessed the HCC risk.

Margin of Exposure

Aflatoxin are genotoxic and carcinogenic substances that include no acceptable limit at any level of intake [20]. According to the European Food Safety Authority (EFSA), the carcinogenic potency of AFB1 is similar to that of the total aflatoxin such as ∑(AFB1, AFB2, AFG1 and AFG2) because AFG1 and AFB2 resulted in development of HCC in experimental rodents [18]. Based on studies in animals, EFSA recommended a benchmark dose lower confidence limit 10% (BMDL10) of 0.4 µg/kg BW per day for the incidence of HCC in male rats due to the exposure to AFB1, used in the MOE approach. The MOE of a substance is the ratio of a toxicological reference point (the dose causing a low assessable response) to its theoretical, predicted or estimated dose or concentration of human intake [21]. In this study, a toxicological reference dose of 0.4 µg/kg BW/d for aflatoxin was used. The 0.4 µg/kg BW/d is a BMDL10 that caused no more than 10% cancer incidence in rodents or human for studies in Africa [20]. An MOE less than 10,000 is addressed as a public health concern with respect to aflatoxins. The MOE was estimated using Eq. 6.

$$\mathrm{MOE} = \frac{\mathrm{BMDL1}}{\mathrm{PDI}} \quad (6)$$

Where, MOE was margin of exposure, BMDL10 was the benchmark dose lower confidence limit and PDI was the probable daily intake of AF in foods.

Hepatocellular carcinoma risk

The HCC risk is based on the carcinogenic potency resulting from the synergism of aflatoxin contamination in foods and HBV infection [18]. In hepatitis B surface antigen-positive individuals ($HBsAg^+$), the AFB1 carcinogenic potency is estimated as 0.3 cases/y/100,000 individuals. In hepatitis B surface antigen-negative individuals ($HBsAg^-$), the AFB1 carcinogenic potency is estimated as 0.01 cases/y/100,000 individual. In this study, prevalence (P) rates of $HBsAg^+$ individuals in the study population included 4.4 and 2.1% in Teso and Busoga Subregions, respectively [22]. The HCC risk (case/y/100,000 individuals) due to hepatitis B was calculated by multiplying the daily exposure by average potency in Eqs. 7 and 8 [21].

$$\text{Liver cancer risk} = \text{Exposure (PDI)} \times \text{Average potency} \quad (7)$$

$$\text{Average potency} = 0.3 \times \mathrm{P} + 0.01 \times (1 - \mathrm{P}) \quad (8)$$

Where, P was the $HBsAg^+$ prevalence rate; average potency for Teso = [0.03 × P] + [0.01 × (1 - P)] = (0.3 × 0.044) + (0.01 × 0.956) = 0.02276 cases/y/100,000 individuals and a verage potency for Busoga = [0.03 × 0.021] + [0.01 × 0.979] = 0.01042 cases/y/100,000 individuals.

Statistical analysis

Data were grouped based on the food matrix (maize grains, maize flours, groundnuts and groundnut pastes). The second grouping was carried out based on the location; where, the samples were collected. Regression equation was used to assess the aflatoxin content calculated from the standard curves by plotting absorbance against total aflatoxin standards using Excel v.19 (Microsoft, USA). Statistical analysis of variance (ANOVA) and Tukey test were used to compare mean differences of aflatoxin contaminations, exposure and cancer risks due to intake of food from selected districts at a 5% level of significance ($p < 0.05$) using R v.4.4.2. Data was reported as lower and upper bound categories.

