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Detection of Aflatoxins and Fungal Contaminants in Packaged and Unpackaged Powdered Milk from Selected Vendors in Katsina Metropolis

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ABSTRACT

The experiment was conducted between June and September 2021 at the Biology Laboratory of Umaru Musa Yar'adua University, Katsina, to study the occurrence of aflatoxins in packaged and unpackaged powdered milk samples using Thin Layer Chromatography (TLC) and to isolate and identify the associated fungal species. Aflatoxin contamination of powdered milk poses a significant health risk, particularly in regions where monitoring and quality control are insufficient. This study aimed to isolate and characterize aflatoxins in packaged and unpackaged powdered milk samples collected from selected vendors in Katsina State. The extracts exhibited variability in texture, predominantly watery with oil suspensions, whereas a smaller subset showed an oily appearance. Aflatoxin G was detected in both packaged and unpackaged powdered milk samples, with blue-green fluorescence observed in samples P_2, P_3, P_5, P_6, P_7, P_{10}, U_1, U_4, U_6, and U_7. Aflatoxin B was identified in samples P_8, U_2, U_3, U_8, U_9, and U_{10}, which exhibited characteristic blue fluorescence under UV light. Morphological analysis of fungal isolates revealed a predominance of *Aspergillus niger* and *Aspergillus flavus*, both characterized by non-branched conidiophores with bulbous ends and conidia arranged in a sunbeam pattern. Notably, *A. Niger* was primarily associated with packaged milk samples, whereas *A. flavus* was frequently detected in unpackaged samples. These findings highlight the health risks associated with aflatoxin contamination in powdered milk and underscore the urgent need for enhanced regulatory oversight. Strengthening surveillance systems, enforcing stringent quality control standards for both packaged and unpackaged dairy products, and aligning local food safety protocols with international standards are critical for protecting consumer health in Katsina State and beyond.

KEYWORDS: Aflatoxin, *Aspergillus niger*, contamination, public health, food safety

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1. INTRODUCTION

Aflatoxin contamination is a critical global food safety concern with far-reaching implications for human and animal health (Huang et al., 2022; Miller, 2018). Aflatoxins, produced by the fungi *Aspergillus flavus* and *A. parasiticus*, are among the most potent mycotoxins, known for their carcinogenic, immunosuppressive, and hepatotoxic effects (Alkuwari et al., 2022; Godswill Awuchi et al., 2020). Among the various aflatoxins, aflatoxin B1 (AFB1) is the most prevalent and toxic (Dai et al., 2017; Stoev, 2023; Tahir et al., 2018), whereas its metabolite, aflatoxin M1 (AFM1), frequently contaminates milk and dairy products, posing elevated health risks, particularly to children and immunocompromised individuals. Globally, AFM1 contamination in milk is widespread, with prevalence rates approaching 80%, underscoring the magnitude of this issue (Jahromi et al., 2025; Salari et al., 2020). Furthermore, aflatoxin contamination extends beyond dairy, affecting staple crops such as maize and groundnuts, which exacerbates food security concerns (Ayalew et al., 2010; FAN et al., 2021). The economic consequences are profound, with losses in the United States alone estimated at USD 500 million annually (Bennett et al., 2003; Kumar et al., 2017; Lindahl et al., 2014). In developing regions, inadequate storage conditions coupled with weak regulatory oversight exacerbate aflatoxin exposure (Anonymous, 2018). In Nigeria, exposure to aflatoxins via staple foods and dairy products remains largely unaddressed, despite evidence of AFM1 contamination in breast milk, cow milk, cheese, and yogurt (Abass et al., 2017; McMillan et al., 2018; Ojochenemi et al., 2016). Chronic aflatoxin exposure has been linked to various health issues, including cancer, liver disease, and growth disorders (Imade et al., 2021; Muhammad et al., 2019). However, there has been limited research specifically focused on powdered milk, which is widely consumed and particularly vulnerable to contamination during production, packaging, and storage. More than 250 mold species produce mycotoxins that pose significant health risks, with aflatoxins being among the most critical in terms of public health impact (Omotayo et al., 2019; Winter and Pereg, 2019). In the dairy sector, molds and yeasts contribute to the spoilage of products, including powdered milk. Notably, species such as *Aspergillus*, *Fusarium*, and *Penicillium* are recognized for their ability to produce mycotoxins, which pose substantial health risks (Abdel-Kader et al., 2024). The presence of these fungi in dairy products can lead to contamination, resulting in acute poisoning, chronic health problems, and even fatalities in extreme cases, underscoring the need for rigorous monitoring and control (Alshannaq and Yu, 2017; Greeff-Laubscher et al., 2019). Existing research emphasizes the importance of developing effective monitoring and control strategies to reduce aflatoxin contamination in food products (Bhatnagar-Mathur et al., 2015; Grace et al., 2015; Nesci et al., 2016). Various approaches have been proposed, including enhanced agricultural practices, improved storage conditions, and the use of biocontrol agents to inhibit the growth of aflatoxin-producing fungi. However, challenges persist in implementing these strategies, particularly in resource-limited regions where agricultural practices may not be effectively regulated. In response to the persistent challenge of aflatoxin contamination in food systems, emerging research highlights the potential of green-synthesized nanoparticles as an innovative mitigation strategy (Bishir et al., 2025). Their biocompatibility and broad-spectrum activity offer a promising avenue for integrating nanotechnology into food safety interventions, particularly in improving storage, processing, and contamination control. Exploring these green nanomaterials provides a forward-looking perspective for developing sustainable approaches to reduce aflatoxin risks in dairy products and other vulnerable foods. This study aimed to investigate the prevalence of aflatoxins in powdered milk, focusing on the impact of packaging on contamination levels and identifying the primary fungal species responsible for aflatoxin production in both packaged and unpackaged milk. By quantifying the frequency and concentrations of aflatoxins in these products, this study aims to provide a deeper understanding of aflatoxin contamination in dairy products. These findings will contribute to the development of enhanced safety and quality control measures for powdered milk, ultimately reducing the public health risks associated with aflatoxin exposure. The significance of this study lies in its potential to address critical public health issues and improve food safety protocols, thereby protecting consumers from the harmful effects of aflatoxins.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted in Katsina to assess the prevalence of aflatoxin contamination in powdered milk, considering the region's agricultural activities and potential exposure to mycotoxins. The socio-economic landscape of Katsina, along with its agricultural importance, makes it a crucial area for investigating food safety issues related to aflatoxins (Wycliff, 2021). Katsina is a significant Local Government Area and serves as the capital of Katsina State in northern Nigeria. Geographically, it is located at coordinates 12.9816° N latitude and 7.6223° E longitude. The city is situated approximately 260 km (160 miles) east of Sokoto and 135 km (84 miles) northwest of Kano, near the border with Niger. As of 2016, Katsina had an estimated population of 429,000

