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AFLATOXIN B1 CONTAMINATION IN PORRIDGE FORMULATIONS FOR CHILDREN AND INGREDIENTS SOURCED FROM SELECTED MARKETS IN RWANDA

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ABSTRACT

Providing safe and nutritious food for children globally is a challenge. In Rwanda, an initiative was introduced in 2018 to tackle chronic malnutrition by offering fortified porridge flour to economically disadvantaged families during critical periods. However, flour-based products in the sub-region have caused public health concerns following aflatoxin B1 (AFB1) contamination. This study analyzed the levels of AFB1 in 197 porridge formulations from health centers, and 248 samples of porridge ingredients from open markets in three districts of Rwanda. Samples were collected between June 2021 and December 2022 and analyzed using ultra-high-performance liquid chromatography. Of the 197 samples from health centers, 97.9 and 89.8% exceeded the European Union maximum limits for baby foods and foods for special medical purposes (0.1 µg/kg), and cereals and nuts (2 µg/kg), respectively with an average contamination level of 2.77 µg/kg (±0.98). Only four samples exceeded the East African Community maximum limits of 5 µg/kg for AFB1. Samples from open markets that exceeded the European Union and East African community limits of 0.1 µg/kg, 2 µg/kg, and 5 µg/kg ranged from 17 to 100%, 0 to 100%, and 0 to 100%, respectively. Site and processing significantly influenced levels of AFB1 contamination in open-market samples. The mean AFB1 levels were 17.85 µg/kg (±70.25) in Burera District, 36.04 µg/kg (±85.59) in Huye District, and 9.01 µg/kg (±18.49) in Nyarugenge District. The average AFB1 levels significantly varied between different products. Peanut samples showed higher contamination levels of 56.79 and 99.08 µg/kg for grain and flour, respectively. Flour samples in general had a higher mean of 51.65 µg/kg (±105.75), compared to grain samples, 16.5 µg/kg (±44). Thus, there are potential health risks associated with chronic exposure to AFB1 in children consuming flour-based foods from health centers and open markets. Interventions to mitigate AFB1 contamination and protect children should focus on food processing practices, implementing strict quality control measures, and raising awareness among stakeholders about the risks of AFB1 in flour-based products provided to children in Rwanda and similar settings.

Key words: Flour-based porridge, contamination, aflatoxin B1, children, Rwanda

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INTRODUCTION

Safe and nutritious foods remain a challenge globally. Children are particularly vulnerable to malnutrition, which often leads to a higher incidence of diarrhea and hindered growth [1]. To ensure their optimal growth, development, and overall health, experts advise exclusive breastfeeding for infants during their first six months [2]. However, after six months, breast milk alone is insufficient to meet a child's nutritional needs [2]. In sub-Saharan Africa (SSA), flour-based porridge is the primary complementary food given to children. Unfortunately, homemade porridges may lack essential nutrients, while other alternatives, like commercially prepared formulations, are costly and out of reach for many low-income families [3].

The chronic malnutrition rate in Rwanda is high, reported at 33.5%, and affects nearly 50% of the most vulnerable children [4, 5]. In 2017, Rwanda made a significant political commitment to tackle persistent malnutrition problems in children. Consequently, in 2018, several interventions, focusing on the first 1000 days of a child were initiated. Health Centers (HCs) in thirteen districts severely affected by chronic malnutrition were empowered to provide nutritional, and health support to mothers and children from low-income households [6], specifically, those classified as “ubudehe 1” and “ubudehe 2” in the Rwandan context. “Ubudehe” is a social stratification system in Rwanda that categorizes households by socio-economic status to identify and support the needs of various population segments, particularly the most vulnerable [7]. A comprehensive understanding of the nuanced distinctions within Ubudehe categories is detailed in Table 1. The support included the issuance of fortified porridge flours during critical phases of pregnancy, lactation, and weaning (6-23 months of age).

Flour-based products in the East African region, where Rwanda belongs, have public health concerns particularly due to aflatoxins [8]. Aflatoxins (AFs) are food contaminants produced by certain fungi, especially *Aspergillus flavus*. These fungi grow on agricultural products before harvest as well as during storage and processing [9]. The degree of AFs contamination can vary depending on several factors including environmental, geographical location, farming methods, and crop susceptibility to fungal infestation [10]. Maize and groundnuts, the main ingredients of complementary foods for children in the region, are particularly prone to AFs contamination [11]. Aflatoxin B1 (AFB1), the most common and potent AF, is associated with adverse health effects in humans and livestock, including acute aflatoxicosis and even death at higher doses [12]. The most severe AFs outbreak occurred in Kenya in 2004, during which 317 cases and 125 deaths were reported [13]. Aflatoxins outbreaks have also been reported in the neighboring countries of Tanzania and Uganda [14, 15].

