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Effect of traditional processing operations on aflatoxin levels in laboratory-contaminated *Asaana* and *Burkina* beverages

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Abstract: Traditional Ghanaian beverages such as *Asaana*, a sweet malted-maize drink, and *Burkina*, a fermented millet beverage, may contain aflatoxins when contaminated grains are used. This study evaluated aflatoxin production in experimentally contaminated maize and pearl millet and assessed the effects of traditional processing on aflatoxin levels during laboratory-simulated beverage production. Cereals were inoculated with a toxigenic Aspergillus flavus isolate and incubated at 30°C for 28 days. Aflatoxin B_1 (AFB_1) and total aflatoxins were quantified by high-performance liquid chromatography after immunoaffinity-column clean-up. In millet, AFB_1 increased to 100.08 µg/kg after incubation. Eight-hour steeping reduced this level to 30.48 µg/kg, corresponding to a 69.5% reduction, while subsequent steaming and fermented-milk addition produced no significant further reductions. Overall, Burkina processing reduced total aflatoxins by approximately 70%. In maize, AFB_1 increased to 137.96 µg/kg. Steeping, germination, and sun-drying reduced AFB_1 to 39.23 µg/kg, representing a 71.6% reduction. Boiling and caramelised-sugar addition produced no significant additional reduction; the *Asaana* base retained approximately 38.36 µg/kg total aflatoxins after 1 hour of boiling. The findings indicate that steeping, germination, and sun-drying were more effective than steaming or boiling in reducing aflatoxin levels. However, substantial residues remained when initial contamination was high. Traditional processing should therefore complement, rather than replace, preventive measures such as good agricultural and storage practices, careful raw-material selection, improved hygiene, and process control.

Keywords: aflatoxin; *Aspergillus flavus*; *Asaana*; *Burkina*; maize; traditional beverages production

1. Introduction

Maize (Zea mays L.) and pearl millet (Pennisetum glaucum L.) are important cereal staples in sub-Saharan Africa and contribute substantially to household food security (Ramashia et al., 2025) and dietary energy (van Ittersum et al., 2025). In Ghana, several traditional cereal-based beverages are produced by micro-scale and artisanal processors. These include *Asaana*, a non-alcoholic, sweet, malted maize beverage, and Burkina, a fermented milk beverage containing steamed millet granules, closely related to the Fulani fermented milk-cereal product *degue*. Both drinks are widely consumed and are commonly sold through informal street and market vending (Baidoo and Parry-Hanson Kunadu, 2018; Osei et al., 2021).

The safety of these beverages depends substantially on the quality of the cereal grains used as raw materials. Inadequate drying, high storage moisture, poor ventilation, insect damage, and unhygienic handling can promote the growth of toxigenic moulds and the production of aflatoxins. (Danso et al., 2018; Kortei et al., 2023). Aflatoxin B_1 (AFB_1), produced mainly by *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*, is the most prevalent and toxic member of the aflatoxin group. Chronic dietary exposure to AFB_1 is associated with hepatotoxicity, immunosuppression, impaired growth, and hepatocellular carcinoma (Li et al., 2022; Prenner and Kulik, 2017; Shan and Shan, 2019). Aflatoxin contamination of cereals and cereal products in West Africa is well documented. (Houissa et al., 2019) reported that 91.4% of Tunisian pearl millet samples contained multiple fungal metabolites, with AFB_1 and ochratoxin A frequently exceeding European Union limits. In Burkina Faso, (Yacine Ware et al., 2017) found high levels of AFB_1, ochratoxin A, and fumonisins in multi cereal infant

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formulas produced under artisanal or semi-industrial conditions. In Ghana, aflatoxins have been detected in maize, rice, sorghum, and millet, and in fermented cereal foods such as kenkey, pito, and brukutu (Agbetiameh et al., 2018; Amoa-Awua et al., 2007; Kpodo et al., 1996; Omari et al., 2022).

More recently, aflatoxins have been reported directly in Ghana and Burkina Faso. (Ofori-Attah et al., 2021) Found AFB_1 concentrations of 1–23 µg/kg in Burkina beverages sold in Accra, with several samples above the commonly cited 20 µg/kg limit for ready-to-eat foods. (Osei et al., 2021) Estimated average annual beverage consumption in Ghana at 22–146 L per capita and aflatoxins were detected in a range of local drinks, with levels from 0.023 µg/kg in Asaana to 5.377 µg/kg in another maize-based beverage. Given these consumption volumes, traditional beverages may contribute substantially to dietary aflatoxin exposure.

Processing can reduce mycotoxin concentrations through several mechanisms. Sorting, washing, density separation, and sieving may remove contaminated or damaged kernels. Steeping may promote the leaching of aflatoxins into the steeping water, while germination and fermentation may contribute to microbial or enzymatic transformation (Adebo et al., 2020; Karlovsky et al., 2016; Kumar et al., 2021; Taylor, 2001; Taylor and Kane, 2005). However, the extent of aflatoxin reduction depends on the initial contamination level, the physical distribution of the toxin within the grain, the duration and conditions of processing, and whether contaminated fractions are discarded. Aflatoxins are also relatively heat-stable under many domestic and artisanal processing conditions; therefore, boiling or steaming alone may not achieve adequate reduction (Kumar et al., 2017; Mutungi et al., 2008; Sabina et al., 2023).

