Food Control 162 (2024) 110463

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Processing complementary foods to reduce mycotoxins in a medium scale Tanzanian mill: A hazard analysis critical control point (HACCP) approach

Francis M. Ngure [a,*], Edna Makule [b], William Mgongo [b], Erica Phillips [c], Neema Kassim [b], Rebecca Stoltzfus [c,d], Rebecca Nelson [e]

[a] Research Consultant, Arusha, Tanzania
[b] Department of Food Biotechnology and Nutritional Sciences, School of Life Science and Bio-Engineering, The Nelson Mandela African Institution of Science and Technology (NM-AIST), P.O.Box 447, Arusha, Tanzania
[c] Division of Nutritional Sciences, Cornell University, Ithaca, NY, 14853, USA
[d] Goshen College, 1700 S. Main Street, Goshen, IN, 46526, USA
[e] School of Integrative Plant Science, Cornell University, Ithaca, NY, 14853, USA

ARTICLE INFO

Keywords:
Aflatoxins
Fumonisins
Food safety
Hazard analysis
HACCP
Maize
Groundnuts
Sorting

ABSTRACT

Designing and implementing processing procedures for producing safe complementary foods in dynamic and unregulated food systems where common food staples are frequently contaminated with mycotoxins is challenging. This paper presents lessons about minimizing aflatoxins (AF) in groundnut flour and AF and/or fumonisins (FUM) in maize and groundnut pre-blended flour for complementary feeding in the context of a dietary research intervention in rural Tanzania. The flours were processed in collaboration with Halisi Products Limited (Halisi), a medium scale enterprise with experience in milling cereal-based flours in Arusha, Tanzania. Using a hazard analysis critical control point (HACCP) approach for quality assurance, two critical control points (CCPs) for AF in processing the pre-blended flour were identified: 1) screening maize before procurement, and 2) blending during the processing of each constituent flour. Blending of maize flour was also identified as a CCP for FUM. Visual inspection during screening and sorting were identified as important control measures for reducing AF, but these steps did not meet the criteria for a CCP due to lack of objective measurement and verifiable standards for AF. The HACCP approach enabled the production of low AF (<5 μg/kg) and FUM (<2 μg/g) flours with low rejection rates for the final products. The paper presents practical lessons that could be of value to a range of commercial processors in similar low- and middle-income contexts who are keen on improving food quality.

1. Introduction

Mycotoxin contamination is a major threat to food safety globally. Mycotoxins contaminate 60–80% of foods globally, with an estimated quarter of the global food supply at higher than the maximum acceptable limits (Eskola et al., 2019). In tropical regions such as sub-Saharan Africa, aflatoxins (AF) and fumonisins (FUM) are among the dominant mycotoxins in dietary staples. Both maize and groundnuts are highly prone to AF contamination, and maize is also widely contaminated with FUM (IARC, 2015). Mycotoxins threaten food security through reduced food quality and reduced competitiveness of agricultural commodities in international trade (Udomkun et al., 2017). Acute exposure to AF causes aflatoxicosis (Azziz-Baumgartner et al., 2005; Kamala et al., 2018; Lewis et al., 2005; Probst et al., 2007), while chronic exposure is a risk factor for liver cancer (IARC 2012), and is associated with child stunting (Gong et al.,2002, 2004). FUM B_1 is classified as a possible human carcinogen (IARC 2002). Chronic FUM exposure is negatively associated with child linear growth (Kimanya et al., 2010; Shirima et al., 2015).

Maize and groundnuts are important sources of dietary AF exposure in Tanzania and the larger East Africa region (Boni et al., 2021; Kimanya et al., 2009, 2010b; Mollay et al., 2020; Mutegi et al., 2018; Mutiga et al., 2015). They are also major ingredients in the preparation of infant complementary foods in Tanzania (Mollay et al., 2021; Ngure et al., 2023). Maize is a staple food for 85–90% of Tanzanians (Wilson & Lewis, 2013, pp. 14–36) and is grown in nearly all agroecological zones in the country (AGRA, 2020). Strong dietary preference for maize over more drought-tolerant traditional cereals such as sorghum and millet has led

* Corresponding author.
E-mail addresses: fmn9@cornell.edu, fmngure@gmail.com (F.M. Ngure).

https://doi.org/10.1016/j.foodcont.2024.110463
Received 27 October 2023; Received in revised form 13 March 2024; Accepted 18 March 2024
Available online 22 March 2024

List of abbreviations

AF	Aflatoxins
CCPs	Critical control points
ELISA	Enzyme-linked immunosorbent assay
FUM	Fumonisins
HACCP	Hazard analysis critical control point
HPLC	high performance liquid chromatography
IYC	Infants and young children
LMICs	Low-and middle-income countries
MC	Moisture content
NM-AIST	The Nelson Mandela African Institution of Science and Technology
PICs	Purdue Improved Crop Storage
QC	Quality control
RUTF	Ready-to-use therapeutic food
SME	Small- and medium enterprise

to maize cultivation even in areas with insufficient rainfall, which makes the crop more susceptible to fungal colonization and mycotoxins contamination (Wilson & Lewis, 2013, pp. 14–36). Poor post-harvest handling of maize and groundnut crops is common in central Tanzania (Seetha et al., 2017), which further exacerbates AF accumulation along value chains. Inadequate storage facilities and poor post-harvest management by small scale farmers causes 15–40% post-harvest loss in maize (AGRA, 2020; Suleiman & Kurt, 2015).

Of 6.2 million metric tons of maize produced in 2018/2019 season in Tanzania, 85% was produced by small scale farmers (AGRA, 2020). The majority of maize in Tanzania (65–80%) is consumed by the household that produced it, while 20–35% enters commercial channels (Wilson & Lewis, 2013, pp. 14–36). White maize in Tanzania is milled to flour primarily by small local mills in both rural and urban areas, and by medium-to large-scale mills in major urban centers. Small scale mills (with a production capacity of <10 tons a day) account for 90% of the country's milled maize. Millers are involved in the penultimate stages of the value chain, the milling of the grain and packaging of the flour. Standardization, grading and enforcement of quality assurance regulations are weak along the market chain (East African Community, 2013). There are few quality checks (e.g., for moisture level, grain quality, or storage pests) and seldom any traceability of origin. Therefore trust, reliable information systems and benefits of economies of scale are not well established.