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The long-term effect of consuming low to moderate amounts of AFs, consistently through staple foods, is not yet fully understood. However, there is compelling evidence linking AFB1 to liver cancer development, particularly in individuals with hepatitis [16]. Unfortunately, many tropical countries, including Rwanda, face significant challenges related to hepatitis viruses [17]. The knowledge that chronic exposure to AFs can cause cancer [18], and weaken immune system function [19] has raised concerns about their potential role in the epidemiology of other health problems, including malnutrition in young children. In most foods, flour-based products are used during child weaning in Rwanda, which increases exposure to AFs [20]. Aflatoxins can also cross the placenta and affect developing fetuses [21], compromising growth in early life. Additionally, breastfeeding and dairy products expose newborns and infants to AFM1, a carcinogenic metabolite of AFB1 [22]. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) jointly recommend a maximum total AFs level of 20 parts per billion (ppb) for various products, including peanuts and maize, to guarantee safety [23]. In the United States, the Food and Drug Administration (FDA) has set similarly low levels, with a limit of 20 ppb for AFB1 [24]. The European Union (EU) followed suit with strict regulations that set limits ranging from 0.1 ppb for AFB1 in baby foods, and foods for special medical purposes to 2 ppb for processed cereals, and nuts intended for human consumption [25].

This study was designed to investigate the levels of AFB1 contamination in flour-based porridge dispensed at HCs and in flour-based porridge and its ingredients sourced from local markets. The work provides evidence regarding the safety of food products supplied as part of existing nutritional programs. Further, the current study provides a baseline to assess future interventions because there have been limited studies on AFs occurrence in Rwanda.

MATERIALS AND METHODS

Study area

The study was conducted in Rwanda, where food samples were collected from three administrative districts. Burera and Huye districts in the North and South, known as the country's food basket, were selected for this research due to their high stunting prevalence (between 33% and 50%), exceeding the national average of 33.5% [4], paradoxically despite their fertile soil. In contrast, Nyarugenge district, chosen as a control group, has stunting rates below the national target of 19% [26]. To ensure a representative sample of the local food supply, two major markets in each district were selected, including border markets with Uganda (North) and Burundi (South) to reflect cross-border trade, and Nyarugenge markets in the capital city, known for local and international products. Additionally, the study involved four health districts (HDs): Kabutare, Ruhango, Kabgayi, and Nyabihu.

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These districts were selected for their logistical convenience and accessibility, which facilitated efficient data collection. In each district, two health centers (HCs) with the highest number of staff were purposively chosen under the assumption that higher staff counts would correspond to more patients attending, ensuring a more comprehensive sampling of food products. However, three of the initially selected HCs were excluded due to a shortage of food products at the time of the study visit. The final sampled HCs were Rango in Kabutare HD, Ruhango in Ruhango HD, Kivumu in Kabgayi HD, and Kora and Bigogwe in Nyabihu HD (Figure 1).