Although aflatoxins have been reported in Ghanaian cereals and cereal-based products, limited information is available on the fate of AFB_1 and total aflatoxins across the individual unit operations used to prepare Asaana and Burkina. In particular, it remains unclear how much of the reduction occurs during cleaning and steeping compared with germination, sun-drying, steaming, boiling, and final formulation. The novelty of the present study lies in tracking aflatoxin concentrations at successive stages of laboratory-simulated Asaana and Burkina production using maize and pearl millet experimentally contaminated with a toxigenic A. flavus isolate. The study had two objectives: first, to characterise aflatoxin production in maize and pearl millet inoculated with A. flavus and incubated under conditions favourable to mould growth; and second, to quantify changes in AFB_1 and total aflatoxin concentrations during selected traditional processing operations used to prepare Asaana and Burkina. The study hypothesised that cleaning, steeping, germination, and drying would reduce aflatoxin concentrations more effectively than subsequent heat treatments, but that processing would not consistently reduce high initial contamination to acceptable levels.

2. Materials and methods

2.1 Study design

The study comprised a survey of artisanal *Asaana* and *Burkina* processors, a laboratory challenge test using maize and pearl millet inoculated with *A. flavus*, laboratory-simulated production of *Asaana* and *Burkina* from the contaminated grains, and HPLC analysis of aflatoxins at selected processing stages.

The study covered two cereal-beverage systems:

1. maize used for laboratory-simulated *Asaana* production; and
2. pearl millet used for laboratory-simulated *Burkina* production.

The contamination model was designed to generate measurable aflatoxin concentrations and to permit comparison across processing stages. It was not designed to estimate the prevalence of aflatoxins in naturally contaminated commercial grains or beverages.

2.2 Survey of artisanal processors

The survey involved 15 *Burkina* processors and 15 *Asaana* processors. Information was collected using structured questionnaires concerning raw material acquisition, storage, processing procedures, hygiene practices, packaging, and product storage.

2.2. Raw materials

Freshly harvested maize and pearl millet were obtained directly from farmers in Techiman, Bono Region, Ghana (approx. 7°35′–8°00′N; 1°49′E–2°30′W). Maize cobs were hand-shelled and cleaned to remove visible

chaff and foreign material. Millet panicles were threshed, and the chaff was removed by winnowing. Grains were sun-dried for 10 days during the harmattan season (average ambient temperature ≈ 33 °C; relative humidity ≈ 25%) until low moisture contents were attained (maize 4.77%; millet 2.1% w.b.). Dried grains were packed in sterile plastic bags, sealed, and transported to the Food Research Institute, Accra, for experimental work. Grains were not sterilised before inoculation. Consequently, they may have contained pre-existing microorganisms or low levels of naturally occurring aflatoxins. The observed aflatoxin concentrations may therefore reflect both the deliberately introduced A. flavus and any pre-existing contamination.

2.3. Isolation and culture of *Aspergillus flavus*

One gram of naturally mouldy yellow maize was transferred to 9 g of saline-buffered peptone and thoroughly shaken to produce a spore suspension. Serial dilutions were prepared, and 1 mL of the diluted suspension was transferred into Petri dishes containing approximately 20 mL of Dichloran Rose Bengal Chloramphenicol (DRBC) agar. The plates were incubated at 30 °C for 7 days and examined daily for fungal growth and spore formation.

Colonies displaying powdery yellow-green spores on the upper surface and reddish-gold pigmentation on the reverse surface were considered consistent with *Aspergillus flavus*. The isolate was subcultured onto Potato Dextrose Agar to obtain a pure culture and incubated at 30 °C for 7 days.

2.4. Experimental contamination of grains

Experimental contamination of maize and millet followed (Gomaa et al., 1997) and (Hsu et al., 2018) with minor modifications. Eight kilograms of millet or maize were soaked in potable water for 5 or 25 minutes, respectively, to increase the moisture content to approximately 15%. Excess water was removed by draining the grains onto a disinfected towel. A spore suspension was prepared by adding 300 mL of sterile water to three sporulated cultures of *A. flavus* and dislodging the spores. Each 8-kg batch of grain received 80 mL of the suspension, which was sprayed uniformly while the grains were mixed thoroughly. The inoculated grains were placed in sterile plastic containers and incubated in the dark at 30 °C for 28 days. The containers were shaken daily to redistribute the spores and promote aeration.

Samples were collected at 0, 7, 14, 21, and 28 days for aflatoxin analysis.

2.5. Laboratory simulation of *Burkina* processing

2.5.1. Process outline

Burkina was prepared from experimentally contaminated millet using the following stages: washing, steeping, wet milling, granule formation, steaming, cooling, preparation of fermented milk, final mixing, and bottling (Figure 4).

2.5.2. Steeping and granule preparation

Contaminated millet was washed three times with potable water and divided into two groups. One group was steeped for 4 hours and the other for 8 hours. After steeping, each group was rinsed once with clean water and wet-milled separately. Water was added to the milled paste to obtain an approximate moisture content of 30%. The paste was mixed by hand, and larger lumps were broken into smaller pieces by pressing and rubbing them through a colander locally known as an “agbaje.” The material was further rubbed and formed into granules approximately 3–5 mm in diameter.

2.5.3. Steaming of granules

Granules from each steeping treatment were steamed for 30 or 60 minutes over boiling water using a covered colander placed on a cooking pot. The steamed granules were cooled in separate clean basins.

2.5.4. Fermented milk preparation

Fermented milk was prepared by mixing 250 g of powdered milk with 2.5 L of water at approximately 25-28 °C, followed by the addition of 500 mL of boiling water. One teaspoon of previously fermented milk with a reported pH of approximately 4 was added as a starter. The mixture was covered, wrapped with a thick cloth, and left overnight for fermentation. The fermented milk was subsequently homogenised and filtered before use.

