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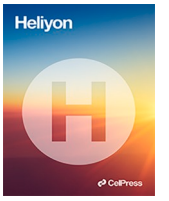

Prevalence of aflatoxin along processing points of locally made complementary food formulae in northern Uganda: Safety and children's exposure across seasons

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ABSTRACT

Aflatoxin contamination along the processing points of locally made complementary food composite needs to be ascertained and minimized to reduce exposure to weaning children. The study established the concentrations of total aflatoxin (TAF) and aflatoxin B1 (AFB_1) along the processing points of locally made malted millet sesame soybean composite (MMSSC) across season one (wet) and season two (dry) and determined children's exposure to them. A total of 363 samples were collected in 2019. TAF and AFB_1 concentrations were determined quantitatively using an enzyme-linked immunosorbent assay (ELISA). Consequently, exposure of individual children was assessed as Estimated Daily Intake (EDI), (ng kg^{-1} bw day^{-1}). All the samples along the processing points had detectable concentrations of TAF and AFB_1 ranging from 0.578 μg kg^{-1} to 1.187 μg kg^{-1} and 0.221 μg kg^{-1} to 0.649 μg kg^{-1} respectively. Contamination was highest in raw materials; soybean (*Glycine max*) > sesame (*Sesamum indicum*), followed by stored composite, freshly prepared composite, and least in millet (*Eleusine coracana*). Contamination varied significantly across seasons with the wet season having higher contamination than the dry season at $P = 0.05$. All samples (100%) were within the European Commission (EC) acceptable maximum tolerable level for TAF and AFB_1 (4 μg kg^{-1} and 2 μg kg^{-1}) respectively for processed foods for general consumption. But were below the EU acceptable maximum tolerable level for TAF and AFB_1 (0.4 μg kg^{-1} and 0.1 μg kg^{-1}) respectively for processed baby foods cereals. However, all were within the United States- Food and Drug Authority (US-FDA) and East African Community (EAC) set maximum acceptable limit of 20 μg kg^{-1} for TAFs, 10 μg kg^{-1} and 5 μg kg^{-1} for TAF and AFB_1 respectively. Conversely, exposure to these toxins was much higher than the Provisional Maximum Tolerable Dietary Intake (PMTDI) of 0.4 ng kg^{-1} bw day^{-1} to 1.0 ng kg^{-1} bw day^{-1}. A significant difference in exposure to both toxins was observed with the weight. The age of 5 months was the most exposed. A concerted effort is needed to reduce children's exposure to MMSSC to TAF and AFB1, taking sesame and soybean as priority ingredients and proper storage based on season to control contamination.

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1. Introduction

Proper and adequate complementary feeding is critical for achieving good nutritional outcomes in children aged 6–23 months. This is because, at this stage of child growth, breast milk becomes inadequate even if a child's mother is well-nourished. Based on this challenge, the World Health Organisation (WHO) in corporation with Ministry of Health (MOH) authorities in various countries have put in place guidelines for implementing safe complementary feeding [1]. Globally, several certified companies produce and market standardized complementary foods designed to achieve good nutrition outcomes in children [2]. However, such industrially produced complementary food products are expensive and are usually inaccessible to rural communities, especially in low-income countries. This is even though improper complementary feeding is a major contributor to poor nutrition outcomes in children in those countries [3]. Households in low-income countries largely use locally made plant-based complementary food formulae [4]. Safety is a critical issue of concern with locally formulated complementary food in low-income countries due to low adherence to sanitary practices [5,6]. One of the greatest concerns is the issue of mycotoxin contamination due to the high prevalence of mycotoxins in dry plant-based food materials that constitute a larger part of the diet and are the principal constituents of the complementary food formulae in such countries [7–9].

Mycotoxins are toxic bi-products of fungal metabolism that contaminate food at any point along the food chain [10]. Mycotoxins such as aflatoxin have both direct and indirect negative impacts on the global economy. Directly, aflatoxin contamination of crop products negatively impacts a country's economy by lowering the quality leading to low sales and even product rejection both in the local and international markets [11]. Indirectly when livestock and humans feed on aflatoxin-contaminated feed or food, they are harmed by the metabolites that may result in morbidity, mortality, and secretion of the bio-transformed metabolites into their milk, meat, and eggs [12]. Additionally, the report indicates that feed business owners and farmers are usually into legal friction as a result of selling contaminated feeds [13]. These significantly lead to national economic loss. For instance, in Canada and the United States USD 5 million loss is registered due to mycotoxin contamination [14]. While 38% of global agricultural losses due to aflatoxin are registered in Sub-Saharan Africa totalling a greater economic loss of USD 450 million [14].

The most common mycotoxins of concern for food safety worldwide are aflatoxins [15,16]. Four types of primary aflatoxins have so far been identified, i.e., AFB_1, aflatoxin B_2 (AF B_2), aflatoxin G_1 (AF G_1), and aflatoxin G_2 (AF G_2) [17]. Two secondary types that are a result of the mammalian metabolism of AFB_1 and AFB_2 are aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2), respectively, and are usually secreted in milk [16].