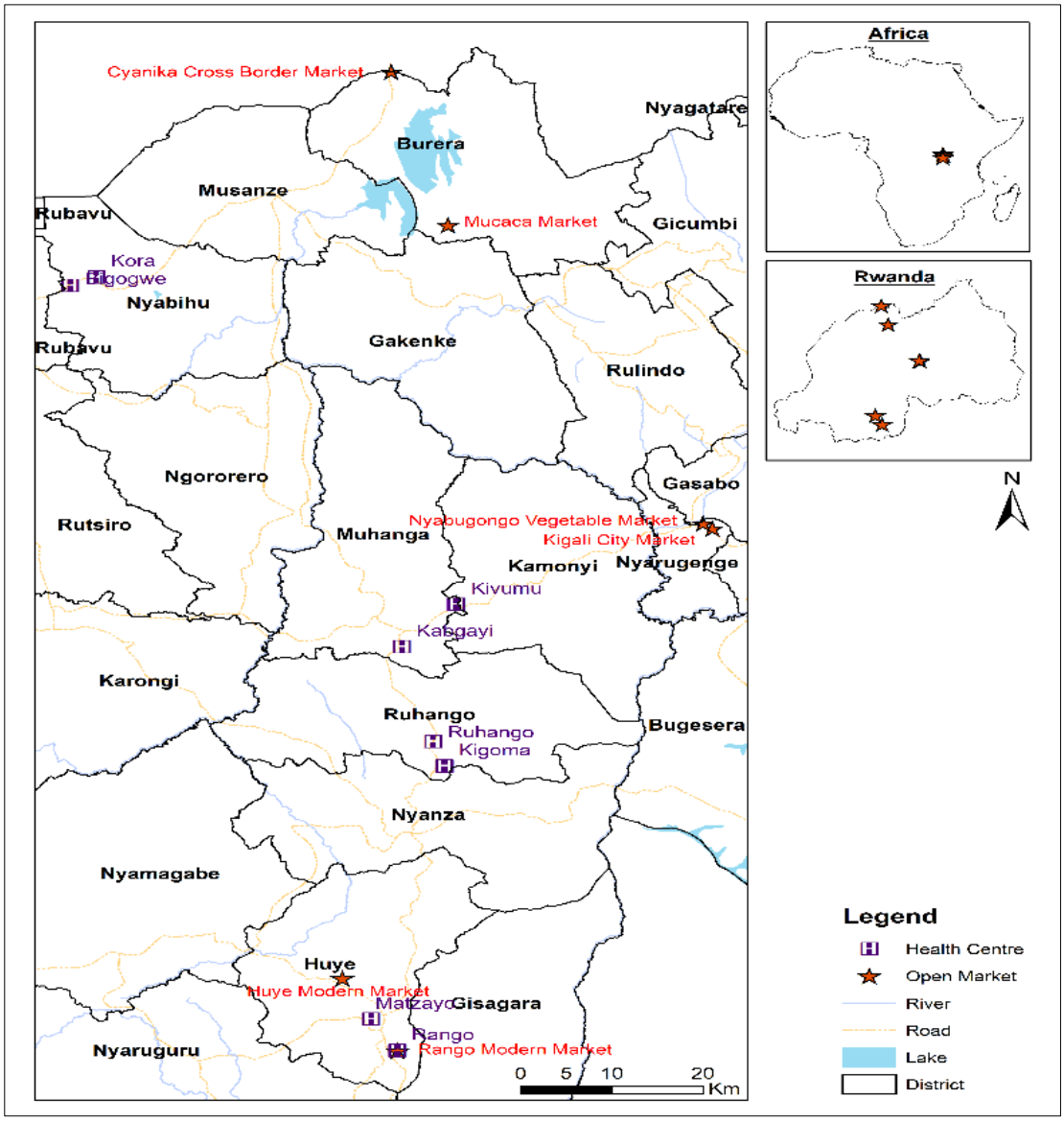

Figure 1: Map of Rwanda showing locations of sampled open markets and Health Centers

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Sampling

Sampling from open markets

Depending on the intended use, local flours are different. For this study, only flours intended for making thin porridge "Igikoma" were collected. These included maize flour, peanut flour, and mixed cereal flours (pre-packed - local brands, and unpacked - mixed by vendors). Individual cereal flours were limited and only a few were sampled - sorghum, soy, wheat, and millet (collectively referred to here as SSWM). As it was challenging for the research team to verify product categories, vendors were requested to provide information related to the flour used in the porridge formulation. Grain samples of maize and peanuts were also collected (to represent consumers who prepare flour after cleaning and sorting).

Sampling was done between June and August 2021. Visits were made to the flour-selling sections of the open market, and samples were obtained from each vendor stall containing the required ingredients. On market days, at least ten samples were purchased, following typical consumer behavior (that is selecting samples from different vendors to ensure a variety of sources). During the sample collection period, samples were not collected when markets were operating with limited vendors or closed due to the COVID-19 pandemic. Therefore, 248 samples were purchased (out of 300 targeted).

Sampling from the Health Centers (HCs)

For the purposes of this study, HC samples are also called "supplementary food", and include formulations made from a mixture of cereals enriched with essential vitamins and minerals. A special formulation was designed for malnutrition patients by adding pre-cooked cereal flours, powdered milk, sugar, and oil. Three types of formulations were considered in this study - coded as A, B, and C.

Samples were collected between June and December 2022, once a month, on the day food was distributed to beneficiaries. Each participating beneficiary was requested to provide a 200 g sample of the flour received (packets of 1.5 kg/packet were issued). Samples were uniquely labeled with identifiers that represented the health facility name, the date, and the batch number. A well-documented chain of custody was established to ensure traceability throughout the process.

Sample collection and analyses

From each market and health center, a sample of 200 g for flour and 500 g for grain was collected and sealed in a sterile plastic bag; (for pre-packed samples, the minimum size available was purchased and sub-sampled to maintain confidentiality). Samples were stored for about three months in a freezer (–20°C) at the biotechnology complex/University of Rwanda soon after collection, to halt fungal growth and additional AFs accumulation. After that, they were transported to

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the International Livestock Research Institute (ILRI) in Nairobi, Kenya, for laboratory analysis.

After removal from the freezer, grain samples were allowed to equilibrate to room temperature before being ground using a Romer Series II® subsampling mill (Romer Labs, Inc., Union, MO, USA). To prevent cross-contamination, the mill was cleaned thoroughly between samples using a vacuum (according to manufacturer's recommendation) and discarding approximately the first 100 g of every ground sample exiting the mill. Each sample underwent preparation by weighing a 5 g portion, which was then mixed with 25 ml of a 70% methanol solution (ACS grade methanol, sourced from Finar Ltd., Gujarat, India or Sigma-Aldrich St. Louis, MO, USA) and water (Milli-Q Water Purifier, Millipore, Bedford, MA, USA). This mixture was agitated at high speed (250 rpm) in a shaker (New Brunswick Scientific, Edison, NJ, USA) for 30 min. Particulate matter was allowed to settle; then, 2 ml of extract was collected in a centrifuge tube. After centrifugation at 3000 rpm for 5 min, 1 ml of extract was diluted in 1 ml 1% acetic acid in a centrifuge tube for analysis. After centrifugation at 3000 rpm for 5 min, an aliquot of about 700 µl was loaded in an umber vial. All extracts were stored in the refrigerator prior to laboratory analyses.

Samples were analyzed using ultra high-performance liquid chromatography (UHPLC system SHIMAZU) using Nexera Liquid Chromatograph LC-30AD with Nexera column oven CTO-30A coupled to a Prominence Fluorescence detector RF-20 AXS and Phenomenex Synergi 2.5u Hydro-RP 100 mm x 3.00 mm column which enables quantification between 0.05 and 500 µg/kg AFB1.