Ideally, mycotoxin levels are managed through a value chain approach that ensures low toxin accumulation before, during and after harvest, followed by removal of toxins during food processing (Dorner, 2008; Massomo, 2020; Nelson, 2016). However, the groundnuts and maize value chains in Tanzania are fragmented and poorly coordinated, with inefficient connections between producers and consumers. Incentives for low mycotoxin food production are lacking. Millers who purchase and process maize for human consumption have an ethical obligation to produce safe food, but this is often at odds with the necessity of operating profitably. It is especially difficult for the small- and medium-sized enterprises (SMEs) that lack the expertise and technological capacity to assess the toxin levels in the grain they purchase and the foods they produce. Most methods for mitigating mycotoxin levels at the milling stage involve sorting out toxic materials and most SMEs cannot afford to discard material from their production systems (Nelson, 2016). The cost of incinerating such materials is high and mechanisms for tracking contaminated grains in contexts such as Tanzania are generally lacking (EAC, 2018; Massomo, 2020). To stay profitable, the food industry focuses on minimizing food losses and the cost of production.

The "hazard analysis and critical control point" (HACCP) approach has been used for several decades as an in-house quality assurance system in food industries (FAO/IAEA, 2001; Henry & Xin, 2014). It involves systematically identifying, evaluating, and controlling food safety hazards to ensure that contamination is prevented, eliminated, or reduced to an acceptable level before a food reaches the consumer. A key step in the HACCP approach is identifying critical control points (CCPs) in the food processing system. A CCP is a step in a food production process at which a control can be applied and is essential to prevent, eliminate or reduce a food safety hazard to acceptable levels (FDA, 2017). After a CCP, there is no corrective action that can reduce or eliminate the hazard, i.e., further processing or cooking does not solve the problem.

The HACCP approach has been used in local processing of a peanut-based ready-to-use therapeutic food (RUTF) in a high income context (Henry & Xin, 2014). However, evidence is scarce on how feasible and acceptable such an approach is for SME food industries in low-middle-income countries (LMICs) where mycotoxin contamination is frequent and often at high levels, and safety regulations are weak or lacking.

This paper documents the processing of raw groundnuts and pre-blended maize and groundnut flours in the context of a research trial in Tanzania (Phillips et al., 2020), using a HACCP approach to reduce mycotoxin levels in the products. The paper explores the following questions, which are relevant to the wider application of this approach; i) What are the CCP(s) in processing a low AF and FUM complementary food using maize and groundnuts? ii) Which of the control measures effectively reduced AF and FUM? iii) How much food was lost with the control measures and identified CCPs in practice? And finally, iv) what is the potential for scaling up such production strategies among SMEs and other millers in LMICs?

2. Methods

2.1. The collaboration

As part of a dietary intervention to reduce mycotoxin exposure in Tanzanian infants, processing of a maize and groundnut pre-blended flour and groundnut flour was conducted at Halisi Products Limited (Halisi) from August 2019 to August 2021. Halisi is a medium-sized milling enterprise in Tanzania with a production capacity of 10–20 tons of flour per day (World Food Programme, 2022). While Halisi had prior experience in processing cereal based blended flours, the specific process of low mycotoxin flour production was a collaboration between Halisi (Arusha, Tanzania), the Nelson Mandela African Institution of Science and Technology (NM-AIST; Arusha, Tanzania) and Cornell University (USA). Researchers from NM-AIST and Cornell provided technical leadership and oversight on training staff, managing maize and groundnuts procurement, overall quality assurance and mycotoxin control. Halisi provided the processing machinery and staff, as well as input on maize and groundnut procurement.

2.2. Processing of complementary feeding flours

The flours were processed from well dried and mature white maize and groundnuts of the *Pendo* variety. All products were processed with adherence to strict food hygiene standards regarding frequent hand washing and sanitary environmental hygiene standards. Figs. 4 and 5 shows the processing steps, critical control points and control measures for each product. Since groundnuts were frequently contaminated with AF and required rigorous sorting, further description of screening and sorting is provided below.

2.3. Screening groundnuts

Screening for quality shelled groundnuts was done at the market or farmer's store to ensure the best quality possible was procured.

Fig. 1. Grade 1; natural color, whole, healthy, smooth, full, "supermarket" or seed grade groundnuts (AF = 2.8 μg/kg).

Fig. 2. Grade 2: Large shriveled groundnuts (AF ranges from 10 to >150 μg/kg).

Fig. 3. Grade 3; AF >150 μg/kg.

Screening involved visual inspection for general quality, the proportion of foreign matter and healthy versus unhealthy (small shriveled and discolored/rotten) kernels, and moldy or musty odors. Moisture content was measured upon receiving the groundnuts at Halisi and before proceeding to sorting. Due to the heterogeneous nature and occurrence of high AF in unsorted groundnuts, AF was not tested at this stage to reduce the cost of quality control.

2.4. Pre-cleaning and sorting groundnuts

All graders and the food production supervisor were thoroughly trained by the investigators on hand/visual sorting of maize and groundnuts prior to commencing the flour processing. The graders were trained on sorting three grades described below, checking each other's work for consistency and the quality of final grades.

The groundnuts were run through a size screening process to remove chaff, foreign materials and all small shriveled (severely water stressed) and broken kernels (grade 3; Fig. 3). Further visual sorting of grade 3 was done to recover the small, whole, and smooth kernels (grade 1; Fig. 1). After size screening, winnowing and extensive visual sorting was done to remove all discolored groundnuts (rotten, with dark spots, greenish, ash-like/grey, moldy and any off color); these kernels were classified as grade 3. Further visual sorting was done to separate medium and large shriveled, water stressed, unhealthy looking, and broken groundnuts (grade 2; Fig. 2) from smooth, medium to large, healthy groundnuts ("supermarket" or seed grade) with normal tan-brown color and no signs of mold spoilage (grade 1). A final inspection and sorting round/pass of grade 1 from each grader was done by the food production supervisor to ensure consistency and that no spoilt groundnuts pass the grader's eye. Each grader received feedback on his/her work for motivation and future improvement.