2.5.5. Final *Burkina* formulation and sampling

For each treatment combination, steamed millet granules were placed into 250-mL bottles. Sugar and fermented milk were added to fill the bottles, and the bottles were closed.

Samples were collected at the raw-material stage before steeping, after steeping, after steaming, and after final formulation. All analyses were conducted in triplicate.

2.6. Laboratory simulation of the production of *Asaana*

2.6.1. Process outline

Asaana was prepared from experimentally contaminated maize using the following stages: washing, steeping, germination, sun-drying, crushing, mash boiling, sieving, caramelised-sugar preparation, final mixing, and bottling (Figure 4).

2.6.2. Steeping, germination, and sun-drying

Contaminated maize was washed and steeped in potable water for 24 hours. The steep water was drained, and the grains were rewashed. The soaked maize was placed in a basket lined with clean fabric, covered, and allowed to germinate for 3 days. The grains were watered at approximately 12-hour intervals. After germination, the wet malt was sampled and then sun-dried.

2.6.3. Crushing, mash boiling, and sieving

The sun-dried malt was crushed using a blender. One cup of crushed malt was mixed with 10 L of water and boiled for 1 or 2 hours. The boiled mash was allowed to cool and was filtered through a strainer with a pore size of approximately 0.5 mm.

2.6.4. Caramelised sugar and final *Asaana* formulation

Caramelised sugar (treacle) was prepared by heating white granulated sugar in a metal saucepan over medium heat, stirring constantly, until a deep brown caramel formed. An equal volume of water was added cautiously in small portions with continuous stirring until the caramel dissolved completely. The treacle was cooled and filtered through a fine mesh. To prepare *Asaana*, treacle and additional white sugar were added to the cooled wort to taste. Treatments included mash boiling with and without treacle addition. The final beverage was bottled in plastic containers.

Samples were collected from the contaminated maize before steeping, after steeping, after wet germination, after sun-drying, after 1 or 2 hours of boiling, and after addition of caramelised sugar where applicable. Analyses of the selected crop species were conducted in triplicate.

2.7. Aflatoxin extraction and HPLC analysis

AFB_1, AFB_2, AFG_1, and AFG_2 were determined by immunoaffinity column clean-up followed by HPLC, based on (Essono et al., 2009; Gnonlonfin et al., 2012). Twenty-five grams of homogenised sample were weighed into a blender jar. Five grams of sodium chloride and 200 mL of 80% methanol in water were added. The mixture was blended for approximately 3 minutes and filtered. Ten millilitres of filtrate were transferred to a beaker and mixed with 60 mL of phosphate-buffered saline.

Approximately 70 mL of the diluted extract were passed through a conditioned immunoaffinity column by gravity at a flow rate of approximately 3 mL/min. The column was washed with approximately 15 mL of water and dried using gentle vacuum or air. Aflatoxins were eluted using methanol in two stages. First, 0.5 mL of methanol was applied to the column and allowed to pass by gravity. After 1 minute, a second portion of 0.75 mL methanol was applied.

The eluate was collected in a 5-mL volumetric flask, made up to volume with water, and mixed. Where necessary, the solution was passed through a 0.45-µm disposable filter before HPLC analysis. HPLC was used to separate and quantify AFB_1, AFB_2, AFG_1, and AFG_2.

Total aflatoxins were calculated automatically as the sum of the four measured analogues. Method performance was consistent with that reported by (Essono et al., 2009; Gnonlonfin et al., 2012) and was suitable for quantifying concentrations at µg/kg levels.

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2.8. Statistical analysis

All analyses were conducted at least in triplicate, and mean values are reported. One-way analysis of variance (ANOVA) was used to compare aflatoxin levels at different processing stages. Differences were considered significant at $p < 0.05$. Statistical analyses were carried out using Minitab® version 17 (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1 Survey findings

The survey, being descriptive, found that *Burkina* and *Asaana* production was mainly carried out by women in household or small-scale settings, with processing skills commonly learned from family members. Ingredients were usually purchased from local markets, although grain-quality assessment varied, particularly among Asaana producers.

Potential post-harvest risks included storing grains on floors or under conditions favouring moisture and mould growth. Processing and storage practices also varied, indicating limited standardization. These findings informed the traditional processing sequences and hence, laboratory simulations presented in Figures 1A and 1B.