residents. The city is at the center of a vibrant agricultural region known for producing a variety of crops, including groundnuts, cotton, millet, and guinea corn. Additionally, Katsina is home to several mills that manufacture peanut oil and steel products. The demographic composition of the city is predominantly Muslim, with the majority of the population belonging to the Fulani ethnic group.

2.2. Sample location and collection

Twenty powdered milk samples were collected from randomly selected vendors in the Kofar Durbi area and the central market of Katsina between June 25th and June 29th, 2021. The sample set consisted of ten packaged powdered milk samples (typically sold in sachets) and ten unpackaged powdered milk samples (measured directly from bulk sources). To prevent contamination and maintain sample integrity, each sample was immediately placed in a separate sterile nylon bag upon collection. The packaged milk samples were labeled systematically as PM_1 through PM_{10}, and the unpackaged samples were designated as UM_1 through UM_{10}. This labeling system facilitated the clear identification and tracking of each sample throughout the study, ensuring accurate correlation during the analysis. All samples were transported immediately to the biology lab at Umaru Musa Yar'adua University, Katsina, for analysis.

2.3. Milk sample processing

Milk extracts were prepared as described by Shamsuddeen et al. (2017) with minor modifications. Briefly, 10 g of each powdered milk sample was placed in a 250 mL conical flask. Then, 50 mL of distilled water and 50 mL of methanol were added. The mixture was shaken for 15 min using an orbital shaker to facilitate toxin extraction. After shaking, the extracts were filtered to remove any solid residues, which were discarded. The resulting filtrates were concentrated using a water bath (Clifton Water Bath 14 L Digital Unstirred NE3-14DT). Finally, the concentrated extracts were transferred to bottles and stored in a refrigerator for further analysis.

2.4. Detection of aflatoxins using thin-layer chromatography (TLC)

The stationary phase for chromatography was prepared using silica-gel crystals. Sixty-five grams of silica gel were accurately weighed and mixed with 150 mL of distilled water to form a slurry, which was then used to coat the chromatographic plates. After the plates dried for 30 min, the samples were spotted onto the plates using a capillary tube (Shamsuddeen et al., 2017). The mobile phase was a methanol-distilled water mixture in a 5:1 ratio. The solvent was introduced into the chromatographic tank, and the retention factor (R*f*) was calculated using the following formula:

R*f*=Distance moved by substance/Distance moved by solvent

Aflatoxins were detected by exposing chromatographic plates to ultraviolet (UV) light. The presence of aflatoxins was indicated by blue or green fluorescence, as described by Shamsuddeen et al. (2017).