The mobile phase of the solvent system was composed of methanol 100% (solvent A) and 0.1% acetic acid nano-pure water (solvent B). The running started isocratic in the proportion of 60% (A) and 40% (B) for 1 min to wash away unwanted impurities, then a linear solvent gradient was applied from the proportion of solvent B from 0% to 100% in 6 min and remained constant at 100% B for 0.5 min and finally returned to baseline (0%) in 0.1 min and was kept at baseline for another 4.4 min for the column to equilibrate. All analyses were performed at a constant flow of 0.4 ml/min after sample injection. Five µl of each AFB1 standard (0, 0.05, 0.5, 50, 500, 1000, 2000, 3000, and 5000 ng/ml) were also injected into the system to prepare a calibration curve.

Prior to analysis, the method was validated to confirm data quality. Spike recoveries and the coefficient of variation (CV) were calculated for each AFB1 standard concentration used, with the acceptable CV level set at 5%. Certified corn and peanut reference material obtained from the Office of the Texas State Chemist (OTC) were used to assess AFs prediction accuracy. In-house analytical method

performance characteristics developed for assessing the accuracy, precision and linearity of each UHPLC run were performed to screen for data integrity. The linearity of the calibration curve was determined by calculating the regression coefficient (r^2). The minimum acceptable level for r^2 was set at 0.98. The accuracy of the method was assessed by using three different known concentrations of certified ground corn and peanut samples of AFs (OTC Aflatoxin Proficiency Testing in Eastern and Central Africa program) for varying sample dilutions and quantification ranges. The three concentrations used were 5 ppb (±40%), 40 ppb (±34%) and 273 ppb (±20%).

To assess compliance and identify areas for improvement in local food safety regulations, AFB1 contamination levels in this study were compared to maximum limits of 5 µg/kg in foodstuffs intended for human consumption within the East African Community (EAC). Comparisons were also made with the EU limits on baby foods, food for special medical purposes or processed cereals, and nuts placed on the market for the final consumer or used as an ingredient in foods (0.1 and 2 µg/kg, respectively).

Statistical analysis

Data was entered in MS Excel® and checked for errors. Analyses included descriptive statistics, analysis of variance (ANOVA), and a t-test, done in Excel. Statistical differences were judged at a 5% significance level. Each sample was run once, and the obtained AFs results were transformed into log10 normalized data prior to variance analysis.

Ethical considerations

Approval to undertake the study was obtained from the Institutional Review Board of the College of Medicine and Health Sciences University of Rwanda (Approval number: IRB/CMHS No 252//2021). We obtained permission from the respective health center administrations and communicated the purpose of the study to health center staff and formulation recipients before sample collection, seeking their consent and cooperation.

RESULTS AND DISCUSSION

Description of the sampled foods

A total of 445 samples were collected (248 from open markets and 197 from HCs). Market samples included mixed flours (26%) (locally manufactured pre-packed, and unpacked blends of different cereals and nuts prepared by vendors) and maize grain (23%), which were mainly obtained from Burera District (Northern Province). Individual cereals included whole maize flour (14%), SSWM flour (11.6%), peanut flour (15%), and peanut grain (8%). Peanut, maize, and mixed flours were found in all the three districts. Neither peanut grain nor SSWM flour

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was found in Burera District as indicated in Table 2. For supplementary foods, product A was the most frequently sampled (60%) due to its widespread distribution, followed by product B (25%) and product C (15%) as detailed in Table 3.

Aflatoxin B1 contamination in sampled foods

The percentage of samples with readings above the EU regulatory limit ranged from 17 to 100%, for baby and young children's foods (0.1 µg/kg) and from 0 to 100%, for processed cereal and nuts (2 µg/kg). The percentage with readings above the EAC regulatory limit ranged from 0 to 100% (5 µg/kg). For grain samples, 100% of peanuts and 1.7% of maize had AFB1 levels above the EAC maximum limit of 5 µg/kg. Almost all HC samples had detectable AFB1 above the EU/MLs but only 2% exceeded the EAC/MLs limits (Table 4).

The overall results of this study suggest that peanut flours and grains contributed the most to the level of AFB1 contamination in samples from open markets. All 58 peanut grain and flour samples were contaminated with AFB1 at levels exceeding the acceptable EAC limits of 5 µg/kg for peanuts. One of these samples was heavily contaminated at 493.28 µg/kg as indicated in Table 4. These findings confirm other studies in the region. For instance, a previous research work [27] found elevated total AFs levels in peanut samples from Nairobi, reaching up to 2277.1 ppb. Both studies indicate that peanuts are particularly susceptible to AFs contamination, emphasizing the need for targeted interventions, and improved agricultural practices in peanut production. Rwanda imports most of its peanuts (as evidenced by vendors in the Rwandan market referring to the peanuts by the country of their origin), which are subject to regulatory controls. In fact, three public institutions regulate AFs in Rwanda. The Rwanda Standard Board establishes national standards that align with regional and international ones. The Rwanda Inspectorate, Competition, and Consumer Protection Authority inspects unprocessed foods and feeds, while the Rwanda Food and Drug Authority oversees processed foods, and feeds to ensure compliance with AFs regulations [28]. Therefore, inadequate regulatory controls, poor storage conditions and the use of low-grade peanuts possibly contributed to the high AFB1 contamination levels recorded in this study. In a related study, an analysis of raw peanut samples that have undergone prior sorting showed significantly lower AFs levels within acceptable limits [29]. Therefore, sorting provides an effective means of decontamination and should be recommended as a prerequisite to limit the risk of AF poisoning. Some studies suggest that heat during cooking may partly remove AFs [30]. However, due to a baby's low body weight and the critical period of rapid growth needed during childhood, baby food manufacturers should avoid including

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contaminated peanuts as the risk may be too high and could have a long-lasting effect on their lives [31].