Results and Discussion

Aflatoxin content in groundnuts and groundnut pastes

Aflatoxins were detected in groundnuts from 32 parishes in Teso and Busoga Subregions. Seventy five percent of the groundnut paste samples were positive for aflatoxins. The level of aflatoxins in groundnuts varied 2.94–3.38 µg/kg (Table 3). The pastes included aflatoxin levels varying 2.80–3.34 µg/kg. The mean level of aflatoxins in the groundnut pastes was not different ($p > 0.05$) from that of the groundnut seeds. The levels of aflatoxins in groundnuts and groundnut pastes were less than the East African Community (EAC) limit of 10 µg/kg. This could be attributed to the storage of groundnuts in their shells, which controlled proliferation of the molds [23]. The contamination of groundnuts by aflatoxins in all 32 parishes could be a result of sprinkling of groundnuts with water prior to shelling. This technique is practiced by most farmers and predisposes the nuts to mold infection and aflatoxin contamination [24]. Kaaya *et al.* (2006) [2] reported aflatoxin levels in groundnuts varying 35.4–52.0 µg/kg and 53.4–65.4 µg/kg from wholesale and retail markets, respectively. Samples less than the LOD were stored in hermetic bags that were air tight and controlled the mold proliferation [6;25]. The level of aflatoxins in the groundnut pastes was not different from that in groundnuts since the pastes included a short storage time [26]. In addition, the paste was prepared instantly for preparing sauce-limiting exposures

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to molds [26]. Lukwago *et al*. (2019) [6] reported a high aflatoxin contamination range of 0–250 µg/kg in groundnut pastes of markets in Uganda. Overall, groundnut pastes and groundnuts included safe levels of aflatoxins.

Aflatoxin contents in maize grains and maize flours

Aflatoxins were detected in 78% of maize grain and 72% of maize flour samples (Table 3). The level of aflatoxin contamination in maize grains ranged 2.87–3.05 µg/kg. Maize flours included aflatoxin levels of 2.11–2.14 µg/kg. The quantity of aflatoxins in the maize grains was higher ($p < 0.05$) than that in maize flours. The levels of aflatoxins in maize grains were higher than that in the flours. This could be explained by the fact that maize flours were processed from dehulled maize [27]. Dehulling decreases aflatoxin levels by nearly 46% [28]. The high aflatoxin levels in flours are due to the differences in processing and storage contributions that predisposed it to mold infections. Differences in aflatoxin contamination in maize grains from harvesting maize between July and September, where rainfall and humidity are high, lead to inappropriate drying [13]. Poorly dried maize, especially from bare grounds, is characterized by mold growth and aflatoxin contamination during storage [29;30]. Samples with values less than the detection limits account for appropriate drying of the crops prior to harvest and the short storage time of the products [23]. Aflatoxin levels as high as 805.5 µg/kg in maize grains and 33.7 µg/kg in maize flours have been reported in Uganda [26;30]. In the current study, aflatoxin levels in maize grains and flours were less than the limit of 10 µg/kg, established by the EAC.

Probable daily intake of aflatoxins in groundnuts and maize products

Groundnuts and groundnut pastes

The PDI of aflatoxin for adults through intake of groundnuts was 0.001–0.005 µg/kg BW/d in the lower and 0.004–0.007 in the upper bound categories. The PDI for children was 0.002–0.017 µg/kg BW/d and 0.012–0.023 µg/kg BW/d (Table 3). The PDI values for adults through consumption of groundnut pastes varied 0.002–0.006 µg/kg BW/d and 0.004–0.006 µg/kg BW/d. The PDI of aflatoxins for children ranged 0.005–0.017 and 0.013–0.019 µg/kg BW/d. Significant differences ($p < 0.05$) were seen in PDI for adults and children in the groundnuts and the groundnut pastes.

The PDI of aflatoxins for children and adults through intake of groundnuts and groundnut pastes were 57–400 times less than the reference dose of 0.4 µg/kg BW/d in the lower and upper bounds, respectively [18]. This is attributed to the intake patterns and the total aflatoxin levels in groundnuts and pastes [31]. The PDI for children was higher than that for adults due to the differences in BW [32]. The smaller the body weight, the more the exposure to aflatoxins as a result of increased surface area to volume ratios [33]. In addition, children are vulnerable to aflatoxin exposure due to differences in physiology and dietary exposures per kg BW, compared to adults [34]. The current findings were similar to exposure values for adult groundnut consumers in Nigeria [19]. However, higher PDI values (0.087–0.2 µg/kg BW/d) were reported for children and adult groundnut consumers in Ghana by Korley *et al.* (2021) [36].