All grades from each 100 kg bag were weighed and recorded to ensure all losses were accounted for. Storage of grade 1 and 2 groundnuts below <5 μg/kg for more than 3 months was done in PICS bags on wooden pallets in a dry, well ventilated, and fumigated warehouse. Prior tests done showed grade 3 groundnuts were frequently contaminated with high levels of AF (>150 μg/kg). These groundnuts were incinerated under the supervision of the food production supervisor (a research staff) and the Halisi director.

2.5. Sampling of maize, groundnuts, and flours

In Tanzania, maize is commonly packaged and transported in 100 kg woven polypropylene bags. To obtain a representative sample, the contents of the bag were emptied onto a clean tarpaulin, mixed thoroughly, and then spread into a uniform shallow layer in a rectangular shape of approximately 1.5 by 3 m. This was then divided into four quadrants and 15–20 sub-samples were drawn by hand from each quadrant to add up to one aggregated sample of approximately 2 kg. The 2 kg sample was mixed well and ground in the hammer mill dedicated for maize milling. The first 0.5 kg was milled to pre-clean the hammer mill, and before the rest of the sample was milled.

Sampling of groundnuts followed a similar procedure, except that the sampling unit was approximately 20 kg for grade 1 derived from sorting market samples and 50–75 kg for Grade 1 from groundnuts sourced from individual farmers. Grade 1 groundnuts were mixed thoroughly and spread into a uniform layer to form a rectangular shape. This was divided into four quadrants and 5 sub-samples were drawn from each quadrant (of 20 kg) to add up to a total 1 kg aggregated sample. 15 to 20 sub-samples were drawn from each quadrant in case of 50–75 kg grade 1 to add up to one aggregated sample of ~2 kg. The aggregated sample was thoroughly mixed and 250g or 500 g was taken for milling and extraction, for grade 1 groundnuts from individual farmers and markets, respectively. The groundnuts were milled in the lab grinder (500A multi-function grain/Herb Grinder, model: RRH-1000G) to obtain a homogeneous fine flour.

Our sampling strategy for maize and groundnuts allowed us to inspect the contents of each bag and draw a representative sample from each bag or unit of sampling. The European Commission recommends five incremental samples, summing to 1 kg of aggregated sample for every 50–500 kg of cereals (European Commision Regulation, 2006). Our sampling strategy was more stringent to ensure reliability, given that the history of the grain was not always known.

After milling, each constituent flour (maize or groundnut) was

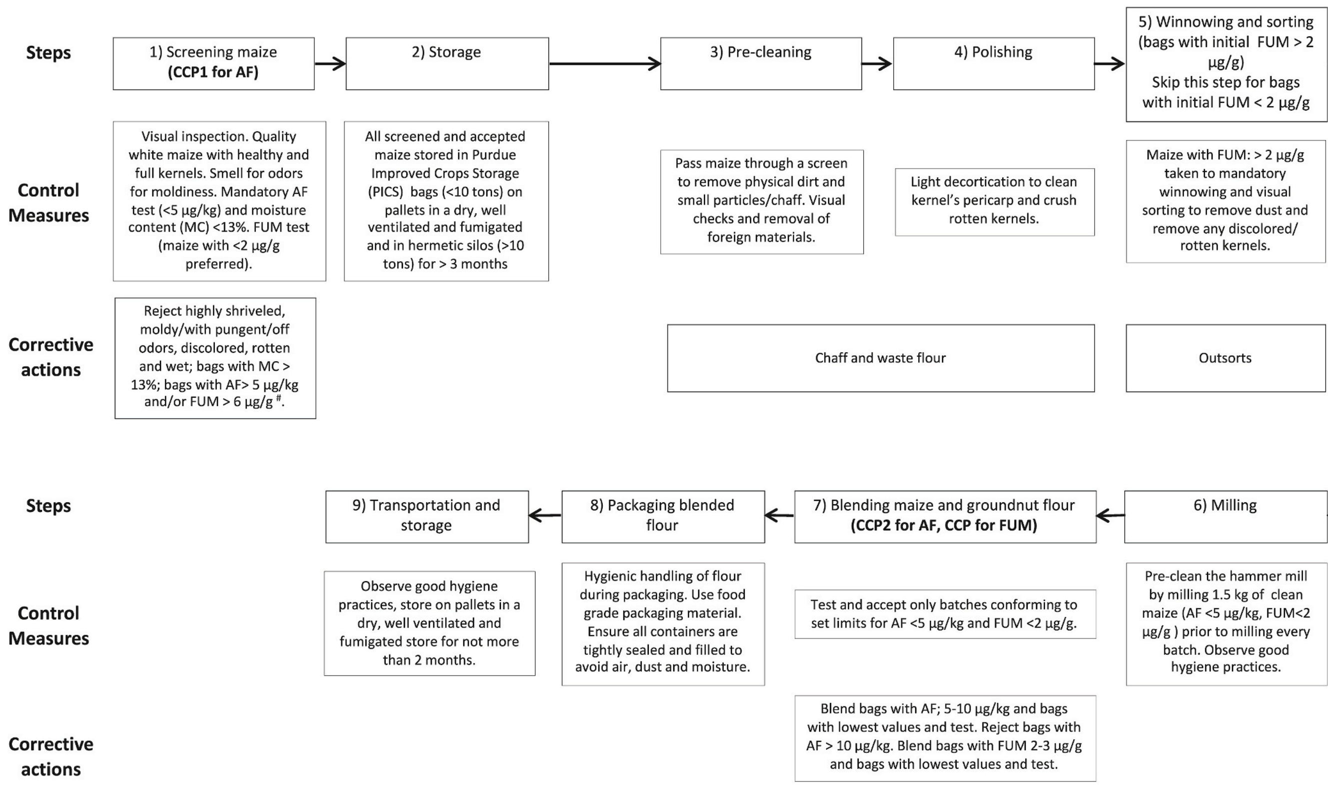

Fig. 4. Flow chart for the processing of 4:1 maize and groundnut blended flour identifying the CCPs for AF and FUM control.