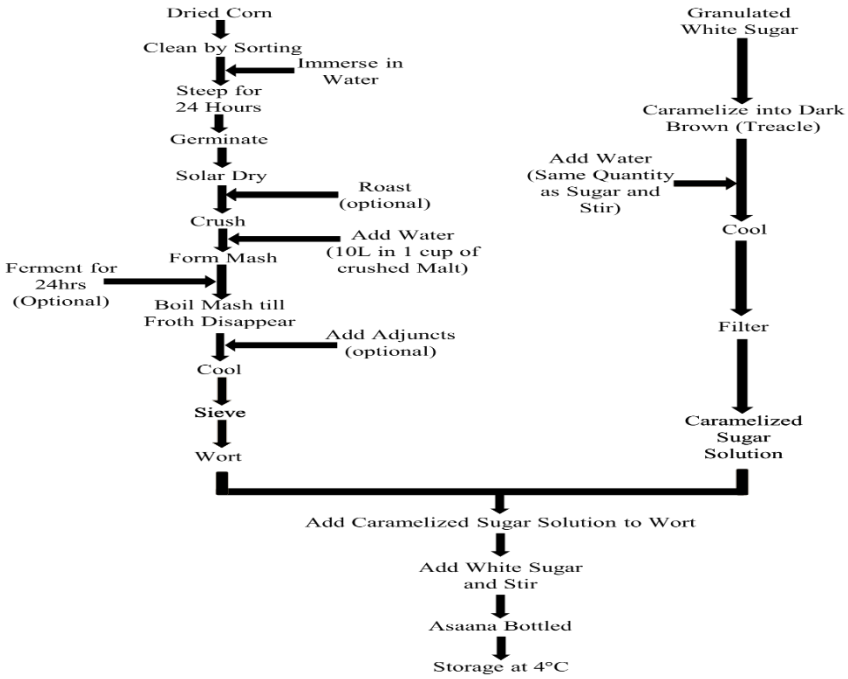


Figure 1A. Traditional and laboratory-simulated processing flow diagram for Burkina: pearl millet → cleaning and washing → steeping for 4 or 8 h → wet milling → granule formation → steaming for 30 or 60 min → cooling → addition of fermented milk and sugar → bottling.

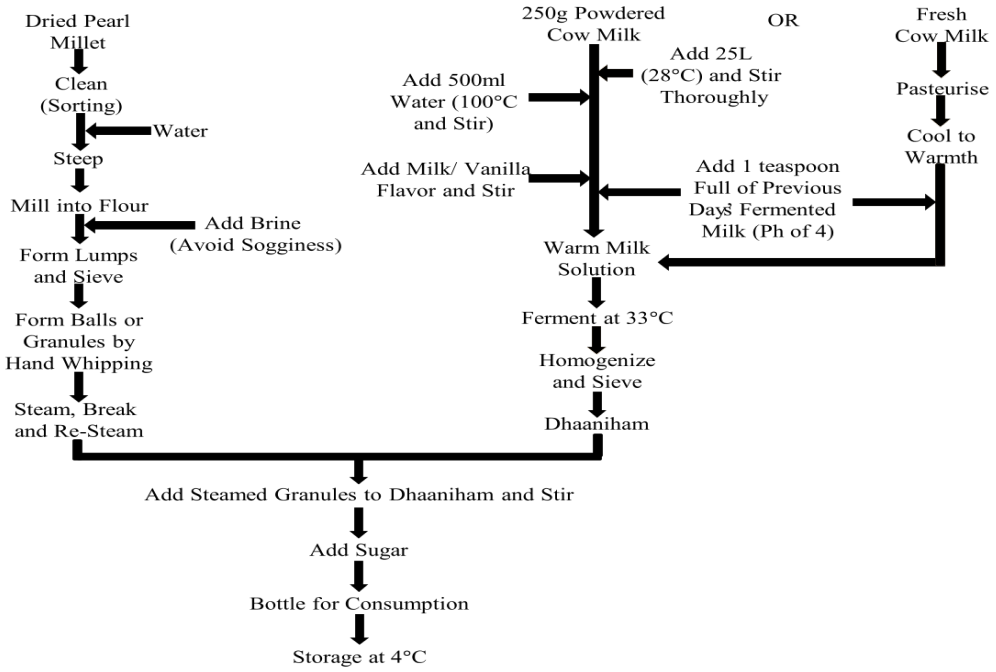
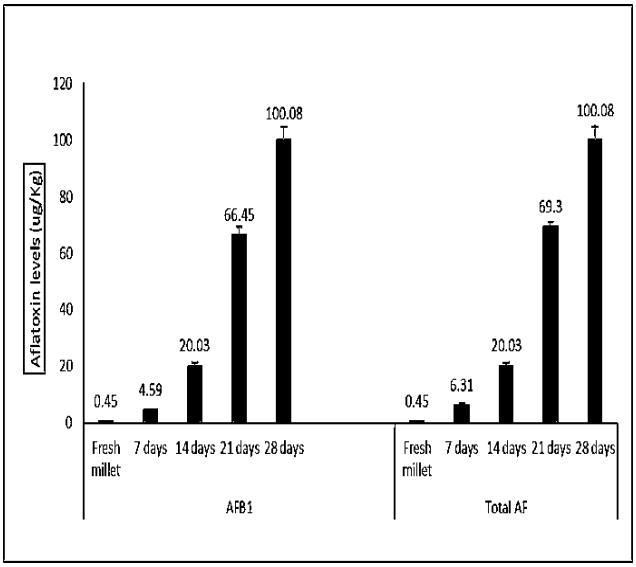


Figure 1B. Traditional and laboratory-simulated processing flow diagram for *Asaana*: maize → cleaning and washing → steeping for 24 h → germination for 3 d → sun-drying → crushing → mash boiling for 1 or 2 h → sieving → addition of caramelised sugar and additional sugar → bottling.

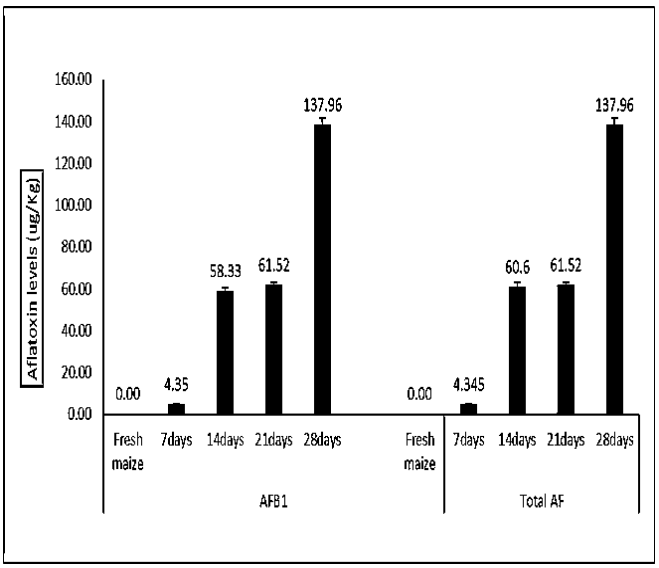
The experimental conditions were selected to reflect the range of practices identified in the survey while allowing evaluation of the effects of individual processing stages under controlled conditions. Sampling points were included at selected stages to determine changes in AFB_1 and total aflatoxin concentrations throughout processing.