Maize grains and flours

Aflatoxin exposure for adults through intake of maize grains were 0.002–0.01 µg/kg BW/d and 0.002–0.011 µg/kg BW/d in the lower and upper bound categories, respectively. The PDI for children ranged 0.004–0.017 µg/kg BW/d and 0.004–0.018 µg/kg BW/d (Table 2). The PDI of aflatoxins for adults was 0.012–0.015 µg/kg BW/d and 0.017–0.022 for children from the intake of maize flours. The PDI for children was significantly ($p < 0.05$) higher than that for the adults. The PDI of aflatoxins for adults and children through consumption of maize flours was higher than that for maize grains. This was explained by the flour consumption rates of higher rates, compared to maize consumption rates [35]. Although, the aflatoxin contamination was slightly higher in maize grains than the flours. Consumption patterns and aflatoxin exposures in maize flours are public-health concerns of the children and adults, who regularly depend on maize porridges and breads [31]. Use of maize flour as a major ingredient in the formulation of baby foods for combating protein-energy malnutrition increases the aflatoxin exposure for children [36]. The aflatoxin exposure results in the present study are similar to those reported for mothers and children of 0.01–1.0 µg/kg BW/d for maize consumers in Kampala, Uganda [31]. High PDI of 0.012–0.065 µg/kg BW/d for adults and children, respectively, were reported in maize consumers in Northern Uganda [13]. Kortei *et al.*(2022) [37] reported high PDI for aflatoxins in children and adult maize consumers in Ghana.

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Table 3. Aflatoxin contents and probable daily intakes in groundnuts and groundnut pastes

Parish	Storage period (months)	Groundnuts						Groundnut paste					
		AF-content (µg/kg)		PDI-Adult[a] (µg/Kg.bw/day)		PDI-Children[a] (µg/Kg.bw/day)		AF-content (µg/kg)		PDI-Adult[a] (µg/Kg.bw/day)		PDI-Children[a] (µg/Kg.bw/day)	
		LB[b]	UB[b]	LB[b]	UB[b]	LB[b]	UB[b]	LB[b]	UB[b]	LB[b]	UB[b]	LB[b]	UB[b]
Ajikidaki	5-6	3.34	3.53	0.005	0.005	0.016	0.017	1.96	3.20	0.003	0.005	0.010	0.016
Akulonyo	3-6	2.69	3.26	0.004	0.005	0.013	0.016	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Arabaka	4-6	3.28	3.37	0.005	0.005	0.016	0.016	3.01	3.48	0.005	0.005	0.015	0.017
Arapai	5-10	3.47	3.51	0.005	0.005	0.017	0.017	1.12	3.24	0.002	0.005	0.005	0.016
Asiranyi	3-5	3.28	3.37	0.005	0.005	0.016	0.016	2.40	2.84	0.004	0.004	0.012	0.014
Atira	3-10	2.76	3.32	0.004	0.005	0.013	0.016	3.35	3.56	0.005	0.006	0.016	0.017
Bubutya	4-6	3.39	3.48	0.005	0.005	0.017	0.017	3.16	3.22	0.005	0.005	0.015	0.016
Bugobi	4-5	2.90	3.30	0.005	0.005	0.014	0.016	3.55	3.58	0.006	0.006	0.017	0.017
Bukaye	4-6	0.38	4.71	0.001	0.007	0.002	0.023	3.32	3.49	0.005	0.005	0.016	0.017
Bukona	3-4	3.30	3.34	0.005	0.005	0.016	0.016	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Bulange	3-4	3.41	3.48	0.005	0.005	0.017	0.017	3.22	3.46	0.005	0.005	0.016	0.017
Busei	2-4	3.30	3.49	0.005	0.005	0.016	0.017	3.34	3.42	0.005	0.005	0.016	0.017
Dakabela	5-10	3.23	3.39	0.005	0.005	0.016	0.017	3.01	3.09	0.005	0.005	0.015	0.015
Dokolo	7-10	3.31	3.52	0.005	0.005	0.016	0.017	2.39	3.20	0.004	0.005	0.012	0.016
Gweri	6-8	1.99	2.41	0.003	0.004	0.010	0.012	2.97	3.55	0.005	0.006	0.014	0.017
Izirangobi	2-3	1.61	2.70	0.003	0.004	0.008	0.013	2.26	2.58	0.004	0.004	0.011	0.013
Kakusi	5-10	2.85	3.65	0.004	0.006	0.014	0.018	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Kalengo	3-6	2.50	3.54	0.004	0.006	0.012	0.017	2.85	3.38	0.004	0.005	0.014	0.016
Kategere	4-6	3.30	3.43	0.005	0.005	0.016	0.017	3.26	3.52	0.005	0.005	0.016	0.017
Kiwanyi	2-4	3.37	3.56	0.005	0.006	0.016	0.017	3.01	3.46	0.005	0.005	0.015	0.017
Magada	3-4	3.07	3.49	0.005	0.005	0.015	0.017	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Nabitende	3-4	2.73	3.24	0.004	0.005	0.013	0.016	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Naibiri	4-6	2.41	3.14	0.004	0.005	0.012	0.015	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Nakalama	3-4	3.16	3.20	0.005	0.005	0.015	0.016	3.31	3.55	0.005	0.006	0.016	0.017
Naluko	3-4	3.41	3.45	0.005	0.005	0.017	0.017	3.19	3.54	0.005	0.006	0.016	0.017
Nambale	4-5	3.21	3.34	0.005	0.005	0.016	0.016	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Nasuti	5-10	3.30	3.59	0.005	0.006	0.016	0.018	2.96	3.71	0.005	0.006	0.014	0.018
Odungura	2-3	3.48	3.63	0.005	0.006	0.017	0.018	2.55	3.58	0.004	0.006	0.012	0.017
Omugenya	6-10	3.31	3.54	0.005	0.006	0.016	0.017	2.84	3.46	0.004	0.005	0.014	0.017
Opurei	5-8	1.99	2.41	0.003	0.004	0.010	0.012	2.82	2.99	0.004	0.005	0.014	0.015
Osuguro	5-8	3.47	3.51	0.005	0.005	0.017	0.017	< 0.002	< 0.002	0.000	0.000	0.000	0.000
Telamot	5-8	2.84	3.30	0.004	0.005	0.014	0.016	1.92	3.83	0.003	0.006	0.009	0.019
Σfx/n[c]		2.94	3.38	0.004	0.005	0.014	0.016	2.80	3.34	0.004	0.005	0.014	0.016