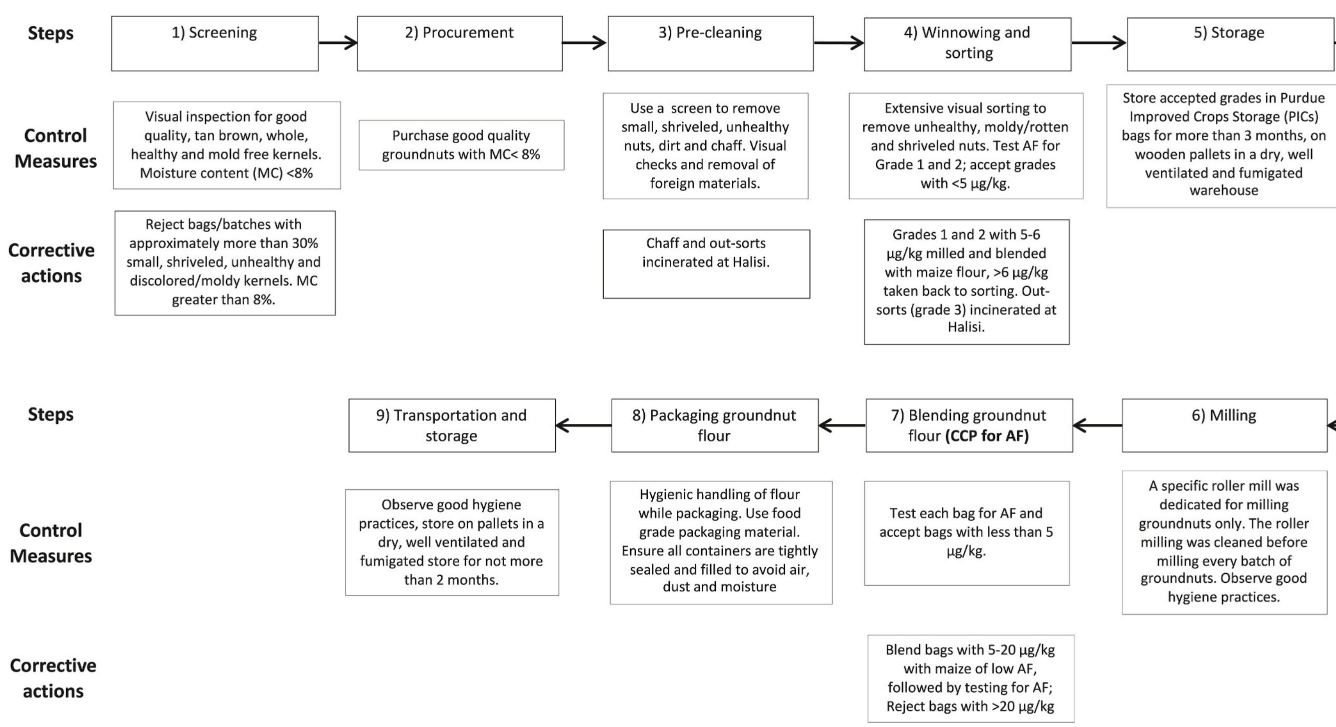

Fig. 5. Flow chart for the processing for groundnut flour identifying the CCP.

spilled into 2 m long, open semi-cylindrical, stainless-steel trough. The flour was mixed by churning the flour from one end to the other, using a food grade plastic container, for at least 5 times. 25–30 sub-samples were drawn randomly along the length of the trough using a serving spoon. The sub-samples were mixed in a separate container to an aggregate sample of ~1 kg. A similar sampling procedure was followed for the pre-blended flour.

The number of bags tested, and assays performed at each step of processing are summarized in Table S1.

2.6. AF and FUM analysis

Since there are no defined maximum acceptable limits for AF and FUM in foods for infants and young children (IYC) in East Africa (East African Community, 2013), the critical limit for AF and FUM in processed flours was set at ≤ 5 μg/kg and ≤2 μg/g, respectively. The critical limits were set as technologically feasible targets and to gurantee the flours were below the maximum acceptable limit for the general population in Tanzania.

Total AF and FUM were analyzed by either Reveal® Q+ quantitative test (Neogen) for most of the samples at Halisi. The Reveal® Q+ is a single lateral flow assay based on a competitive immunoassay format intended for quantitative testing of AF or FUM in grain and grain products. Occasionally, some samples were analyzed at NM-AIST using a quantitative competitive enzyme-linked immunosorbent assay (ELISA) by Helica Biosytems Inc., (Fullerton, CA) following the manufacturers protocols for each type of flour. According to the manufacturers' protocols total ELISA correlates well with high performance liquid chromatography (HPLC) for analysis of AF in maize. Low matrix ELISA method has been validated for commodities with a high matrix effect such as groundnuts. Neogen method for AF has been validated for numerous matrices including maize and groundnuts, and so has Neogen FUM assay for maize. The detection range for Neogen and ELISA allowed for measurements of AF and FUM within the desired range and critical limits for the study.

For the Neogen Reveal Q+ rapid assays, two sub-samples of 2 g ± 0.1 each were randomly drawn from the homogeneous aggregated sample of maize, groundnut or pre-blended flour processed from market sourced maize and groundnut. For a batch of maize or groundnuts, especially from an individual farmer, whose products consistently showed levels below 5 μg/kg for at least 10 bags, one extract was run to due to the cost implications and scarcity of test strips. The flour samples were placed in 15 ml Falcon tubes for extraction. Solvent (10 ml of 65 % analytical grade ethanol) was added into each of the 15 ml Falcon tubes, which were then vortex-mixed for 3 min. After allowing the sample to settle, the extract was filtered with a Whatman #1 paper. The same extract was used for both AF and FUM Neogen assays.

For ELISA assays, a 5-g sub-sample of flour was randomly drawn from an aggregated sample (described in the previous section) of either maize, groundnuts, or specific flour for AF or FUM extraction. Extraction for AF involved thorough mixing of the sub-sample with 25 ml of solvent (75% methanol for maize flour: 80% methanol for groundnuts and pre-blended flour). FUM was extracted for maize and pre-blended flour using 25 ml of 90% methanol.

Each batch of samples included a maize reference sample for AF provided by Neogen for quality control (QC). The QC reference material allowed comparison of results across the test assays and the two methods. The results of a given set of tests were accepted if the reference value fell within one standard deviation of the mean AF for the reference material (i.e., 6.1 ± 1.1 μg/kg). Since there was no reference material available for FUM selected samples were occasionally run using Neogen and compared with ELISA results for consistency.