3.2. Aflatoxin production in experimentally contaminated grains

The evolution of AFB_1 and total aflatoxins in millet and maize during 28 days of incubation at 30 °C after inoculation with *A. flavus* is summarised in Figure 1(A and B).



A



B

Figure 2. Time course of aflatoxin B_1 (AFB_1) and total aflatoxins in experimentally contaminated millet (A) and maize (B) stored at 30°C for 28 d. Values are means ($n \geq 3$); error bars indicate standard deviations. AFB_1 and total aflatoxins increased significantly ($p < 0.05$) after 14 days of incubation of both grains; AFB_1 dominated the aflatoxin profile throughout the 28-day incubation.

Although millet was carefully selected from the field, it contained detectable AFB_1 at 0.45 µg/kg on day 0, indicating pre-harvest or early post-harvest contamination. After adjustment to 15% moisture and inoculation, AFB_1 increased significantly ($p < 0.05$) each week, reaching 100.08 µg/kg by day 28. Total AF showed a similar trend, confirming AFB_1 as the dominant analogue (Figure 2A).

Freshly dried maize initially contained no detectable aflatoxins. After inoculation and incubation, AFB_1 was first detected at 4.35 µg/kg on day 7 (not significantly different from 0 at $p > 0.05$), then increased significantly to 58.33 µg/kg (day 14), 61.52 µg/kg (day 21) and 137.96 µg/kg (day 28) (Figure 2B). Total AF followed the same pattern. It could also be observed that both cereals had total AF level to be the same as that of AFB_1 and it for the reason being that AFB_2, AFG_1 and AFG_2 were below detection

Both cereals, therefore, supported substantial aflatoxin production under favourable storage conditions, with final AFB_1 concentrations far exceeding EU maximum levels of 5–10 µg/kg for unprocessed cereals, underscoring the importance of adequate drying and storage. The increase observed in millet also indicates that contamination may be present before processing, even when grains appear visually acceptable.

3.2. Effect of *Burkina* processing on aflatoxin levels

3.2.1. Effect of Steeping

Steeping had a marked effect on aflatoxin levels in contaminated millet. As shown in Table 2, AFB_1 decreased from 100.08 µg/kg (no steeping) to 87.84 µg/kg after 4 hours of steeping (12.2% reduction; $p > 0.05$) and to 30.48 µg/kg after 8 hours of steeping (69.5% reduction; $p < 0.05$). Total AF decreased by approximately 67% after 8 hours.

Table 1. Effect of steeping on AFB_1 and total aflatoxin levels in millet used for *Burkina* production.

Steeping time	AFB_1 (µg/kg)	Total AF (µg/kg)	% reduction in AFB_1 vs 0 h
0 h	100.08	100.08	–
4 h	87.84	92.14	12.2
8 h	30.48	33.07	69.5

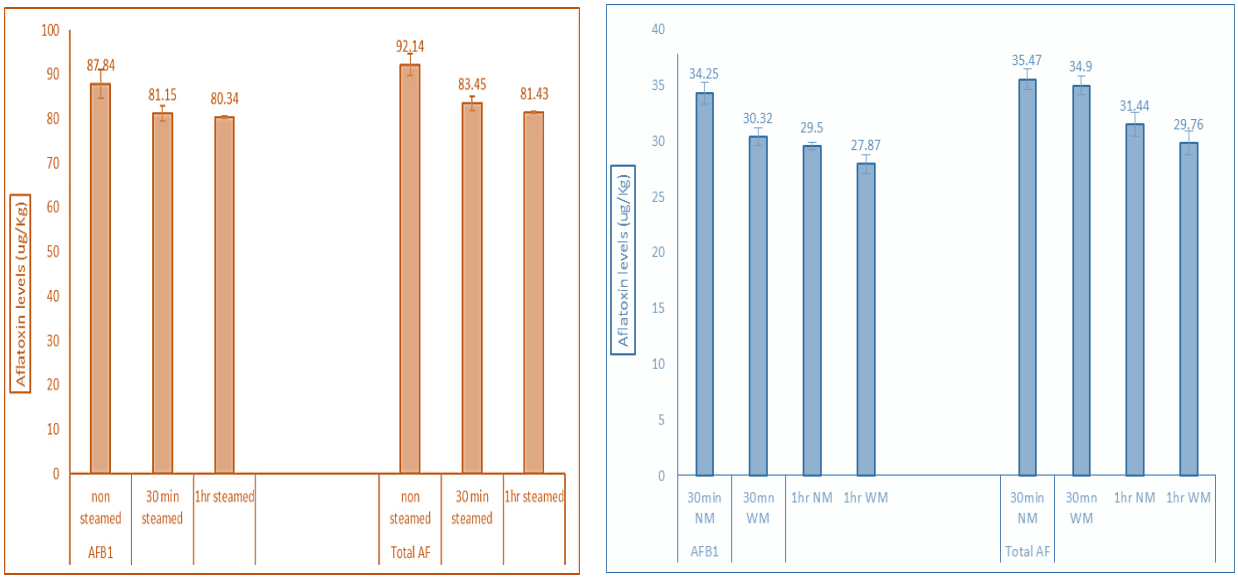
These findings are consistent with previous reports that washing, steeping and sorting can remove large proportions of aflatoxins (Karlovsky et al., 2016; Vijaya, 2018). The observed reduction may have resulted from the removal of damaged or heavily contaminated kernels during washing, leaching of aflatoxins into the steeping water, dislodgement of fungal spores and surface contaminants, and possible microbial or enzymatic transformation during soaking (Aasa et al., 2026; Yang and Yang, 2019).