AF: Total aflatoxin; a: PDI-Adult and PDI-Children; Probable Daily intake for adult and children respectively; b: LB and UB; Lower and Upper 95% CI of Mean; c: Σfx/n; Total mean

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Risk characterization

Margin of exposure

Groundnuts and groundnut pastes

The MOE for adults due to aflatoxin exposure in groundnuts was 31.10–105.91 in the lower and 73.91–185.76 in the upper bound categories. The MOE values for children ranged 9.90–33.73 and 23.53–59.15, while those for adults ranged 58.16–99.50 and 72.64–179.74, respectively (Table 4). The MOE for adults was significantly ($p < 0.05$) higher than that for children. The MOE of aflatoxin for adults and children through intake of groundnuts and groundnut pastes were less than the safe lower limit of 10,000 [18], revealing that regular consumption of groundnut products was likely to cause a potential health risk. The MOE less than 10,000 for adults and children in the groundnuts and groundnut pastes was the result of aflatoxin contamination due to poor processing and storage conditions [23]. No difference ($p > 0.05$) was seen between the MOE in groundnuts and groundnut pastes because the paste was only stored for a short time before use. The present findings were similar to those reported by Qin *et al.* (2020) [21] for groundnut consumers in China. Korley *et al.* (2021) [36] reported MOE less than the threshold margin of 10,000 for children and adult consumers of groundnuts in Ghana; however, their values (2000–4597) were higher, showing a higher risk for consumers of groundnuts in this study.