2.7. Data analysis

Descriptive and summary statistics for the AF and FUM data were analyzed using STATA 15.1. Half the value of the limit of detection (LOD) was used for AF or FUM values less than the LOD. The arithmetic mean and standard deviation of AF and FUM at various processing and testing steps were calculated. For the sub-set of 28 bags of maize analyzed to show FUM reduction at various processing steps, the data was first transformed using natural logarithm before paired *t*-test mean comparison. Geometric means (GM) and 95% confidence intervals (95% CI) are presented for the sub-set data.

3. Results

3.1. Identifying critical control points

Following the Codex Alimentarius decision criteria (Henry & Xin, 2014), two CCPs for AF control were identified in processing of pre-blended flour (Table S2, Fig. 4). Screening maize during procurement was identified as CCP1 for AF. Ten percent of bags with AF > 5 μg/kg (Table 1) were rejected during screening. This step ensured that bags of maize with high AF levels that could not be reduced to acceptable levels in subsequent processing steps were eliminated. Several bags of maize rejected at this step had AF levels as high as >150 μg/kg. AF testing of 314 bags of maize flour confirmed that AF was below 5 μg/kg. During the first 6 months of processing, maize with higher FUM (>3 μg/g) was rejected at the screening stage (Table 1), but due to shortage of low-AF maize beginning February 2020, such maize was processed by light decortication (polishing), winnowing and visual sorting to reduce FUM.

Blending of maize flour (AF of 5–10 μg/kg) with lower AF maize flour, and subsequently with groundnut flour was identified as CCP2. Blending could only correct or reduce the hazard to acceptable levels if the initial contamination of maize was relatively low (in this case less than 10 μg/kg). Blending was also identified as a CCP in FUM control. Any bag of maize that marginally exceeded 2 μg/g was blended with other bags with very low FUM. Blending of maize with groundnut flours further reduced the FUM levels since groundnuts are not contaminated with FUM.

Blending was identified as the only CCP in processing of groundnut flour (Table S3, Fig. 5). Eight percent of groundnut flour bags were blended with maize of very low AF (n = 13; range = 5.1–20 μg/kg) and another 8% (13 bags) were rejected due to high AF (>20 μg/kg) that could not be reduced to acceptable levels through blending (Table 3). Two percent of pre-blended flour bags were rejected due to high AF (n = 4; range = 13–22 μg/kg). Blending of high with low FUM bags ensured each final unit of product had less than 2 μg/g. Therefore, no pre-blended flour was rejected due to high FUM (Table 3).

Overall, 10% of the total weight of maize was lost during pre-cleaning, winnowing, and sorting (Table 4). Five percent of the weight was lost through sampling and the removal of chaff and foreign material. Another 3% was lost to winnowing and sorting. About a third of the weight of groundnuts was lost to winnowing and sorting (Table 4). Higher losses were incurred by sorting market samples (31%), while 27% was lost by sorting groundnuts sourced directly from farmers.

3.2. Fumonisins reduction attributed to pre-cleaning, polishing, winnowing, and sorting in a sub-set of maize

The mean FUM levels before and after processing of a sub-set of

Table 1
AF and FUM levels and rejection rates for maize.

Number of bags tested	n (%) or concentration
Bags tested for AF at step 1 (screening, CCP1)	357
Rejected at step 1 (screening, CCP1): AF > 5 μg/kg	35 (10%)
Rejected due to high FUM (>3 μg/g)	8 (2%)
Bags tested for AF after step 6 (milling)	314 (88%)
Mean (SD) μg/kg	2.0 (1.0)
Median (range) μg/kg	2.1 (0.1–4.7)
Bags tested for FUM at step 1 (screening)	272
FUM at < 2.0 μg/g	104 (38%)
FUM at > 2.0 μg/g	168 (62%)
Rejected at step 1 (screening): FUM >3 μg/g[a]	19 (7%)
Bags tested for FUM after step 6 (milling)	85
Mean (SD) μg/g	1.2 (0.6)
Median (range) μg/g	1.2 (0.2–2.8)

[a] Due to shortage of low AF maize in local markets, maize with FUM at > 3 μg/g was processed and tested for FUM, beginning February 2020.

maize were significantly different (n = 28 bags). The geometric mean (95% CI) of FUM in maize before processing (step 1) was 2.5 μg/g (1.9–3.8 μg/g) compared to 0.4 μg/g (0.3–0.6 μg/g) for the milled maize (step 6) after pre-cleaning, polishing, winnowing, and sorting ($p < 0.05$; *t*-test comparison for natural log transformed data). FUM was reduced by 95% in 6 of the 28 bags with a mean of 3.3 μg/g to a mean of 0.15 μg/g in processed maize flour.

Samples of the chaff and waste flour from pre-cleaning and polishing step, as well as out-sorts from sorting maize were tested for FUM. The highest concentration of FUM was removed through chaff and waste flour (*Pumba)* coming out of pre-cleaning and polishing of maize (step 3 and 4); at >14 μg/g, and out-sorts; step 5 (mean of 6.4 μg/g). Further dilutions and assays were not possible due to shortage of ELISA kits, and therefore the exact average FUM value in *Pumba* could not be determined.

4. Discussion

Processing of low AF and FUM flour from maize and groundnuts in a resource limited context where quality assurance systems and regulation are generally lacking is a challenging task. However, applying the HACCP approach within an SME context in Tanzania enabled the processing of low mycotoxin complementary feeding flours with minimal rejection rates for the final products. Screening maize before procurement (CCP1) reduced subsequent losses and rejection of maize during processing, especially at CCP2, where blending of flours was not a corrective action in cases where maize had high AF levels.

Although part of good practice in flour processing, none of the other steps, including pre-cleaning, winnowing, and sorting were identified as a CCP for AF in maize. Due to the cryptic nature of *Aspergillus flavus* infection of maize kernels (Shu et al., 2017), these steps may not always reduce AF. For example, visual and/or density sorting does not reliably reduce AF for heterogeneous hybrid maize (Mutiga et al., 2014; Ngure et al., 2021). Furthermore, any bag that exceeded the critical limit for AF at these prior steps would be detected at CCP2 and corrected by either blending or rejecting the bag. Although pre-cleaning, winnowing, and sorting may not consistently and effectively reduce AF in maize, these control measures were important in reducing FUM and ultimately losses of maize flour. This is consistent with other studies showing that sorting can reduce FUM (Aoun et al., 2020; Mutiga et al., 2014; Ngure et al., 2021; van der Westhuizen et al., 2010).