Maize flours and grains were contaminated at acceptable levels of 1.48 µg/kg, (±1.67) and 1.66.11 µg/kg, (±7.61), respectively, within local and international limits (2 µg/kg and 5 µg/kg), while only one out of the 59 samples of maize grain had a higher amount (58.54 µg/kg) above local limit for AFB1 (Table 4). These findings show the results of many efforts implemented by the Rwandan government through the Rwanda Agriculture Board (RAB) to decrease AFs levels in maize. Rwanda Agriculture Board trained farmers to use Good Agricultural Practices (GAP), such as avoiding direct contact between harvested maize and soil and using adequate methods for drying and storing. In addition, since 2019, researchers have evaluated the effectiveness of Aflasafe-RW01, a specific Aspergillus strain, in biological control in Rwanda. Maize remains a staple food and a primary ingredient in many child-oriented foods in many countries, including Rwanda [32]. Over extended periods, a substantial quantity of maize flour/meal is consumed (100 - 400 g per day), resulting in significant exposure to consumers [33]. Therefore, even low contamination levels are of concern because of chronic poisoning by AFB1. However, the occurrence of AFB1 contamination exceeding EAC/MLs is lower in this study than in most recent studies in the region. In a previous study [34], AFs were detected in 100% of the samples, with levels ranging from 2.14 to 411 µg/kg. In another study [35], the range of AFs contamination in maize and maize products was 1.6 to 86.6 ng/g.

The results in Table 4 indicate a detectable but low level of contamination (0.11 µg/kg ± 0.31) of AFB1 in sorghum, soy, wheat, and millet samples (SSWM) in this study. Even though the collected samples of SSWM were few, these results are particularly reassuring. They show that sorghum, soy, wheat, and millet in Rwanda present less aflatoxin ingestion risk for young children. The present findings reaffirm previous research indicating that SSWM grains exhibit lower susceptibility to AFs contamination when compared to other cereals [36], possibly due to a high concentration of anti-nutritional factors (ANFs). However, a study conducted in Cote d'Ivoire suggests that finger millet and sorghum may be similarly susceptible to AFs contamination as other flour-based products [37]. Several authors also found that AFs levels in nuts and cereals increased during storage but not in soybeans due to the natural presence of antioxidant-like flavonoids, which suppress AFs formation [38].

AFB1 contamination levels detected in mixed flours, averaging 4.99 µg/kg (±9.47) (Table 4), are nearly equivalent to the EAC limit of 5 µg/kg, possibly due to the presence of peanuts in some of the samples. The AFB1 levels found in mixed

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flours varied from 0.00 to 9.47 µg/kg (apart from six samples containing peanuts which exceeded the EAC/MLs). Mixed flours were the most commonly sampled of all open market products. The current study found that AFB1 contamination increased as the product complexity (the number of ingredients) increased. Based on these study findings, products with higher fortification may be more susceptible to fungal infestation and AFs contamination due to the enriched nutrient environment that supports fungal growth. However, further research is necessary to confirm this hypothesis.

The supplementary foods from HCs exhibited detectable levels of AFB1. Although the contamination levels were lower, 98% (193/197) of the samples exceeded the EU regulatory limit of 0.1 µg/kg for baby foods, and foods for special medical purposes. While these levels were slightly higher than the general EU limit of 2 µg/kg, they remained below the EAC limit of 5 µg/kg. Results in Table 4 indicate that only 2% (4/197) of the samples exceeded the EAC limit. These findings show that manufacturers of these supplementary formulations in Rwanda adhere to GMPs hence the attainment of values within the allowable limits established by regulatory bodies in the region. The potential adverse health effects of these findings on children, however, remain unclear. This situation brings to light the need for further research into the AFB1 dose-response relationship. Such studies can help policymakers to determine the appropriate regulations for food intended for children.

Aflatoxin B1 contamination in marketed food products

The mean AFB1 concentration was 17.85 µg/kg (±70.25) (median of 0.41 µg/kg) in the Burera, 36.04 µg/kg (±85.59) (a median of 2.7 µg/kg) in Huye, and 9.01 µg/kg (±18.49) (median of 0.9 µg/kg) in Nyarugenge. Peanut samples showed higher levels of contamination in all the provinces, compared to other foods sampled in the open markets (Figure. 2). The analysis of variance showed that the difference between locations was statistically significant (p=0.001). Post-hoc analyses revealed that the average AFB1 in samples from Huye was significantly higher than those from Burera and Nyarugenge (Figure. 3).