Maize grains and flours

The MOE of aflatoxins for adult through consumption of maize grains was 37.25–175.38 in the lower and 38.66–179.43 in the upper bound categories. The MOE for children varied 22.53–106.06 and 23.38–108.51 (Table 4). The MOE for adults through intake of maize flours was 26.71–33.64 and 26.81–33.93, while those for children ranged 18.25–22.99 and 18.32–23.18. A significant difference ($p < 0.05$) was seen between the MOEs for children and adults in the flours and the maize grains. The recorded MOEs for children and adults due to aflatoxin exposure in maize grains and flours were less than 10,000. This revealed that regular consumers of low-grade maize and flours were at a high risk of aflatoxicosis. Moreover, children might be at a greater risk because they were introduced to weaning foods such as maize porridges at a rather early age [37]. Wokorach et al. (2021) [13] reported similar findings for maize and other grain consumers in Northern Uganda. However, Kortei *et al.* (2022) [37] reported MOE in a range of 2.67–6.25 for children and adult maize consumers in Ghana. A high liver cancer risk was observed in the current study. An MOE less than 10,000 for aflatoxins in foods is addressed as a public-health concern. Regular monitoring of aflatoxin contamination in maize and training of farmers and dealers on handling of grains to limit the health effects of aflatoxins should be prioritized as a moderation strategy.

Hepatocellular carcinoma

Groundnuts and groundnut pastes

The HCC risk for adults due to aflatoxin exposure in groundnuts ranged 0.94–12.34 cases/100,000 individuals/y in the lower and 6.76–12.94 cases/100,000 individuals/y in the upper bound categories. The HCC for children varied 2.94–38.74 and 21.23–40.62 cases/100,000 individuals/y (Table 5). The risk values due to aflatoxin exposure in groundnut pastes were 3.67–11.88 and 8.05–13.55 cases/100,000 individuals/y for the adults and 12.48–37.30 and 20.24–42.56 cases/100,000 individuals/y for the children. The HCC risk for children was significantly ($p < 0.05$) higher than that for the adults.

According to the United States Environmental Protection Agency (US-EPA), the acceptable additional CR is 1 cancer case/y/100,000 individuals with a tolerable risk range of 1–10 cases [39]. Results of this study suggested that the HCC risk for children was 2–3 times higher than the tolerable limit, according to EPA. Korley *et al*. (2021) [36] reported HCC risks of 63.4 and 26.9 cancers/y/100,000 individuals correspondingly for children and adult groundnut consumers in Ghana. Similarly, Kooprasertying *et al.*(2016) [40] reported values of 36.9 and 15.2 cancers/y/100,000 individuals for male and female groundnut consumers in Thailand.

Maize grains and flours

The HCC risk for adult due aflatoxin exposure in maize grains ranged 3.59–23.31 cases/100,000 individuals/y in the lower and 3.67 to 24.25 cancer cases/100,000 individuals/y in the upper bound categories. The risk values for children ranged 5.93–38.55 and 6.07–40.09 cases/100,000 individuals/y (Table 5). The HCC risk values ranged 21.32–33.61 and 21.50–34.07 cases/100,000 individuals/y for adults and 31.19–49.18 and 31.47–49.86 cases/100,000 individuals/y for children through the intake of maize flours. A significant difference ($p < 0.05$) in risks between children and adults was reported. The cancer risk for children and adults due to aflatoxin exposure in maize flours was high, compared to that in maize grains. Kortei *et al.* (2022) [37] reported an HCC risk of 43.6–99.0 cancer cases/y/100,000 for children and adult maize consumers in Ghana. Cancer risks of 1.50–6689.4 cases/y/100,000 have been reported for maize consumers [41]. The quantity of consumed flours is higher than that of consumed maize grains although the aflatoxin content for maize grains was higher [40]. The reported HCC revealed that children could be at a high health risk since maize flours are extensively used in the formulation of complementary foods.

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Table 5. Estimated margin of exposure values of aflatoxins from groundnuts and maize