Of all the six (6) aflatoxin types, AFB_1 was originally considered to be the most toxic and placed among group 1 carcinogens by the International Agency for Research on Cancer (IARC) [18]. However, it was later found that when AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 coexist, the mixture becomes more toxic than the individual toxins, and as such, the mixture has also been included among group 1 carcinogens [19]. Considering the fact that locally formulated complementary food utilized in local settings in low-income countries is largely plant-based, contamination by AFM_1 and AFM_2 and subsequent exposure of children to a mixture of AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 would be unlikely. Therefore, AFB_1 remains the most toxic fungal contaminant of plant-based complementary foods in this context.

Several human health conditions including hepatocellular carcinoma, aflatoxicosis, haemorrhage, hepatotocosis, nephroptosis, mutagenesis, immunosuppression, birth defects, and stunting have been associated with aflatoxin exposure [20,21]. Given the multiplicity of adverse health conditions associated with aflatoxin exposure, various regulatory bodies such as the EU, US-FDA, and EAC have set limits for acceptable aflatoxin contamination levels in food ranging from 0.1 to 20 μg kg^{-1} [22]. To further guarantee safety, a PMTDI of 1.0 ng kg^{-1} bw day^{-1} and 0.4 ng kg^{-1} bw day^{-1} for children and adults who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively have been adopted [23,24].

Several studies have examined aflatoxin contamination of locally processed complementary foods and exposure of children in low-income countries [9,25–28]. A critical examination of those studies indicates that results are largely based on data derived from already prepared complementary food samples. However, limited information exists on the contamination status of the ingredients used in the preparation of the complementary food formula as well as contamination status along the processing-storage pathway. Due to the fact that aflatoxin contamination of food can occur at any point along the production-consumption continuum [29], this lacuna makes it difficult to use the results of the aforementioned studies to identify critical points at which aflatoxin contamination of locally-formulated complementary food formula can be controlled. On the other hand, whereas results of exposure assessment already available provide indications of the risks of aflatoxin exposure through feeding children on locally formulated food composite, such information does not provide a comprehensive indication of the exposure risks because of a lack of information on critical handling aspects such as the length of storage of the food product once prepared. The objective of this study, therefore, was to compressively examine seasonal variation in aflatoxin contamination along the processing-storage-consumption continuum of MMSSC, a model product developed previously by Alowo et al. [30] and exposure of children aged 2–23 months to aflatoxin as a function of product, storage and season was also examined. Northern Uganda particularly Amuru and Nwoya districts where the study was conducted has bimodal seasons wet and dry seasons. The wet periods ranged from the end of March to the beginning of November while the dry season is from the end of November to early March.

2. Materials and methods

2.1. Study area

The study was conducted in the Acholi sub-region in Amuru (02°50′N 33°05′E) and Nwoya (02°38′N 32°00′E) districts, Northern

Uganda which is recovering from 20 years of war. The two districts were selected to follow up on a study conducted by Alowo et al. [30] The study population consisted of caregivers (mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father) of children (3–23 months).

2.2. Samples

A total of 363 samples were collected in 2019. The wet season samples (208) were collected in June 2019 and the dry season samples (155) were collected in early March 2019. The samples were purposively collected from all the caregivers of children who were trained in the preparation of locally made MMSSC complementary food composite. Wet season samples included millet (n = 46), sesame (n = 41), soybean (n = 45), freshly processed composite (n = 32), and composite stored for 30 days (n = 44). Consequently, samples for the dry season consisted of millet (n = 46), sesame (n = 35), soybean (n = 1), and freshly processed composite (n = 30) composite stored for 30 days (n = 43).

2.3. Collection of samples

Representative samples were collected and put in a sterile sealable sample bag. Two sample replicates of 250 g each were collected and labelled with a sample identification number, location, and date. Samples were then transported at 4 °C in cool to the laboratory for further processing. Using a laboratory grinder (KA M20 Batch Mill/Grinder, 20,000 rpm, Max. Vol 250 ml, 1,603,603), samples were finely ground, and the sample holder was cleaned and disinfected with 70% ethanol after every grinding process to avoid cross-contamination. One hundred grams (100 g) were removed from each sample in another sterile sample bag, labelled, and stored at 4 °C until further analyses.

2.4. Aflatoxin's extraction and analysis

Extraction was done following the manufacturer's instructions (TAF Assay-low matrix and AFB_1 Assay-low matrix HELICA Biosystems, Inc 2019). Concisely, 50% methanol extraction solvent was prepared by the addition of 50 ml analytical grade methanol to 50 ml double distilled water. 20 g of finely ground samples were extracted by adding it to 100 ml of 50% methanol and mixed for 10 min and centrifuged at 3500 rpm for 5 min, the supernatant was collected and proceeded to the ELISA assay. The resultant ratio of the sample to the extraction solvent was then 1:5 (w/v).