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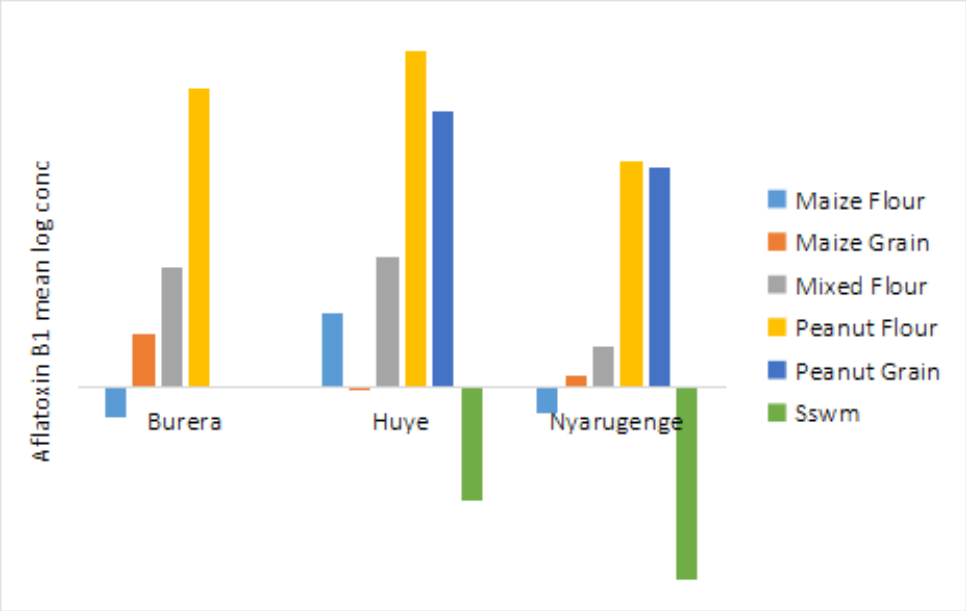

Figure 2: Mean log concentration of Aflatoxin B1 in all marketed food products by sample type and sample source, in Rwanda

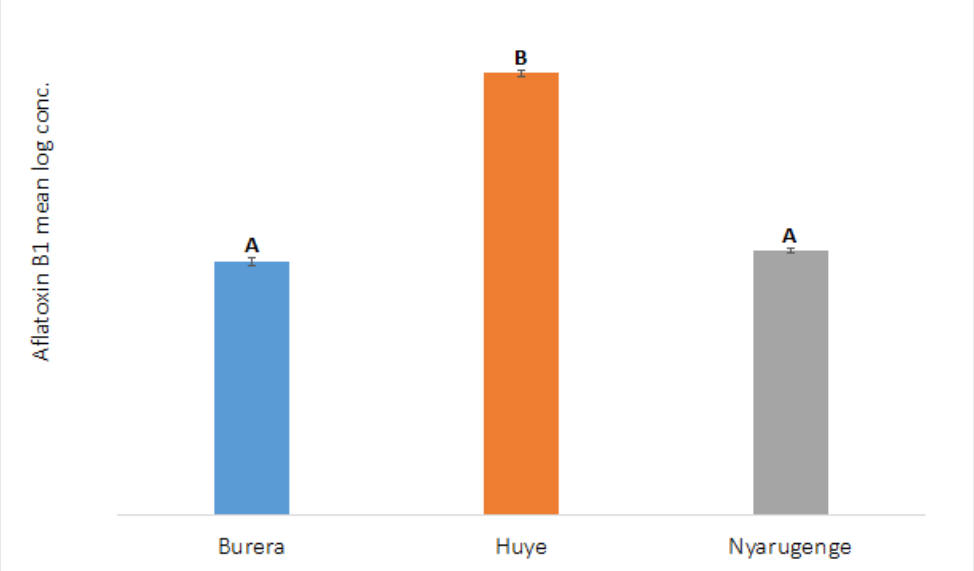

Figure 3: Aflatoxin B1 levels as the mean values of all samples from different districts

*Bars with different letters are statistically different

Location significantly influenced AFB1 contamination levels in open-market samples in our study. Even though peanut samples consistently showed higher contamination levels across all districts, the overall mean AFB1 levels varied by district, with Huye exhibiting the highest contamination levels. Several factors may contribute to this, including the market infrastructure in Huye, local climate conditions, and the types of containers used to store the products. However, it's

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important to note that these are speculative reasons, and further research is needed to fully understand the factors driving the variation in contamination levels among districts. This finding aligns with many studies conducted in the region, where significant regional differences in AFs contamination were observed in maize and peanut samples in Tanzania [30], and Rwanda [39]. Similarly, studies in Kenya reported variations in AFs contamination levels in maize grain samples across different regions [40].

Comparison of aflatoxin B1 contamination levels between mixed flours in pre-packed and unpacked samples

The 51 (unpacked) mixed flour samples had a higher mean of 5.18 µg/kg, (±10.38) (median of 1.37 µg/kg) compared to the 15 pre-packed mixtures, which had a mean of 4.31 µg/kg, (±5.51) (median of 1.32 µg/kg). However, this difference was not statistically significant (p=0.704) (Figure 4).