Prior research had identified physical attributes that predict FUM contamination (Aoun et al., 2020; Mutiga et al., 2014; Ngure et al., 2021; Stafstrom et al., 2021), with visible moldiness and low test weight maize predicting high FUM. Sensory inspection of maize during screening reduced procurement of maize with unacceptable levels of FUM.

Multiple stages of AF and FUM testing assured the food products met the research trial limit of 5 μg/kg for total AF and the maximum acceptable limit of 2.0 μg/g for FUM in Tanzania. Testing ~5% of the processed flours using ELISA confirmed the validity of prior tests done using the Neogen Reveal Q+ rapid assays on groundnuts and maize.

Visual inspection of groundnuts prior to procurement, pre-cleaning and visual sorting were important control measures in lowering AF levels and reducing subsequent losses of groundnuts during processing. Ideally, screening (step 1) and visual sorting of groundnuts (step 4, Table 2) would qualify as CCPs. However, in this study, these were only identified as control points since there was no objective way of measuring and verifying the AF levels in groundnuts in each of these steps. Testing each bag during screening and visual sorting would have been laborious and expensive. Only the best groundnuts (grade 1 and occasionally grade 2) from visual sorting were subjected to AF testing and processing to minimize AF and the cost of QC, which depended on expensive and difficult-to-source lateral flow assays. Due to the high frequency of AF contamination and the heterogeneous nature of its distribution in groundnuts, visual sorting did not always guarantee that all bags of grade 1 would have less than 5 μg/kg after milling and mixing. At the blending step, all bags were tested for AF and remedial action was taken, so this step met the CCP criteria. Sorting based on kernel size and visual appearance is known to be effective in reducing AF in groundnuts (Aoun et al., 2020; Dorner, 2008; Whitaker et al., 2005).

Groundnuts for export to international market are subjected to stringent visual sorting to yield the best quality grade. Because out-sorted fractions from processing of high quality products are often returned to the domestic food system (Matumba et al., 2015), sorting and grading increase the risk of low-income consumers being exposed to higher levels of aflatoxin (Gelli et al., 2020). To avoid the risk of concentrating AF in the food system, the researchers in this study supervised complete incineration of out-sorts at Halisi. More sustainable and environmentally friendly management options to utilize out-sorts in such contexts, such as production of fuel briquettes, should be explored.

The quality control process was associated with high losses (31% on average) in groundnuts, with those procured from local markets in Arusha being the most contaminated. It was common for traders to blend groundnuts from different farmers or markets and quality was highly compromised. Visual sorting of the market samples was extremely laborious, such that sorting took much longer for market-sourced groundnut than for those sourced from individual farmers. Due to sub-optimal drying and storage conditions along the market chain, the market samples were often darker in color and more shriveled than freshly threshed groundnuts from farmers, which were easy to sort visually.

After the first four months of processing, a change in procurement strategy from local markets to sourcing from individual farmers provided better quality groundnuts. This strategy encouraged interactions with farmers that provided insights on the history of both maize and groundnuts before procurement. Risk factors for AF exposure and accumulation, such as drought during the crop growth and grain-filling stages and pre- and peri-harvest rainfall were considered to help reduce the risk of procuring contaminated grain. Vertical integration, in which a processor connects with and influences producers to reduce pre- and post-harvest contamination, can be an important step in reducing risk and loss to a processor endeavoring to produce low-toxin products.

As an in-house approach for a food processor in a low-resource, unregulated and dynamic food system, the HACCP approach has its limitations. The wider value chain features for both of maize and groundnuts presented substantial challenges such as seasonality, the

Table 2
AF levels in raw groundnuts after pre-cleaning, winnowing and visual sorting.

Number of bags tested	n (%) or concentration
Bags tested for AF (Step 4, after winnowing and sorting)	585
Milled and blended with maize flour (5–6 μg/kg)	13 (2%)
Returned for repeat sorting (6 to > 150 μg/kg)	77 (13%)
Bags milled into groundnuts flour (AF=<5 μg/kg)	495 (85%)
Mean (SD) μg/kg	2.2 (1.2)
Median (range) μg/kg	2.4 (0–4.9)

Table 3
AF and FUM levels of processed flours and rejection rates after blending; CCP2 for AF/CCP for FUM in pre-blended flour (step 7), and CCP for AF in groundnut flour (step 7).

Levels or number of bags	Pre-blended flour		Groundnut flour
	AF (μg/kg)	FUM (μg/g)	AF (μg/kg)
Mean (SD)	3.5 (1.1)	0.8 (0.5)	3.3 (1.1)
Median (range)	3.7 (0.3–4.9)	0.9 (0–1.9)	3.6 (0.4–4.9)
n (100 kg bags)	262	221	131
Rejected bags	4 (2%)		13 (8%)
Bags blended with maize flour			13 (8%)
Total number of bags	266		157

Table 4
Percent losses during processing of maize and groundnuts.

Processing step	Mean (SD)	Range
Sampling and pre-cleaning maize	5 (3)	0–18
Winnowing and sorting maize	3 (2)	0–12
Total maize loss	10 (4)	3–28
Sorting groundnuts from:		
Local markets in Arusha	31 (12)	14–50
Individual farmers in Kongwa	27 (7)	1–52

Maize: n = 225 batches (13 batches by processing month and on average 100 kg bags for the rest). The weight was not recorded for pre-cleaning and winnowing for one batch.
Groundnuts: n = 8 batches (total = 2008 kg) from local markets; n = 311 bags of 100 kg each from local farmers in Kongwa.

lack of standardization and quality control, and the lack of incentives to produce and sell high-quality product. Food processors lack control over many of the stages of the value chain at which toxins accumulate. For example, in Tanzania a food processor cannot get a certificate of analysis for maize or groundnuts from local suppliers or farmers, since they do not have capacity to acquire or generate such, as is the case in developed economies (Henry & Xin, 2014). A more holistic value chain approach is critical in reducing losses, the cost of production and ensuring that SMEs can be profitable while producing clean flour. A well-designed procurement strategy and extensive quality assurance plan can significantly reduce the cost of producing safe complementary feeding flours.