Parish	Groundnut				Groundnut paste				Maize grain				Maize flour			
	Adult		Children		Adult		Children		Adult		Children		Adult		Children	
	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]
Ajikidaki	72.83	77.07	23.19	24.54	76.32	124.50	24.30	39.64	38.76	40.31	23.44	24.38	33.64	33.93	22.99	23.18
Akulonyo	78.55	94.76	25.01	30.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.93	28.16	18.40	19.24
Arabaka	76.47	78.47	24.35	24.99	73.58	85.24	23.43	27.14	58.03	78.04	35.09	47.19	28.40	29.15	19.41	19.92
Arapai	73.35	74.31	23.36	23.66	62.38	179.74	19.86	57.23	0.00	0.00	0.00	0.00	27.24	27.62	18.61	18.87
Asiranyi	76.47	78.47	24.35	24.99	90.14	106.76	28.70	34.00	39.22	39.75	23.72	24.04	28.20	28.32	19.27	19.35
Atira	76.99	92.73	24.52	29.53	72.22	76.73	23.00	24.43	39.66	39.86	23.99	24.11	27.21	27.21	18.59	18.59
Bubutya	73.95	75.94	23.55	24.18	80.03	81.42	25.49	25.93	37.79	38.66	22.85	23.38	28.20	28.46	19.27	19.45
Bugobi	77.65	88.42	24.73	28.16	71.94	72.64	22.91	23.13	37.67	42.01	22.78	25.40	29.56	29.72	20.20	20.31
Bukaye	31.10	185.76	9.90	59.15	73.85	77.44	23.52	24.66	125.84	128.56	76.10	77.74	0.00	0.00	0.00	0.00
Bukona	77.10	78.10	24.55	24.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.38	29.63	20.07	20.25
Bulange	73.92	75.63	23.54	24.08	74.45	79.80	23.71	25.41	38.95	40.85	23.56	24.70	0.00	0.00	0.00	0.00
Busei	73.66	77.90	23.46	24.81	75.24	77.20	23.96	24.58	45.84	46.87	27.72	28.35	0.00	0.00	0.00	0.00
Dakabela	75.90	79.60	24.17	25.35	83.24	85.60	26.51	27.26	109.88	110.77	66.45	66.99	28.34	28.50	19.37	19.47
Dokolo	73.21	77.83	23.31	24.78	79.06	105.93	25.18	33.73	38.55	40.98	23.31	24.78	26.77	27.13	18.29	18.54
Gweri	105.91	128.28	33.73	40.85	72.10	86.18	22.96	27.44	0.00	0.00	0.00	0.00	28.14	28.29	19.23	19.33
Izirangobi	91.32	148.87	29.08	47.40	99.50	113.35	31.69	36.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kakusi	69.82	88.79	22.23	28.27	0.00	0.00	0.00	0.00	38.07	39.14	23.02	23.67	0.00	0.00	0.00	0.00
Kalengo	71.22	99.66	22.68	31.73	75.73	89.70	24.11	28.56	39.66	39.86	23.99	24.11	27.21	27.21	18.59	18.59
Kategere	74.97	78.01	23.87	24.84	73.05	78.96	23.26	25.14	0.00	0.00	0.00	0.00	29.95	30.21	20.46	20.64
Kiwanyi	72.22	76.37	23.00	24.32	74.21	85.26	23.63	27.15	37.93	39.90	22.94	24.13	26.75	26.99	18.28	18.44
Magada	73.53	83.63	23.41	26.63	0.00	0.00	0.00	0.00	37.25	40.28	22.53	24.36	0.00	0.00	0.00	0.00
Nabitende	79.04	93.64	25.17	29.82	0.00	0.00	0.00	0.00	37.69	41.15	22.80	24.88	0.00	0.00	0.00	0.00
Naibiri	80.40	105.41	25.60	33.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.77	27.13	18.29	18.54
Nakalama	80.54	81.57	25.65	25.98	72.50	77.76	23.09	24.76	37.73	39.78	22.82	24.06	26.71	26.81	18.25	18.32
Naluko	74.70	75.45	23.79	24.03	72.52	80.47	23.09	25.63	40.22	65.58	24.32	39.66	28.14	28.29	19.23	19.33
Nambale	77.01	80.11	24.52	25.51	0.00	0.00	0.00	0.00	175.38	179.43	106.06	108.51	27.27	27.52	18.64	18.81
Nasuti	71.65	77.90	22.82	24.81	68.50	86.10	21.81	27.42	46.78	47.40	28.29	28.66	0.00	0.00	0.00	0.00
Odungura	71.01	73.91	22.61	23.53	70.09	98.53	22.32	31.38	38.07	39.14	23.02	23.67	27.21	27.21	18.59	18.59
Omugenya	72.59	77.75	23.12	24.76	73.77	89.94	23.49	28.64	37.60	39.11	22.74	23.65	27.75	27.90	18.96	19.06
Opurei	105.91	128.28	33.73	40.85	85.95	91.36	27.37	29.09	41.50	41.72	25.09	25.23	0.00	0.00	0.00	0.00
Osuguro	73.35	74.31	23.36	23.66	0.00	0.00	0.00	0.00	43.61	44.22	26.37	26.74	28.20	28.32	19.27	19.35
Telamot	77.48	90.44	24.67	28.80	58.16	123.46	18.52	39.31	58.65	59.16	35.47	35.78	28.82	33.65	19.69	23.00
$\Sigma fx/n^b$	76.06	90.54	24.22	28.83	75.36	93.92	24.00	29.91	52.81	56.10	31.94	33.93	28.12	28.58	19.22	19.53