Analyses of TAF and AFB_1 were done by low matrix competitive solid phase inhibition, a competitive enzyme-linked immunoassay intended to quantitatively analyse AFB1, B_2, G_1, and G_2 in grains, nuts, cottonseeds, cereals, and other commodities including animal feeds. With a detection limit of <1 ppb. But then, the limit of detection (LOD) is commodity specific and should be measured for each different commodity. Concisely sample extracts were analysed as described by the manufacturer's protocol. The optical density (OD) of each sample in a microwell was read at a 450 nm filter using a microtiter ELISA plate reader (Thermo-fisher 357-Ty, Multiskan-FC, Shangai, China) and then entered into Excel Program. Using the manufacturer's spreadsheet, the dose-response curve was constructed by the mean of OD values stated as a percentage of the OD of zero standards (0.0) against the aflatoxin content of the standard (%B/Bo). Whereby %B is the percentage binding for each standard and sample while Bo is the zero standards set as 100% binding.

As described in the extraction protocol, the samples were diluted in a 5:1 ratio during extraction. Therefore, the concentrations of aflatoxin were multiplied by 5 to indicate parts per billion (ppb) of the samples. The LOD used to determine the sensitivity of the method was determined by calculating the mean concentration of 10 blank samples plus three standard deviations. The limit of quantification (LOQ) was calculated as the mean value plus 10 standard deviations. The limit of LOD and LOQ values were estimated to be 0.021 and 0.069 μg kg^{-1}, respectively. Concentrations of both the TAF and AFB_1 in the samples were measured by interpolation of the standard curve by constructing a dose-response curve using the average of OD values expressed as a percentage (%B/B0) of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknown TAF and AFB_1 concentrations were measured by interpolation from the standard curve. The concentrations of the standards were labelled on the vial (0.0, 0.02, 0.05, 0.1, 0.2, 0.4). However, the sample was diluted at a 5:1 ratio by extraction solvent as instructed in the extraction procedure and so the level of aflatoxin shown by the standard was multiplied by 5 to indicate the ng per gram (ppb) of the samples. Linear regression and coefficient of determination (R^2) were calculated to assess the linearity of the calibration curve using six points of the standard curve. The R^2 values for all the curves (R^2 0.989–0.996) were within acceptable values.

2.5. Estimation of TAF and AFB_1 dietary intake

In this study, deterministic methods were performed by combining normalized MSSC daily intake per body weight data. Whereby, the MSSC intake data was collected using a modified food frequency questionnaire, and 24 h Recall for three consecutive days and the average was used as previously described by Huong et al. [31]. Children's weights were measured using a precise weighing balance. Exposure of individual children to TAF and AFB_1 was assessed as Estimated Daily Intake, EDI (ng kg^{-1} bw day^{-1}) = (TAF or AFB_1 concentration x consumption/day/body weight) [31].

2.6. Statistical analysis

Using SPSS version 21, data were analysed using descriptive statistics and inferential statistics. Specifically, means, standard

deviation, standard error, and median were analysed for the dependent variables. The significance of the means was tested using a one-way analysis of variance (ANOVA) and Duncan post-ANOVA test for mean comparison in the case of TAF and AFB_1 concentrations across the five processing points. The *t*-test was used to assess differences in mean for EDI TAF and EDI AFB_1 between stored and processed products. A pairwise correlation analysis was also performed to test for the significance of the correlation between aflatoxin exposure indicators; age and weight.

3. Results

3.1. TAF concentrations at processing points

Overall TAF concentration was highest in soybean (1.174 ± 0.994 μg kg^{-1}) followed by sesame (1.024 ± 0.849 μg kg^{-1}), stored processed products (0.856 ± 0.172 μg kg^{-1}), freshly processed products (0.807 ± 0.236 μg kg^{-1}) and millet (0.709 ± 0.258 μg kg^{-1}) was least contaminated. The concentration of TAF significantly differed at processing points in the wet season. The highest concentration was observed in sesame (1.199 ± 1.123 μg kg^{-1}) followed by soybean (1.187 ± 1.001 μg kg^{-1}), freshly processed (0.912 ± 0.257 μg kg^{-1}), stored products (0.865 ± 0.216 μg kg^{-1}) and least in the millet (0.754 ± 0.312 μg kg^{-1}), (Table 1). However, there was no significant difference in TAF concentration between the soybean, freshly processed, and processed stored products. Although, TAF concentration differed significantly between sesame and millet.

In the dry season, TAF was highest in stored processed products (0.847 ± 0.112 μg kg^{-1}) followed by sesame (0.819 ± 0.157 μg kg^{-1}), freshly processed products (0.696 ± 0.531 μg kg^{-1}) and least in the millet (0.665 ± 0.183 μg kg^{-1}). There was no significant difference in TAF concentration between the millet and freshly processed. However, TAF was significantly higher in the stored products in the dry season. Soybean was not included in the analysis in the dry season because there was only one sample. Overall, there was no significant difference between freshly processed products and stored products.