The processing of the flours in this study took place within a well-resourced, short-term research effort that explicitly targeted AF and FUM reduction. While this is a special case, the study provides important lessons that can contribute to enhancing safe flour processing in a more typical setting. It is important to focus on attributes that helped reduce the risk of procuring contaminated maize and groundnuts. In 2020, maize sourced from markets in this study was frequently contaminated with AF, presumably due to the drought conditions. Middlemen commonly blended maize, complicating efforts to evaluate its quality. Sourcing maize directly from farmers enabled the researchers to not only probe farmers about specific risk factors, as noted above, but also to source from inherently lower-risk production environments. Cooler, relatively higher altitude areas (e.g., Karatu District) have lower AF risk but higher FUM risk. This was a favorable balance in a high-risk year, since FUM could be successfully reduced by pre-cleaning, polishing, winnowing, and sorting.

Procuring groundnuts directly from farmers in Kongwa District reduced time and losses during sorting. During the groundnuts sowing season (November and December), the farmers sought better quality groundnuts as seeds, despite the higher price. The best timing for procuring groundnuts in Kongwa District was June and July (1–2 months after harvesting) since the crop was well dried and prices were still low. In spite of the COVID 19 pandemic, an affordable, uninterrupted supply of complementary feeding flours was made possible, by planning and projecting the monthly needs throughout the research trial, seeking grain in a timely manner, and subsequent hermetic storage of well dried crops.

In processing such flours a few important caveats should be noted. First, losing nearly a third of groundnuts in processing such food is not feasible for SMEs that have small profit margins. Alternative and novel use of poor grades of groundnuts and out-sorts should be explored. Further research is recommended on value addition through a rigorous grading scheme that provides grades for alternative use such as poultry/animal feed and fuel. Second, in a drought year, grain supplies are likely to be limited and of poor quality. It may be impossible for all millers, even those with the best of intentions, to find sufficient quantity and quality of grain to produce foods with low levels of mycotoxin contamination. In the long run, it will be essential to support farmers to improve soil health so that crops are less susceptible climate- and soil-related stresses that lead to mycotoxin accumulation.

5. Conclusion

Using a HACCP approach in such a challenging context enabled successful processing of low AF and FUM complementary feeding flours. Rigorous sorting of groundnuts was found to be effective in reducing AF, though with high losses. There is a need for processors to adopt procurement and screening strategies that ensure they receive low AF maize prior to processing since sorting is not effective in reducing AF in maize in this context. Our work demonstrates significant reduction in FUM through pre-cleaning, polishing, winnowing, and sorting that can be adopted in small- and medium-scale processing in similar settings.

Application of the principles laid out in this paper can help food processors in similar contexts effectively process low AF and FUM flours from maize and groundnuts. Since there are no clear incentives for low mycotoxins food production in low-income settings, application of these principles will require increased awareness and training among millers, as well as support and oversight from regulatory agencies along food and feed value chains. We acknowledge that this rarely happens in many mycotoxin-prone settings. A sensible first step would be to implement national surveillance systems using geographic information systems that track risk factors and inform sampling efforts to help anticipate mycotoxin outbreaks that require intervention.

Funding

This study was funded by the Bill & Melinda Gates Foundation, as part of a larger study, under Grant Number OPP1155626. The supporting source was not involved in study design, implementation or data analysis and interpretation.

CRediT authorship contribution statement

Francis M. Ngure: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Edna Makule:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **William Mgongo:** Writing – review & editing, Methodology, Investigation, Data curation. **Erica Phillips:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **Neema Kassim:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Rebecca Stoltzfus:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Rebecca Nelson:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank Mrs. Sarah William Kessy, the Director Halisi Products Limited, and the staff for the collaboration and support in processing the complementary flours. The authors also thank John Mshanga for the technical support at NM-AIST.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.foodcont.2024.110463.

References

AGRA. (2020). *Analysis of supply- chain models*. https://agra.org/wp-content/uploads/2020/10/Markets-Analysis-of-Supply-Chain-Models-Tanzania.pdf.

Aoun, M., Stafstrom, W., Priest, P., Fuchs, J., Windham, G. L., Williams, W. P., & Nelson, R. J. (2020). Low-cost grain sorting technologies to reduce mycotoxin contamination in maize and groundnut. *Food Control, 118*(February), Article 107363.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H. S., Kieszak, S., Njapau, H., … Bowen, A. (2005). Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environmental Health Perspectives, 113*(12), 1779–1783.

Boni, S. B., Beed, F., Kimanya, M. E., Koyano, E., Mponda, O., Mamiro, D., … Mahuku, G. (2021). Aflatoxin contamination in Tanzania: Quantifying the problem in maize and groundnuts from rural households. *World Mycotoxin Journal, 14*(4), 553–564.

Dorner, J. W. (2008). Management and prevention of mycotoxins in peanuts. *Food Additives & Contaminants Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 25*(2), 203–208.

EAC. (2018). Disposal and alternative use of aflatoxin contaminated food. EAC policy brief on aflatoxin prevention and control. Policy Brief No. 8 https://www.eac.int/documents/category/aflatoxin-prevention-and-control.

East African Community. (2013). East African standard: Maize grains Specification. EAS 2:2013. https://members.wto.org/crnattachments/2017/TBT/BDI/17_5584_00_e.pdf.

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S., & Krska, R. (2019). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. *Critical Reviews in Food Science and Nutrition*, 1–17.

European Commision Regulation. (2006). Commision Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union, 70*(401), 12–34.

FAO/IAEA. (2001). Manual on the application of the HACCP system in mycotoxin prevention and control. *FAO Food & Nutrition Paper, 73*, 124.

Gelli, A., Donovan, J., Margolies, A., Aberman, N., Santacroce, M., Chirwa, E., … Hawkes, C. (2020). Value chains to improve diets: Diagnostics to support intervention design in Malawi. *Global Food Security, 25*(September 2019), Article 100321.

Gong, Y. Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P., Hall, A., & Wild, C. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: Cross sectional study. *British Medical Journal, 325*(7354), 20–21.

Gong, Yun yun, Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., … Wild, C. P. (2004). Postweaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, west Africa. *Environmental Health Perspectives, 112*(13), 1334–1338.

Henry, C. J. K., & Xin, J. L. W. (2014). Application of hazard analysis critical control point in the local manufacture of ready-to-use therapeutic foods (RUTFs). *Food and Nutrition Bulletin, 35*(2), S57–S63.