MOE; Margin of Exposure; a: LB and UB; Lower and Upper 95% CI of Mean; b: $\Sigma fx/n$; Total mean

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Table 6. Liver cancer incidence for adults and children through intake of groundnuts and maize

Parish	$HBsAg^+$ prevalence rate (%)	Groundnut				Groundnut paste				Maize grain				Maize flour			
		Adult		Children		Adult		Children		Adult		Children		Adult		Children	
		LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]	LB[a]	UB[a]
Ajikidaki	4.4	11.83	12.51	37.14	39.29	6.95	11.33	21.82	35.58	22.62	23.52	37.40	38.90	26.88	27.11	39.34	39.67
Akulonyo	4.4	9.53	11.54	29.94	36.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.37	33.85	47.38	49.53
Arabaka	4.4	11.62	11.92	36.49	37.45	10.65	12.34	33.43	38.74	11.49	15.39	18.99	25.44	31.28	32.11	45.77	46.99
Arapai	4.4	12.27	12.43	38.54	39.05	3.97	11.48	12.48	36.07	0.00	0.00	0.00	0.00	33.02	33.48	48.32	48.99
Asiranyi	4.4	11.62	11.92	36.49	37.45	8.49	10.05	26.65	31.57	22.94	23.25	37.94	38.45	32.20	32.34	47.13	47.32
Atira	4.4	9.77	11.74	30.70	36.87	11.88	12.62	37.30	39.62	22.88	22.99	37.83	38.02	33.52	33.52	49.05	49.05
Bubutya	2.1	8.48	8.71	26.63	27.34	7.91	8.05	24.84	25.27	16.65	17.04	27.54	28.18	22.63	22.84	33.11	33.42
Bugobi	2.1	7.26	8.26	22.79	25.95	8.87	8.95	27.84	28.11	15.28	17.06	25.27	28.20	21.67	21.79	31.71	31.89
Bukaye	2.1	0.94	11.78	2.94	36.98	8.31	8.72	26.10	27.37	5.01	5.12	8.28	8.46	0.00	0.00	0.00	0.00
Bukona	2.1	8.25	8.35	25.89	26.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.73	21.92	31.81	32.08
Bulange	2.1	8.51	8.71	26.74	27.36	8.06	8.64	25.32	27.14	15.76	16.52	26.05	27.32	0.00	0.00	0.00	0.00
Busei	2.1	8.26	8.73	25.95	27.43	8.34	8.56	26.19	26.88	13.74	14.05	22.72	23.23	0.00	0.00	0.00	0.00
Dakabela	4.4	11.45	12.01	35.96	37.72	10.65	10.95	33.45	34.40	8.23	8.30	13.61	13.72	32.01	32.18	46.84	47.09
Dokolo	4.4	11.71	12.45	36.77	39.09	8.45	11.32	26.53	35.54	22.23	23.64	36.77	39.09	33.61	34.07	49.18	49.86
Gweri	4.4	7.06	8.54	22.16	26.81	10.51	12.56	33.00	39.44	0.00	0.00	0.00	0.00	32.23	32.41	47.17	47.43
Izirangobi	2.1	4.04	6.76	12.68	21.23	3.67	12.24	17.78	20.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kakusi	4.4	10.10	12.94	31.72	40.62	0.00	0.00	0.00	0.00	23.30	23.95	38.52	39.61	0.00	0.00	0.00	0.00
Kalengo	4.4	8.86	12.55	27.83	39.42	10.10	11.97	31.72	37.58	22.88	22.99	37.83	38.02	33.52	33.52	49.05	49.05
Kategere	2.1	8.25	8.59	25.91	26.97	8.14	8.80	25.58	27.65	0.00	0.00	0.00	0.00	21.32	21.50	31.19	31.47
Kiwanyi	2.1	8.43	8.91	26.46	27.99	7.52	8.64	23.62	27.13	16.13	16.97	26.68	28.05	23.86	24.07	34.92	35.23
Magada	2.1	7.67	8.73	24.08	27.42	0.00	0.00	0.00	0.00	15.97	17.27	26.40	28.55	0.00	0.00	0.00	0.00
Nabitende	2.1	6.83	8.09	21.46	25.42	0.00	0.00	0.00	0.00	15.62	17.06	25.82	28.21	0.00	0.00	0.00	0.00
Naibiri	2.1	6.04	7.85	18.95	24.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.73	24.06	34.73	35.21
Nakalama	2.1	7.89	8.00	24.79	25.11	8.27	8.87	25.98	27.87	16.18	17.05	26.75	28.20	24.02	24.11	35.15	35.28
Naluko	2.1	8.53	8.62	26.80	27.07	7.98	8.86	25.07	27.82	9.31	15.19	15.40	25.12	22.76	22.88	33.31	33.49
Nambale	2.1	8.04	8.36	25.24	26.25	0.00	0.00	0.00	0.00	3.59	3.67	5.93	6.07	23.40	23.61	34.24	34.56
Nasuti	2.1	8.25	8.98	25.91	28.19	7.41	9.27	23.28	29.12	13.59	13.77	22.47	22.76	0.00	0.00	0.00	0.00
Odungura	4.4	12.34	12.84	38.74	40.31	9.02	12.68	28.33	39.82	23.30	23.95	38.52	39.61	33.52	33.52	49.05	49.05
Omugenya	4.4	11.71	12.55	36.79	39.42	10.06	12.25	31.60	38.45	23.31	24.25	38.55	40.09	32.69	32.86	47.84	48.09
Opurei	4.4	7.06	8.54	22.16	26.81	9.98	10.60	31.33	33.29	21.86	21.98	36.15	36.34	0.00	0.00	0.00	0.00
Osuguro	4.4	12.27	12.43	38.54	39.05	0.00	0.00	0.00	0.00	20.62	20.91	34.10	34.58	32.20	32.34	47.13	47.32
Telamot	4.4	10.04	11.70	31.53	36.74	6.79	13.55	21.34	42.56	15.42	15.55	25.49	25.71	26.98	31.45	39.49	46.02
$\Sigma fx/n$[b]	3.25	8.90	10.19	27.96	32.00	6.31	7.92	20.02	24.29	13.06	13.80	21.60	22.81	20.35	20.67	29.78	30.25