3.2. AFB_1 concentrations at process points

Generally, concentration was highest in soybean (0.649 ± 0.256 μg kg^{-1}) followed by stored processed product (0.457 ± 0.265 μg kg^{-1}), sesame (0.380 ± 0.279 μg kg^{-1}), freshly processed products (0.377 ± 0.244 μg kg^{-1}), and the least were in millet (0.347 ± 0.333 μg kg^{-1}). However, there was no significant difference in AFB_1 concentration between freshly processed products, and sesame.

In the wet season, AFB_1 concentration was highest in soybean (0.649 ± 0.038 μg kg^{-1}) followed by stored processed product (0.512 ± 0.036 μg kg^{-1}), freshly processed products (0.406 ± 0.048 μg kg^{-1}), sesame (0.261 ± 0.048 μg kg^{-1}) and the least were in millet (0.222 ± 0.04 μg kg^{-1}) (Table 2). However, there was no significant difference in AFB_1 concentrations between sesame and millet, freshly processed products, and stored processed products. On the other hand, in the dry season AFB_1concentration was highest in sesame (0.485 ± 0.031 μg kg^{-1}) followed by millet (0.438 ± 0.040 μg kg^{-1}), stored processed products (0.408 ± 0.044 μg kg^{-1}), and the least was in the freshly processed product (0.377 ± 0.040 μg kg^{-1}). Despite this, there was no significant difference in AFB_1 concentration in processed and stored products across the seasons but overall, stored products had significantly high concentrations.

Table 1
TAF concentrations at process points of locally made malted millet sesame soy composite across the two seasons.

Process points	N (%)	Mean ± SD (μg kg^{-1})	Median	LL	UL
Wet season					
Soybean (raw material)	45 (21.6)	1.187 ± 1.001^{ab}	0.899	0.590	4.940
Sesame (raw material)	41 (19.7)	1.199 ± 1.124^{a}	0.860	0.430	4.870
Millet (raw material)	46 (22.1)	0.754 ± 0.313^{b}	0.734	0.080	1.350
Freshly processed product	32 (15.4)	0.912 ± 0.257^{ab}	0.908	0.540	1.530
Stored processed product	44 (21.2)	$0.8653. \pm 217^{ab}$	0.826	0.490	1.490
Dry season					
Soybean (raw material)	1* (0.6)				
Sesame (raw material)	35 (22.6)	0.819 ± 0.157^{ab}	0.904	0.550	0.980
Millet (raw material)	46 (29.7)	0.665 ± 0.183^{a}	0.634	0.080	1.350
Freshly processed product	30 (19.4)	0.696 ± 0.531^{a}	0.594	0.090	3.430
Stored processed product	43 (27.7)	0.847 ± 0.112^{b}	0.833	0.610	1.270
Overall TAF concentrations					
Soybean (raw material)	46 (12.7)	1.187 ± 1.001^{a}	0.900	0.590	4.940
Sesame (raw material)	76 (20.9)	1.025 ± 0.849^{ab}	0.875	0.430	4.870
Millet (raw material)	92 (25.4)	0.709 ± 0.258^{c}	0.695	0.080	1.350
Freshly processed product	62 (17.1)	0.807 ± 0.424^{bc}	0.685	0.090	3.430
Stored processed product	87 (23.9)	0.856 ± 0.172^{bc}	0.830	0.490	1.490

The values reported in the tables are mean ± standard deviation, median, and upper and lower limits. Means with a different subscript letter(s), a, ab, b, bc, and c indicate significant differences from each other (Duncan's test, $P < 0.05$). LL = lower limit, UL = upper limit, * Sample not included in the analysis. The proportion of the composite was: malted millet (76%), malted sesame (3.8%), and soybean (20.2%).

Table 2
AFB_1 concentrations at process points of locally made malted millet sesame soy composite across two seasons.

Process points	N (%)	Mean ± SD (μg kg^{-1})	Median	LL	UL
Wet season					
Soybean (raw material)	45 (21.6)	0.649 ± 0.256^{a}	0.659	0.06	0.122
Sesame (raw material)	41 (19.7)	0.261 ± 0.307^{b}	0.089	0.00	0.1.05
Millet (raw material)	46 (22.1)	0.221 ± 0.277^{b}	0.050	0.01	1.040
Freshly processed products	32 (15.4)	0.401 ± 0.272^{c}	0.514	0.010	0.810
Stored processed products	44 (21.2)	0.505 ± 0.236^{c}	0.523	0.060	0.860
Dry season					
Soybean (raw material)	1*(0.6)				
Sesame (raw material)	35 (22.6)	0.485 ± 0.186^{a}	0.552	0.09	0.870
Millet (raw material)	46 (29.7)	0.429 ± 0.258^{a}	0.523	0.010	1.600
Freshly processed products	30 (19.4)	0.350 ± 0.209^{a}	0.339	0.030	0.680
Stored processed products	43 (27.7)	0.408 ± 0.287^{a}	0.438	0.010	0.830
Overall AFB_1 concentrations					
Soybean (raw material)	46 (12.7)	0.649 ± 0.256^{a}	0.659	0.060	1.220
Sesame (raw material)	76 (20.9)	0.380 ± 0.279^{bc}	0.380	0.000	1.050
Millet (raw material)	92 (25.4)	0.347 ± 0.333^{b}	0.465	0.010	2.000
Freshly processed products	62 (17.1)	0.377 ± 0.244^{bc}	0.435	0.010	0.810
Stored processed products	87 (23.9)	0.457 ± 0.265^{c}	0.500	0.01	0.86

The values reported in the tables are mean ± standard deviation, median, and upper and lower limits. Means with a different subscript letter(s), a, ab, b, bc, and c indicate significant differences from each other (Duncan's test, $P < 0.05$). LL = lower limit, UL = upper limit. * Sample not included in the analysis. The proportion of the composite was: malted millet (76%), malted sesame (3.8%), and malted soybean (20.2%).