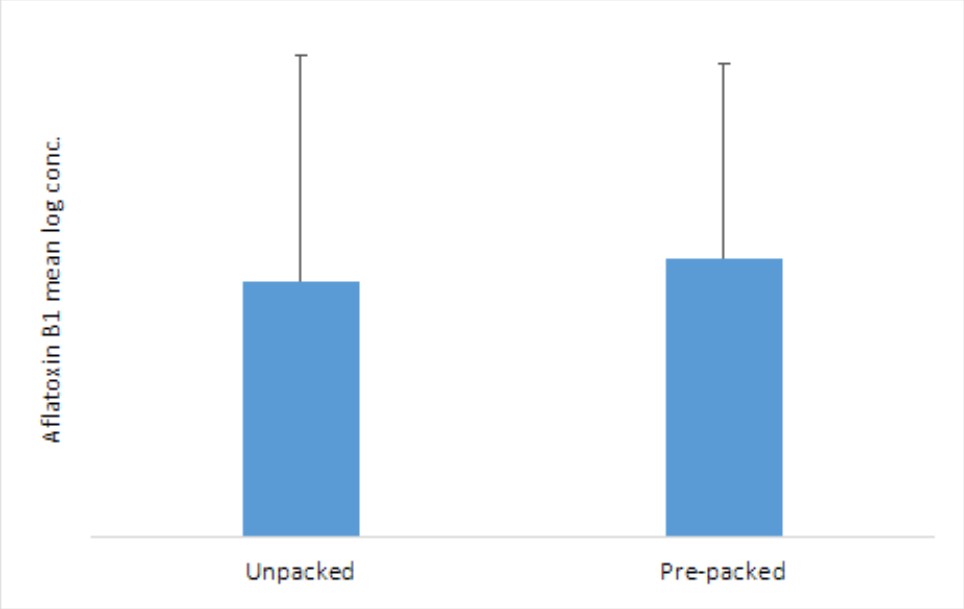

Figure 4: Aflatoxin B1 levels as the mean values of all mixed flour samples (pre-packed and unpacked) from different locations
Levels are not statistically different (p=0.704)

The study examined how packaging affects the susceptibility of mixed flour samples to AFB1 contamination. Although the raw materials undergo quality assessment for pre-packed products in Rwanda, the analysis showed no significant differences in the AFB1 levels between pre-packed and unpacked mixed flour samples, indicating that packaging the product may not significantly influence susceptibility to AFs contamination. Further research and investigation may be necessary to explore other factors contributing to AFs contamination levels in mixed flours. Findings differ from another study, which found that packaging

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significantly affected AFs contamination levels in peanut samples [41]. Other factors, such as the materials used for packaging, the quality of raw ingredients and storage conditions, may play a more crucial role in AFs contamination of food products.

Comparison between grain and flour samples

The 72 flour (maize and peanut) samples had a greater mean 51.65 µg/kg, (±105.75) (median of 4.17 µg/kg) as compared to the 78 grain (maize and peanut) samples 16.5 µg/kg, (±44) (median of 0.64 µg/kg). This difference was statistically significant at p=0.002 (Figure 5).

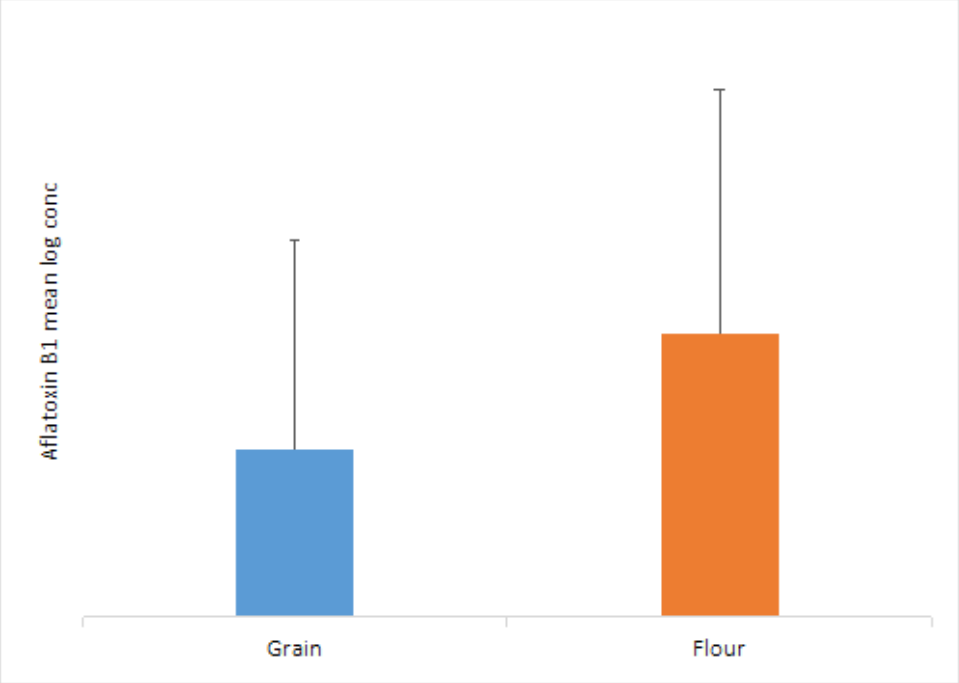

Figure 5: Aflatoxin B1 levels as the mean values of all flour and grain samples from different locations

This study compared AFB1 contamination levels between grain and flour samples. The analysis demonstrated that flour samples had significantly higher mean values than grain samples. This indicates that milling increases susceptibility of grain products to AFs contamination. The results underscore the importance of effective processing methods and quality control measures to minimize AFs contamination in food products. The significant difference in AFB1 contamination levels between grain and processed samples observed in our study is consistent with a similar study, which reported elevated AFs levels in maize products, including flour and porridge, compared to raw maize grain [30]. These findings highlight the vulnerability of processed products to AFs contamination at various stages of production and processing. However, Temba and Bakari [42] found that AFs formation in maize grain is three times more likely than in maize flour, because the

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nutrients in grain samples are less accessible to fungi, increasing nutrient stress and thereby triggering AFs production.