Liver cancer risk from Lower and upper 95% CI of mean AF exposure (cases/100,000 persons/year) = Exposure x Average potency; $HBsAg^+$ prevalence rate was obtained from *Ministry of Health National Sero-epidemiological Survey of 2019; a: LB; Lower Bound; UB; Upper Bound; b: $\Sigma fx/n$; Total mean

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Conclusion

Maize and groundnut products in Eastern Uganda are safe for consumption based on their low levels of aflatoxins that were less than EAC standards. Exposure to aflatoxins in children was significantly ($p < 0.05$) higher than that in adults. Aflatoxin exposure in groundnuts and maize grains were correspondingly lower than those in pastes and flours. The MOE of aflatoxin was lower than the acceptable safe margin of 10000, signifying heath concerns to regular consumers of groundnuts and maize products. Liver cancer risk was greater than the EPA established tolerable range of 1–10 cases/100,000 individuals/y. In addition, unacceptably high liver cancer risks were reported due to aflatoxin exposure in maize and groundnut products. This study highlights needs of regular monitoring of aflatoxin contamination in maize and groundnut products and training of farmers and dealers on the appropriate handling of grains as a moderation strategy to decrease the associated health risks.

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Author contribution

RM, PO and MB conceptualized and developed the study and did data curation. RM carried out the experiments and wrote the original draft of the manuscript. PO and MB edited and revised the manuscript. All authors read and approved the manuscript for submission.

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