3.3. Exposure to TAF and AFB_1

As indicated in Fig. 1, on average the most exposed age to both TAF and AFB_1 across wet and dry season across wet and dry was 5 months (37.51 ng kg-^{1} bw day-^{1}). The mean age for the children was 15.693 ± 6.92 months and the typical weight was 11.2 ± 3.243 kg.

As illustrated in Table 3, the mean EDI for TAF in the freshly processed products was higher in the wet season than in the dry season (14.646 ± 2.110 ng kg^{-1} bw day^{-1} and 13.587 ± 2.206 ng kg^{-1} bw day^{-1}) respectively. A similar scenario was observed in the stored processed product where the mean EDI for TAF in the wet season was higher than in the dry season (18.078 ± 2.147 ng kg^{-1} bw day^{-1} and 16.861 ± 1.900 ng kg^{-1} bw day^{-1}) respectively. Equally, the mean EDI for AFB_1 in freshly processed products was higher in the dry season than in the wet season (8.140 ± 1.578 ng kg^{-1} bw day^{-1} 6.657 ± 1.383 ng kg^{-1} bw day^{-1}). While the mean EDI for AFB_1 in the wet season was her in the stored processed product than in the dry season 10.665 ± 1.698 ng kg^{-1} bw day^{-1} and 7.806 ± 1.294 ng kg^{-1} bw day^{-1} respectively. However, there was no significant difference in the TAF and AFB_1 EDI resulting from consumption of the freshly processed and stored processed MMSSC, $P < 0.05$ as indicated in Table 4.

3.4. Correlation between EDI for total and AFB_1 across the season, processing points, and age and weight of children

As indicated in Table 5, in both the freshly processed and stored products, there was a weak negative correlation between EDI for total and AFB_1 and age. However, these were not significant at a $P = 0.05$ level of significance in both seasons. Yet, the weight of the children significantly ($P = 0.024$) correlated negatively to EDI for TAF in the wet season in the stored processed products.

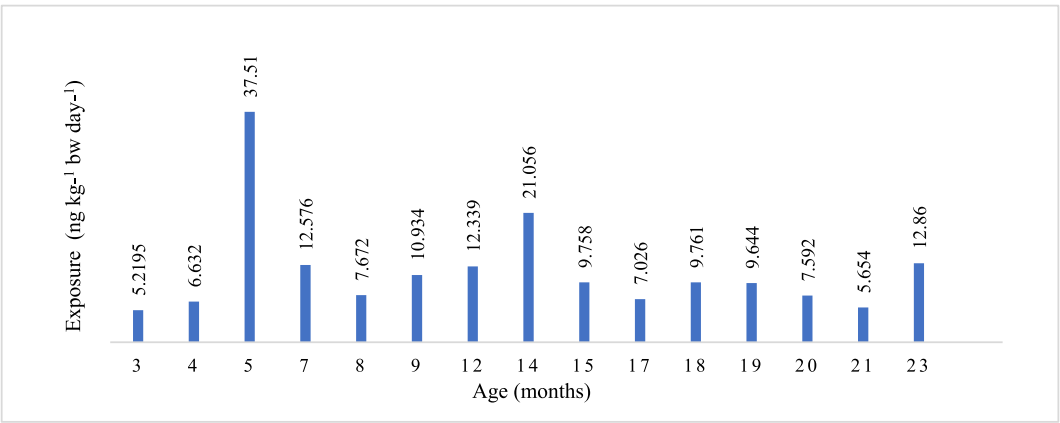

Fig. 1. Average exposure (ng kg^{-1} bw day^{-1}) of children with age (months).

Table 3
Children's Exposure to TAF and AFB_1 resulting from consuming freshly processed and stored processed MSSC.

Parameters	Freshly processed products	Stored processed products
	Mean ± SD	Mean ± SD
Age (months)	15.947 ± 6.897	15.432 ± 7.029
Weight (kg)	11.242 ± 3.263	11.157 ± 3.265
EDI TAF S-1 (ng kg^{-1} bw day^{-1})	14.646 ± 13.009	18.078 ± 12.702
EDI AFB_1 S-1 (ng kg^{-1} bw day^{-1})	6.657 ± 8.522	10.665 ± 10.045
EDI TAF S-2 (ng kg^{-1} bw day^{-1})	13.587 ± 13.599	16.861 ± 11.240
EDI AFB_1 S-2 (ng kg^{-1} bw day^{1})	8.140 ± 9.729	7.806 ± 7.762

The daily intake was calculated based on the mean concentration reported with consumption data of processed products. EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively), EDI AFB_1 S-1 and EDI AFB_1 S-2 (Estimated Daily Intake for AFB_1 in the wet season and dry season respectively).