This study has both strengths and limitations. Its strengths lie in thoroughly examining AFB1 contamination across various food types commonly consumed by children in Rwanda. The study collected samples from open markets and health centers, resulting in a robust dataset for analysis. Additionally, the focus on regional differences enhances the understanding of contamination distribution. However, one limitation is the sample size, which may impact the representativeness of the results for the general population. Moreover, regional variations in dietary practices, and contamination sources could restrict the generalizability of the study to other regions. Therefore, when applying the results to areas with different dietary practices and contamination sources, caution should be exercised as these factors could influence AFs exposure and the outcomes.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The study's main findings reveal a widespread presence of AFB1 in various food samples, with peanuts identified as a significant source of contamination. Despite efforts to reduce AFs levels, maize products are a cause for concern due to their widespread consumption, and the risk of chronic exposure to AFB1. Additionally, supplementary foods, often used in nutrition programs and crucial for vulnerable populations, showed detectable but generally acceptable levels of AFB1, highlighting the importance of adhering to regulatory standards in food processing. Effectively addressing AFs contamination in peanut production is essential for improving public health and food safety. Maintaining regulatory standards in food processing is crucial to reduce AFs exposure and protect public health. The study recommends aligning local food regulations with EU standards, implementing targeted interventions and training programs for agricultural practices, and enhancing quality control measures in food processing to address AFs contamination effectively. Collaboration among stakeholders is also vital. Future studies should prioritize further exploration of AFs contamination sources, interventions' effectiveness, and long-term health effects to inform evidence-based policies and strategies for AFs control.

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Competing interests

The authors declare that the research was conducted without any commercial or financial relationships that might be construed as conflicts of interest.

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Table 1: The categories of households in Rwanda based on 2015 categorization [7]

Ubudehe	Characteristics
Category 1	Very poor and vulnerable citizens who are homeless and unable to feed themselves without assistance.
Category 2	Citizens who are able to afford some form of rented or low-class owned accommodation, but who are not gainfully employed and could only afford to eat once or twice a day.
Category 3	Citizens who are gainfully employed or are even employers of labor. This category included small farmers who had moved beyond subsistence farming, or owners of small and medium-scale enterprises.
Category 4	Citizens classified under this category are Chief Executive Officers of big businesses, employees who had full-time employment with organizations, industries or companies, government employees, owners of shops or markets and owners of commercial transport vehicles or trucks.

Table 2: Type, source, and number of samples collected in open markets in Rwanda, between June and August, 2021

Commodity type	Burera	Huye	Nyarugenge	Total
Maize flour	12	12	12	36
Maize grain	30	14	15	59
Mixed flours	7	31	28	66
Peanut flour	9	12	16	37
Peanut grain	0	13	8	21
SSWM[a]	0	14	15	29
Total	58	96	94	248

[a]SSWM = Sorghum, soy, wheat, and millet flour

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Table 3: Type and number of samples collected in health centers in Rwanda, between June and December, 2022

Supplementary foods[b]	Sample (n)	(%)
A	119	60.41
B	49	24.87
C	29	14.72
Total	197	100

[b]A, B, and C refer to the three supplementary foods dispensed to sick and vulnerable populations during the critical phases of pregnancy, lactation, and weaning

Table 4: Aflatoxin B1 (AFB1) content of samples collected from six open markets and five

Sample type	Sample n (% of total)	Mean AFB1 content ± SD	Median	AFB1 content Range	% that exceed the three regulatory limits		
					>0.1[c]	>2[d]	>5[e]
Maize flour	36 (14.52)	1.48±1.67	0.69	0 - 4.48	72	30.5	0
Mixed flour	66 (26.61)	4.99±9.47	1.35	0 - 63	83	40.9	24
Peanut flour	37 (14.92)	99.08±131.53	53.27	1.34 - 493.28	100	89	78
Maize grain	59 (23.79)	1.66±7.61	0	0 - 58.54	47	15	1.7
Peanut grain	21 (8.47)	56.79±70.42	29.07	16.41 - 334.42	100	100	100
SSWM	29 (11.6)	0.11±0.31	0	0 - 1.55	17	0	0
Supplementary foods	197 (100)	2.7±0.98	2.74	0 – 9.38	97.9	89.8	2

[c]AFB1 maximum limits on baby foods, food for special medical purposes and processed cereal-based foods intended for infants and young children (EU No 915/2023) [25]
[d]AFB1 maximum limits on cereals and peanuts, including processed products derived from cereals and peanuts placed on the market for the final consumer or use as an ingredient in food (EU No 915/2023)[25]
[e]AFB1 maximum limits on foodstuffs intended for human consumption in the East African Community (EAC/TF/405/2013) [43]

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