3.2.2 Effect of steaming and fermented-milk addition

Steaming of millet granules had only a limited additional effect (Figure 3A). After 8 hours of steeping, AFB_1 fluctuated between 30.48 and 34.25 µg/kg for 0–30 minutes of steaming and decreased slightly to 29.50 µg/kg after 60 minutes of steaming; these changes were not statistically significant ($p > 0.05$). Total AF trends were similar. The limited effect of steaming is consistent with the thermal stability of AFB_1 under conventional moist-heat conditions. Thus, steaming for up to 1 hour under domestic or artisanal conditions should not be regarded as a reliable aflatoxin-control step. Its principal contribution is more likely to be related to texture development and microbiological safety.

Addition of fermented milk to steamed granules produced only small, non-significant changes in aflatoxin concentrations (Figure 3B). For 30 minutes of steamed granules (8 hours steeped), AFB_1 decreased from 34.25 µg/kg without milk to 30.32 µg/kg with milk. For 60 minutes of steamed granules, AFB_1 decreased from 29.50

to 27.87 µg/kg upon addition of fermented milk. Total AF showed a similar slight decrease from 31.44 to 29.76 µg/kg.



A **B** Legend: NM=No Milk; WM= With Milk

Figure 3. Effect of steaming and fermented milk addition on AFB_1 and total aflatoxins in millet during laboratory-simulated production in *Burkina*. (A) Aflatoxin levels in granules from 8 h-steeped millet after 0, 30 and 60 minutes of steaming. (B) Aflatoxin levels in final *Burkina* with or without fermented milk, prepared from 8 h-steeped millet granules steamed for 30- or 60-min. Steaming of millet granules had only a limited additional effect (Figure 2A). After 8 hours of steeping, AFB_1 fluctuated between 30.48 and 34.25 µg/kg for 0–30 minutes of steaming and decreased slightly to 29.50 µg/kg after 60 minutes of steaming; these changes were not statistically significant ($p > 0.05$). Total AF trends were similar. The modest changes observed confirm that steaming for up to 1 hour under domestic conditions does not substantially degrade aflatoxins, consistent with the known thermal stability of AFB_1.

3.3 Effect of Asaana processing on aflatoxin levels

3.3.1. Effect of steeping and germination

Steeping and germination (malting) of contaminated maize also resulted in substantial reductions in aflatoxin levels (Table 2).

Table 2. Effect of steeping and germination on AFB_1 and total aflatoxin levels in maize used for *Asaana* production.

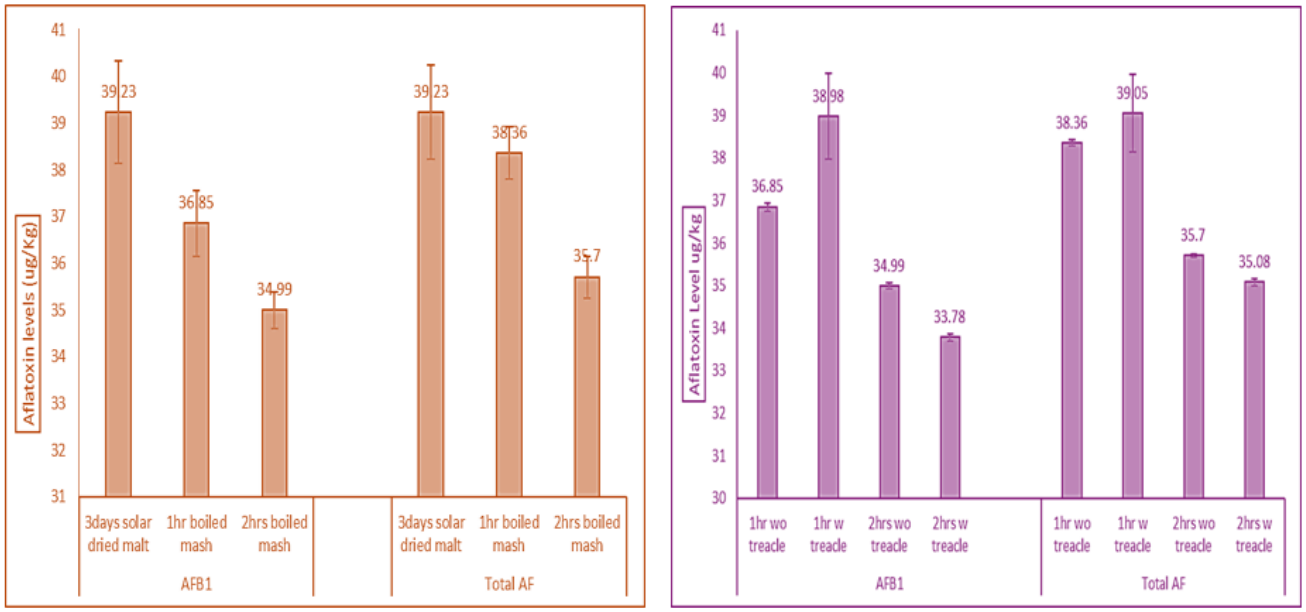
Processing stage	AFB_1 (µg/kg)	Total AF (µg/kg)	% reduction in AFB_1 vs initial
Contaminated maize (day 28)	137.96	137.96	–
After 24 hours of steeping	61.06	61.36	55.7
After 3 days of germination	51.84	53.98	62.4