Table 4
Differences in TAF and AFB_1 EDI resulting from the consumption of freshly processed food and the stored processed.

EDI	Stored product Mean ± SD (μg kg-1)	Mean ± SE (μg kg-1)	Processed Product Mean ± SD (μg kg-1)	Mean ± SE (μg kg-1)	Mean difference (μg kg-1)	t-stat	p-value
TAFL S-1	18.078 ± 12.702	18.078 ± 12.702	14.646 ± 13.009	14.646 ± 2.110	3.432	1139	0.259
AFB1 S-1	10.665 ± 10.045	10.665 ± 10.045	6.657 ± 8.522	6.657 ± 1.383	4.007	1.843	0.070
TAFL S-2	16.861 ± 11.240	16.861 ± 11.240	13.587 ± 13.599	13.587 ± 2.206	3.275	1.116	0.268
AFB1 S-2	7.806 ± 7.762	7.806 ± 7.762	8.142 ± 9.729	8.142 ± 1.578	−0.334	0.163	0.871

EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively), EDI AFB_1 S-1 and EDI AFB_1 S-2 (Estimated Daily Intake for AFB_1 in the wet season and dry season respectively).

Table 5
Correlation between EDI for total and AFB_1 a cross season, process levels, age, and weight of children.

EDI of TAF and AFB_1 in two seasons	Age				Weight			
	Stored Product		Processed Product		Stored Product		Processed Product	
	Correlation	*P*-value	Correlation	*P*-value	Correlation	*P*-value	Correlation	*P*-value
EDI TAF S-1	−0.075	0.667	−0.091	0.586	−0.382	0.024*	−0.179	0.281
EDI AFB_1 S-1	−0.009	0.960	−0.041	0.807	−0.212	0.222	−0.132	0.429
EDI TAF S-2	0.089	0.611	−0.115	0.492	−0.258	0.135	−0.133	0.425
EDI AFB_1 S-2	0.107	0.535	−0.106	0.527	−0.085	0.624	−0.231	0.163

EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively), EDI AFB1 S-1 and EDI AFB1 S-2 (Estimated Daily Intake for AFB1 in the wet season and dry season respectively).

4. Discussions

TAF and AFB_1 contamination were observed throughout the process points with the highest contamination observed in the raw materials (sesame and soybean). The higher TAF and AFB_1 contamination of sesame and soybean than millet or processed product could be differences in crop structure and or pre and postharvest management according to the respective food types [32–34]. Similarly, other studies reported differential contamination by aflatoxins due to differences in postharvest practices of many staple crops including oil seeds [35,35]. According to Marshall et al. contamination along processing points could be reduced by the use of innovative technologies such as sorting produce using fluorescence devices and secondary processing [36]. Equally, Özer et al. argued that ensuring good agricultural practices and proper storage may not guarantee the reduction of mycotoxin [37], but Boudergue et al. reported that decontamination approaches such as sorting, sieving, washing, and heat treatment may ensure reduction [38]. Additionally, Özer et al. noted that chemical treatment using acids, alkalis, oxidizing agents, and microbial agents such as enzymes may also be employed to minimize contamination [37]. Thus, it may not be reasonable to conclude that once the raw materials are free from contaminants than the processed products are safe. This is because contamination can occur at any stage along the processing chain including at storage. However, combinations of decontamination methods may be appropriately applied to guarantee minimal contamination to acceptable levels.

TAF contamination was most common in the raw materials followed by the stored products and the least contaminated was the freshly prepared products in the wet season. Whereby overall, the mean TAF concentration was highest in soybean followed by sesame, stored processed products, freshly processed products, and least in millet. In the same vein, the report showed that depending on the

availability of favorable conditions, fungal growth, and toxin production may occur at any stage of the food chain [39]. The low prevalence of both the total and AFB_1 in millet is comparable to a study by Akello et al. who found that small cereals were less prone to aflatoxin contamination [40]. The literature further explained that measures to control/prevent fungal infection at pre-harvest are critical for sustainably minimizing aflatoxin contamination at the post-harvest level [41]. This could be the reason for the high contamination of the raw materials than the processed and stored products since contamination of the product could have most likely happened in the field. A previous study also indicated that high aflatoxin contamination of the product may occur due to late detection and removal of contaminated ones in the processing continuum and, their spread along the chain can be exacerbated and promoted by poor storage conditions [42]. This is supported by Casquete et al. whose findings showed that the proliferation of *A. flavus* and AFB_1 production was significantly resulting from optimum temperature, pH, temperature, and water activity of the substrate during storage [43]. Furthermore, they observed a higher level of aflatoxin contamination in the stored processed product could be due to poor storage conditions that might have exacerbated and promoted contamination [43]. Therefore, to minimize contamination, there is an urgent need to sort raw material before storage of grains.