International Agency for Research on Cancer. (2012). Aflatoxins IARC Monographs. *International Agency for Research on Cancer, 100F*, 225–248.

Kamala, A., Shirima, C., Jani, B., Bakari, M., Sillo, H., Rusibamayila, N., … Simba, A. (2018). Outbreak of an acute aflatoxicosis in Tanzania during 2016. *World Mycotoxin Journal, 11*(3), 311–320.

Kimanya, M. E., De Meulenaer, B., Roberfroid, D., Lachat, C., & Kolsteren, P. (2010a). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition & Food Research, 54*(11), 1659–1667.

Kimanya, M. E., De Meulenaer, B., Roberfroid, D., Lachat, C., & Kolsteren, P. (2010b). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition & Food Research, 54*(11), 1659–1667.

Kimanya, M. E., Meulenaer, B. De, Baert, K., Tiisekwa, B., Van Camp, J., Samapundo, S., … Kolsteren, P. (2009). Exposure of infants to fumonisins in maize-based complementary foods in rural Tanzania. *Molecular Nutrition & Food Research, 53*(5), 667–674.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., … Gupta, N. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives, 113*(12), 1763–1767.

Massomo, S. M. S. (2020). Aspergillus flavus and aflatoxin contamination in the maize value chain and what needs to be done in Tanzania. *Scientific African, 10*, Article e00606.

Matumba, L., Van Poucke, C., Monjerezi, M., Njumbe Ediage, E., & De Saeger, S. (2015). Concentrating aflatoxins on the domestic market through groundnut export: A focus on Malawian groundnut value and supply chain. *Food Control, 51*.

Mollay, C., Kassim, N., Stoltzfus, R., & Kimanya, M. (2020). Childhood dietary exposure of aflatoxins and fumonisins in Tanzania: A review. *Cogent Food & Agriculture, 6*(1), Article 1859047.

Mollay, C., Kassim, N., Stoltzfus, R., & Kimanya, M. (2021). Complementary feeding in Kongwa, Tanzania: Findings to inform a mycotoxin mitigation trial. *Maternal and Child Nutrition, 17*(4), 1–10.

Mutegi, C. K., Cotty, P. J., & Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960-to date). *World Mycotoxin Journal, 11*(3), 341–357.

Mutiga, S. K., Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (2015). Assessment of aflatoxin and fumonisin contamination of maize in western Kenya. *Phytopathology, 105*(9), 1250–1261.

Mutiga, S. K., Were, V., Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (2014). Extent and drivers of mycotoxin contamination: Inferences from a survey of Kenyan maize mills. *Phytopathology, 104*(11), 1221–1231.

Nelson, R. (2016). Sorting technologies to rehabilitate toxic maize. *Outlooks on Pest Management, 27*, 247–251.

Ngure, Francis M., Kassim, N., Phillips, E. L., & Turner, P. C. (2023). Infant and young child feeding practices and mycotoxin contamination of complementary food ingredients in Kongwa District, Tanzania. *Current Developments in Nutrition, 7*(2), Article 100030.

Ngure, F. M., Ngure, C., Achieng, G., Munga, F., Moran, Z., Stafstrom, W., & Nelson, R. J. (2021). Mycotoxins contamination of market maize and the potential of density sorting in reducing exposure in unregulated food systems in Kenya. *World Mycotoxin Journal, 14*(2), 165–178.

Phillips, E., Ngure, F., Smith, L. E., Makule, E., Turner, P. C., Nelson, R., … Kassim, N. (2020). Protocol for the trial to establish a causal linkage between mycotoxin exposure and child stunting: A cluster randomized trial. *BMC Public Health, 20*(1), 1–11.

Probst, C., Njapau, H., & Cotty, P. J. (2007). Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. *Applied and Environmental Microbiology, 73*(8), 2762–2764.

Seetha, A., Munthali, W., Msere, H. W., Swai, E., Muzanila, Y., Sichone, E., … Okori, P. (2017). Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. *Mycotoxin Research, 33*(4), 323–331.

Shirima, C. P., Kimanya, M. E., Routledge, M. N., Srey, C., & Kinabo, J. L. (2015). A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. *Environmental Health Perspectives, 123*(2), 173–179.

Shu, X., Livingston, D. P., Woloshuk, C. P., & Payne, G. A. (2017). Comparative histological and transcriptional analysis of maize kernels infected with *Aspergillus flavus* and *Fusarium verticillioides*. *Frontiers in Plant Science, 8*(2075).

Stafstrom, W., Wushensky, J., Fuchs, J., Xu, W., Ezera, N., & Nelson, R. J. (2021). Validation and application of a low-cost sorting device for fumonisin reduction in maize. *Toxins, 13*(9).

Suleiman, R., & Kurt, R. (2015). Current maize production, postharvest losses and the risk of mycotoxins contamination in Tanzania. In *2015 ASABE international meeting* (p. 1). St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Udomkun, P., Wiredu, A. N., Nagle, M., Bandyopadhyay, R., Müller, J., & Vanlauwe, B. (2017). Mycotoxins in sub-saharan Africa: Present situation, socio-economic impact, awareness, and outlook. *Food Control, 72*, 110–122.

van der Westhuizen, L., Shephard, G. S., Rheeder, J. P., Burger, H.-M., Gelderblom, W. C. A., Wild, C. P., & Gong, Y. Y. (2010). Simple intervention method to reduce fumonisin exposure in a subsistence maize-farming community in South Africa. *Food Additives & Contaminants Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 27*(11), 1582–1588.

Whitaker, T. B., Dorner, J. W., Lamb, M., & Slate, A. B. (2005). The effect of sorting farmers' stock peanuts by size and color on partitioning aflatoxin into various shelled peanut grade sizes 1. *Peanut Science, 32*(2), 103–118.

IARC. (2015). CHAPTER 1. Human exposure to aflatoxins and fumonisins. In C. Wild, J. Miller, & J. Groopman (Eds.), *Mycotoxin control in low- and middle-income countries* (pp. 1–5).

Wilson, R. T., & Lewis, J. (2013). *The maize value chain in Tanzania*. FAO. https://www.fao.org/sustainable-food-value-chains/library/details/en/c/285408/.

World Food Programme. (2022). Tanzania nationwide mills census report. Retrieved from https://www.wfp.org/publications/tanzania-nationwide-mills-census-report-2022.