AFB_1 decreased from 137.96 µg/kg in contaminated maize to 61.06 µg/kg after 24 hours of steeping (55.7% reduction; $p < 0.05$) and further to 51.84 µg/kg after 3 days of germination (62.4% total reduction; $p < 0.05$). Total AF decreased from 137.96 to 61.36 µg/kg after steeping and to 53.98 µg/kg after germination (55.5% and 60.9% reductions, respectively; Table 2). These findings reinforce the role of steeping and germination as key mycotoxin mitigation steps. The reduction during steeping may be attributed to leaching, washing, removing contaminated particles, and discarding the steep water. Germination may have provided an additional reduction through continued hydration, physical separation of contaminated material, metabolic activity, and possible microbial or enzymatic transformation. However, the additional reduction attributable to germination was modest compared with the initial effect of steeping.

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3.3.2. Effect of Sun-Drying, Boiling and Sieving

After germination and drying, malt contained 39.23 µg/kg AFB_1 (Figure 4A). After germination and sun-drying, the malt contained 39.23 µg/kg AFB_1. This represented a further decrease from the 51.84 µg/kg measured after germination and indicates that drying contributed to the overall reduction. The reduction may have resulted partly from the removal of contaminated material during handling and partly from physical changes in the malt during drying.



A B Legend: w = with; wo = without

Figure 4. Effects of boiling and treacle addition on AFB_1 and total aflatoxin levels during laboratory-simulated production of *Asaana*. (A) Aflatoxin levels in mash after boiling for 1 or 2 h following malt drying. (B) Aflatoxin levels in final *Asaana* product after treacle addition to the wort boiled for 1 or 2 h and subsequently sieved.

Boiling produced only a limited additional reduction. AFB_1 decreased from 39.23 µg/kg in the dried malt to 36.85 µg/kg after 1 hour of boiling and to 34.99 µg/kg after 2 hours (Figure 4A). Neither reduction was statistically significant ($p > 0.05$), and total aflatoxin concentrations followed a similar pattern. Thus, boiling for 1–2 hours under the conditions used produced at most approximately an 11% additional reduction.

The sieved wort after 1 hour of boiling contained 38.36 µg/kg total AF (Figure 4B), corresponding to an overall 72.2% reduction compared with the contaminated maize (137.96 µg/kg; Table 2). As observed for millet, the bulk of the reduction was achieved during steeping and germination rather than during boiling. Sieving may have removed some suspended or particulate material, but it did not eliminate the residual aflatoxins dissolved or distributed in the wort.

3.3.3. Effect of caramelised sugar

The addition of caramelised sugar had no appreciable effect on aflatoxin concentrations. After 1 hour of boiling, AFB_1 concentrations were 36.85 µg/kg without treacle and 38.98 µg/kg with treacle. After 2 hours, the corresponding values were 34.99 and 33.78 µg/kg. These differences were not statistically significant ($p > 0.05$), and total aflatoxin concentrations were similar.

The results indicate that caramelisation and treacle addition at levels used for acceptable colour and flavour did not substantially degrade or bind aflatoxins. The main functions of caramelised sugar in *Asaana* are therefore sensory rather than detoxification-related. This finding further demonstrates that later formulation steps cannot compensate for the use of heavily contaminated maize.

3.4 Comparison of aflatoxin reduction in *Burkina* and *Asaana*

The overall results are summarized in Table 3

Table 3 summarizes AFB_1 concentrations and percentage reductions at key processing stages for both beverages.

Table 3. Summary of AFB_1 levels and percentage reductions at key stages of *Burkina* and *Asaana* production.

Product	Stage	AFB_1 (µg/kg)	Total AF (µg/kg)	% reduction vs contaminated grain
Burkina	Contaminated millet	100.08	100.08	–
	Final product after 1 h steaming	27.87	29.76	70.3
Asaana	Contaminated maize	137.96	137.96	–
	Final product after 1 h of boiling	36.85	38.36	72.2

Burkina processing reduced total aflatoxins from 100.08 µg/kg in contaminated millet to approximately 29.76 µg/kg in the final product, representing a 70.3% reduction. *Asaana* processing reduced total aflatoxins from 137.96 µg/kg in contaminated maize to approximately 38.36 µg/kg in the sieved wort after 1 hour of boiling, corresponding to a reduction of approximately 72.2%. Despite these substantial percentage reductions, both beverages retained measurable aflatoxin residues. When starting concentrations were approximately 100-140 µg/kg, final concentrations of approximately 30-40 µg/kg remained. These concentrations exceed most international guidance values for AFB_1 in ready-to-eat products, which are often within the range of 5-10 µg/kg, with 20 µg/kg used as an upper limit in some regulatory contexts (Aflasafe, 2020; Almonds Board of, 2022; European, 2025; European, 2023). This shows that percentage reduction alone is not an adequate indicator of food safety. A process may remove approximately 70% of the toxin while still producing a beverage with an unacceptable residual concentration if the raw material is highly contaminated. The starting contamination level must consequently be considered when evaluating the effectiveness of a traditional processing method.