The highly contaminated raw materials were soybean and sesame which could be explained by the fact that oil seed plants promote the proliferation of *A. flavus* and subsequent toxin production. This was evidenced by several studies, for instance, Rajasekaran et al. found that total lipid content significantly enhances AFB_1 production in cotton while in the field [44]. Comparably, Lui et al. revealed that growth and toxin production were significantly negatively affected when oily grains and cereals such as soybean and peanuts were defatted and vice versa when corn oil was reintroduced [45]. Additionally, the same study showed a positive correlation between AFB_1 concentrations and an increase in substrate-soluble sugars such as glucose, maltose, fructose, sucrose raffinose, and stachyose [45]. Similarly, Singh and Sinha's report showed that the total starch and amylopectin content of the substrate were positively correlated with AFB_1 levels [46]. However, on the contrary, other substrate components such as Tocopherols have been shown to negatively influence the production of aflatoxins [47]. But soybean that was heavily contaminated compared to millet has been shown to have higher tocopherol content compared to millet [48]. Therefore, susceptibility to fungal infestation and subsequent production of aflatoxins may vary among crop produce depending on their nutrient content, crop structure, and harvest management [33,49,50].

Overall, the low contamination observed in freshly processed products could be explained by the fact that processing reduces fungal contaminants [50–52]. For instance, previous studies indicated a reduction of fungal load in processed cereals. Equally malting, a process that was employed in this study has been sought to reduce mycotoxins concentration [52], which could be due to the low release of the toxins into the food but rather remain in the mycelium during processing [47].

Based on the season, both total and the mean TAF concentration was highest in the wet season (wet) than in the dry season (dry). Samples collected in the wet season were during the wet season whereas those collected in the dry season were during the dry season. High concentrations of both TAF and AFB_1 observed in the wet than in the dry season is analogous to a study by Taheri et al. who registered high concentrations of TAF in winter than in summer (1.99 ng g^{-1} and 0.82 ng g^{-1}) respectively [53]. That study also reported a high level of AFB_1 during winter to be above the globally acceptable limit than during summer (7.4% and 3.4%, respectively). Likewise, Bashiry et al. noted that AFB_1 occurrence was higher (60%) in the cold season than in the warm season [54]. According to Atongbiik et al. high concentrations observed in the wet season may indicate the availability of favorable environmental conditions (water activity of between 0.93 and 0.99 and temperature of 0–33 °C) for the proliferation of aflatoxin-producing *Aspergillus* species [55]. Contrarily, Damianidis et al. findings showed significantly high aflatoxin contamination in the dry seasons possibly due to night heat stress [56]. Conversely, AFB_1 concentration in the wet season was highest in soybean followed by stored products, freshly processed products, sesame, and then millet. In the dry season, the mean concentration of AFB_1 was highest in sesame followed by stored product, millet, and processed product (not determined for soybean). Generally, the mean concentration varied, with concentrations of both TAFs and AFB_1 being higher in the wet season than in the dry season. This could have been due moist environment during the wet season that favored fungal growth [39,57]. In agreement with the current finding, Negash reported that contamination may vary from year to year [17]. These may result from the availability of favorable factors such as optimal growth conditions, pest invasion, and poor pre- and post-harvest management [8]. Specifically, the production of aflatoxin by *A. flavus* varies depending on growth conditions and the availability of another microorganism that degrades the produced toxins [56].

The current study demonstrated that 100% of the locally made malted millet-sesame-soy composite had detectable levels of TAF and AFB_1. Additionally, 100% of the composite analysed seemed to be contaminated by TAF and AFB_1 above the EU maximum allowable limits of 0.10 μg kg^{-1} and 0.40 μg kg^{-1} for TAF and AFB_1 respectively, especially for processed baby foods made from cereals [58]. Although, contamination levels for both TAF and $AFB_1$of the raw materials were within the EU acceptable levels of 2 μg kg^{-1} for AFB_1 and 4 μg kg^{-1} for TAF for processed foods for other human consumption and 8 μg kg^{-1} unprocessed foods [54]. In terms of compliance with the EAC and USFDA standards, 5 μg kg^{-1} and 10 μg kg^{-1} for AFB_1 and TAF and 20 μg kg^{-1} for TAF in food and feeds respectively [20], the studied product met the standard. Nevertheless, due to frequent daily consumption, children consuming this product could be exposed to a high load of aflatoxins.

Globally, infants and young children are more vulnerable to health risks of aflatoxins exposure than adults because of their increased special food intake mainly cereals for each kg/body weight [59]. The present study showed that exposure to both TAF and AFB_1 was higher when stored products were consumed than freshly processed products in both seasons. However, there was no significant difference in both the TAF and AFB_1 EDI resulting from the consumption of freshly processed food and stored processed food in both seasons ($P = 0.05$). This could be due to the short storage period (one month used in the current study) that limits the proliferation of toxigenic fungi and results in toxin production.

The study also demonstrated that on average when both the processed and stored products are consumed across the wet and dry seasons, the most exposed age was 5 months followed by 14 months. The least exposed group was 3 months. This could be because this age group complementary composite is introduced and frequently taken, compared to the younger (3 months) and older ones and who

may depend on breast milk and family food respectively. Contrarily, Bashiry et al. found the most exposed age to be 6–12 months with an average EDI of 153 ± 0.13 μg kg^{-1} bw day^{-1} [54]. This was higher than the overall level of exposure in the current study. Eshete et al. on the other hand found that in Nigeria, children below 12 months were less exposed in comparison with older children [26]. This could have been due to a myriad of factors such as the quantity and frequency of consumption of contaminated weaning foods given to this age group in addition to proper hygiene care rendered to this age group to avoid cross-contamination and consequent exposure. Several other studies have also reported levels higher than PMTDI. For instance, an average exposure level of 68 ng kg^{-1} bw day^{-1} due to the consumption of cereal was reported [22]. Another finding also indicates higher aflatoxin exposure levels (763.6–1901.1 ng kg^{-1} bw day^{-1}) of children who consumed stored maize grains [60]. Similarly, in Tanzania, a very high aflatoxin exposure level of 1337 ± 392.5 ng kg^{-1} bw day^{-1} was registered [58].

Generally, exposure levels in these studies were above the aflatoxin PMTDI of 1.0 ng kg^{-1} bw day^{-1} and 0.4 ng kg^{-1} bwday^{-1} for children who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively [24]. Aflatoxin exposure levels in other continents apart from Africa have also been explored and found on average relatively lower levels such as 0.93–2.45 ng kg^{-1} bw day^{-1} in Europe, and 2.7 ng kg^{-1} bw day^{-1} in the USA [61]. This variation could be because countries with high levels of exposure may rely much on local production of complementary food compared to those with low levels of exposure who could mainly depend on industrialized exported products whose production and processing strictly adhere to recommended safety practices. Literature affirms that locally produce foods had reasonably high levels of aflatoxin compared to the imported ones possibly due to contaminants in the raw materials and along the chain due to negligence for safety among households [7,25]. This calls for urgent action to ensure the safety of locally made complementary food to minimize exposure of infants and young children owing to the health implication of aflatoxins.

Exposure to both TAF and AFB_1 was higher in the stored products than in freshly processed products in both seasons. Although no significant difference was observed in both the TAF and AFB_1 EDI resulting from the consumption of the freshly processed food and the stored processed food, in both seasons ($P > 0.05$). There was a weak negative correlation between EDI for total and AFB1 and age in both the freshly processed and stored products. However, these were not significant in both seasons. This finding suggests that exposure of children to total and AFB_1 is independent of the season.

4.1. Study limitations

The study was conducted in a rural domestic environment. This is important to the public health of rural communities that cannot access and afford standardized complementary foods that are produced under controlled safety conditions. Thus, the study contributed to the body of knowledge safety of complementary food produced in a rural setting with minimal controlled conditions. However, the study had the following methodological limitations: The ELISA method used for the determination of Aflatoxins in the current study is a routine test with a detection limit lower than other methods such as HPLC so the accuracy may be lower. However, in resource-limited settings, ELISA is used and it provides reliable results. Additionally, the study only examined aflatoxins contamination of samples obtained from the trained group so our findings may not be generalized to the entire community. Further study is thus needed to compare the aflatoxin contamination of samples collected from both the trained and untrained groups. As well, the number of soybeans samples in season 2 was limited due to the dry season when the food grains produced in the households were inadequate, therefore it could not be statistically analysed.

5. Conclusion

This study has demonstrated that TAF and AFB_1 contamination in MMSSC varied along processing points from raw materials, stored, and freshly prepared composite in descending order. The variation in TAF and AFB_1 along the processing points was higher in the wet than dry season except in the stored composite. Estimated Daily intake for both total and AFB_1 exceeded the Codex PMTDI with no variation in the wet and dry seasons. Based on food safety standards, all the raw materials, processed, and stored products met the EU, US-FDA, and EAC standards limit for general consumption but were below EU standards for processed cereal baby food. Therefore, a concerted effort is needed to reduce contamination and exposure of the children consuming locally made MMSSC to aflatoxin and AFB_1 taking sesame and soybean as priority ingredients and formulated composite based on the season.

Ethical statement

This study was reviewed and approved by the Gulu University Research Ethical Committee (GUREC 02719) and the Uganda National Council of Science and Technology (SS 4958). All participants (legal guardians) provided informed consent to participate in the study. All participants (legal guardians) provided informed consent for the publication of their anonymized images. Caregivers' (legal guardians) permission was sought to collect weight and exposure data of the children.

Author contribution statement

Eunice Achiro: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Lawrence Okidi: Performed the experiments; Analysed and interpreted the data; Wrote the paper.

Richard Echodu, Simon Peter Alarakol: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the

paper.

Juliet Anena: Performed the experiments; Wrote the paper.

Duncan Ongeng: Conceived and designed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18564.

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