3.5 Integrated interpretation and food-safety implications

The findings, under experimental conditions, indicate that the most effective aflatoxin-reducing operations occurred early in both production processes. Cleaning and steeping were particularly important, while germination and subsequent sun-drying provided additional reductions during *Asaana* production.

In contrast, steaming, boiling, fermented-milk addition, and caramelised-sugar addition produced limited or statistically non-significant further reductions. The dominance of AFB_1 in the total aflatoxin profile throughout contamination and processing is also important. Because AFB_1 is both the most prevalent and the most toxic aflatoxin analogue, its concentration largely determined residual risk.

The persistence of AFB_1 after steaming and boiling is consistent with evidence that conventional heat treatments do not reliably destroy aflatoxins (Dhanshetty et al., 2021; Kumar et al., 2017; Mutungi et al., 2008; Namaumbo et al., 2023; Sabina et al., 2023). These findings have practical implications for small-scale processors. First, the prevention of contamination before processing remains the most important control measure.

Appropriate drying, moisture protection, pest control, safe storage, sorting of damaged grains, and careful raw-material selection are essential. Second, where contaminated material cannot be avoided, enhanced cleaning and adequately extended steeping may provide relatively low-cost opportunities for reducing aflatoxin levels. Third, thermal operations should be optimised primarily for microbiological safety, texture, and shelf-life rather than being relied upon to destroy aflatoxins.

The results are consistent with the principle that mycotoxin exposure should be minimised primarily through prevention and control of contamination in raw materials, with processing serving as a supplementary rather than a stand-alone intervention.

3.6 Limitations and implications for future research

The findings should be interpreted within the laboratory challenge-test design. The grains were intentionally inoculated with a single *A. flavus* isolate and incubated under conditions favourable to mould growth. Natural contamination is more heterogeneous and may involve multiple fungal strains, other fungal species, uneven toxin distribution, and co-occurring mycotoxins. The percentage reductions observed in this study therefore should not be interpreted as direct estimates of the reductions that would occur in every naturally contaminated grain lot or commercial beverage.

In addition, although the HPLC procedure was based on published immunoaffinity-column clean-up and HPLC methods, complete method-performance information was not available in the surviving records. Recovery, limits of detection, limits of quantification, calibration performance, and precision were not fully documented. This limitation does not invalidate the documented analytical observations, but it restricts independent evaluation of the analytical performance for the present dataset.

Future studies should include complete method validation or verification and should evaluate naturally contaminated maize and millet obtained from different production and storage environments. Additional work should also assess the effects of multiple fungal strains, co-occurring mycotoxins, seasonal conditions, processor-level variability, and commercial-scale production practices. Such studies would help determine whether the reductions observed under laboratory conditions are reproducible in field and artisanal settings.

4. Conclusions

Under the laboratory conditions used in this study, experimental contamination of maize and pearl millet with *Aspergillus flavus* resulted in substantial increases in AFB_1 and total aflatoxin concentrations during 28 days of incubation at 30 °C. Cleaning and steeping were the principal aflatoxin-reducing operations observed. Germination and subsequent sun-drying provided additional reductions during *Asaana* preparation. The wet germinated malt contained 51.84 µg/kg AFB_1, which decreased to 39.23 µg/kg after sun-drying. Steaming of millet granules, boiling of maize malt mash, fermented-milk addition, and caramelised-sugar addition produced limited or statistically non-significant additional reductions. When the starting grains contained high experimentally induced aflatoxin concentrations, measurable residues remained after processing. Traditional processing under the conditions evaluated should therefore not be regarded as sufficient to guarantee aflatoxin safety. The findings support the importance of controlling contamination before processing through appropriate drying, storage, sorting, raw-material selection, and hygienic handling. These conclusions are limited to the laboratory challenge-test conditions and should be validated using naturally contaminated grains and commercially produced *Asaana* and *Burkina*. Authors would recommend that good farming and storage practices should be promoted to prevent mould growth and aflatoxin contamination before grains reach processors. Small-scale processors should be encouraged to carefully sort raw materials and remove mouldy, damaged, discoloured, and insect-infested grains before processing. Clean water and properly maintained equipment should be used for cleaning and soaking grains, while germinated maize should be thoroughly dried before crushing and mashing. All processing equipment, including mills, colanders, cooking pots, sieves, bottles, and storage containers, should be properly cleaned and rinsed before use. Raw materials should be stored in cool, dry, and well-ventilated environments and kept off the floor to minimize the risk of contamination. Furthermore, simple HACCP-based procedures should be adapted and implemented in small-scale *Asaana* and *Burkina* production to improve food safety. Further studies should investigate naturally contaminated maize and millet obtained from different agroecological zones and assess the occurrence of other mycotoxins that may co-occur with aflatoxins, including fumonisins, ochratoxin A, and zearalenone. Future research should also clearly report the number of biological and analytical replicates to ensure transparency, reproducibility, and scientific reliability.

CRediT authorship contribution statement

Diana Narkie Frempong: conceptualization, methodology, and writing of original draft; Paa-Nii Torgbor Johnson: conceptualization, supervision, review, and editing; Leonardo Abormegah and Frances Nathan-Mensah: investigation and data collection. The final manuscript has been reviewed and approved by all authors.

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Declaration of competing interests

The authors declared no conflict of interest.

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AI disclosure statement

Artificial intelligence tools, including ChatGPT (OpenAI), were used to assist with language editing and enhance sentence clarity. The authors carefully reviewed and verified the entire manuscript and accept full responsibility for the accuracy, originality, and integrity of the work, including the appropriateness and scientific soundness of all statements and citations.

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