2.5. Isolation of fungi from powdered milk

All glassware and culture materials were sterilized before use. Fungi were isolated using Sabouraud dextrose agar (SDA), prepared according to the manufacturer's instructions, and autoclaved at 121°C for 15 min. Powdered milk samples were serially diluted (10^{-1} to 10^{-1}), and 0.1 mL aliquots were inoculated onto SDA plates using the spread-plate technique (Bazana et al., 2022). The plates were incubated at room temperature for seven days and monitored for fungal growth. Distinct colonies from well-diluted plates were subcultured onto freshly prepared SDA to obtain pure isolates. Fungi were morphologically identified based on colony characteristics.

2.6. Slide culture technique

For microscopic examination, fungal isolates were cultured on SDA blocks using the modified slide culture method described by Nugent et al. (2006) and Riddell, (1950). Cultures were incubated at room temperature for up to 14 days to allow for the full development of fungal features.

2.7. Microscopic examination

Fungal structures were stained with lactophenol cotton blue and examined under a Euromex iScope series microscope (1053-PLPOLRi model) fitted with an HD Ultra camera. Observations were made at 10× and 40× magnifications to assess the conidial arrangement, conidiophores, and other diagnostic features.

2.8. Data analysis

Data obtained from TLC and fungal isolation were entered into Microsoft Excel and analyzed using R statistical software (version 4.5.1). Descriptive statistics were used to summarize the frequency of aflatoxin detection, fluorescence type, and fungal species distribution. For categorical comparisons, such as the association between packaging type

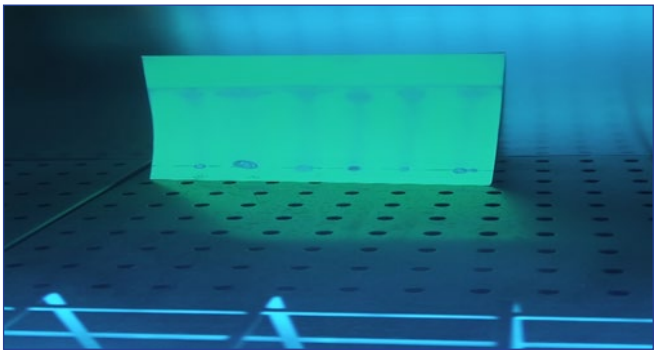

Figure 1: Thin Layer Chromatography (TLC) plate illuminated under UV light (365 nm) showing aflatoxin detection in powdered milk samples

(packaged vs. unpackaged) and aflatoxin detection (present vs. absent), a chi-square test of independence was performed. The retention factor (R*f*) values were treated as continuous data. To compare the mean R*f* values between toxin types (aflatoxin B vs. G) and packaging categories, independent samples t-tests were conducted. Statistical significance was set at $p<0.05$ for all tests.

3. RESULTS AND DISCUSSION

3.1. Nature of extracts from milk samples

The analysis of the powdered milk samples revealed a range of colors and textures in the extracts obtained after filtration and concentration via a water bath. The extracts displayed varying characteristics, with colors ranging from pale yellow to milky white. The textures varied considerably, with some samples exhibiting an oily consistency and others presenting a more aqueous or sticky oily suspension (Table 1).

3.2. Thin-layer chromatography and aflatoxin detection

The results obtained from TLC (Table 2) indicated the presence of aflatoxins in several powdered milk samples.

Table 1: Nature and color of the milk extracts

Sample (s)	Color of the extract	Texture of the extract
P_1	+++	Watery with oil suspension
P_2	++	Watery with oil suspension
P_3	++	Watery with oil suspension
P_4	++++	Oily
P_5	+	Watery with oil suspension
P_6	++	Watery with oil suspension
P_7	+	Watery
P_8	++	Watery with oil suspension
P_9	+++	Watery
P_{10}	++++	Watery with oil suspension
U_1	+	Watery
U_2	+++	Watery
U_3	+	Watery
U_4	++	Watery with oil suspension
U_5	++++	Oily
U_6	+++	Watery
U_7	+++	Watery
U_8	++	Watery with oil suspension
U_9	+++	Watery
U_{10}	+	Watery

Key- P_1 to P_{10}: packaged milk samples; U_1 to U_{10}: unpackaged milk samples; +: Whitish; ++: Pale yellow; +++: Milky; ++++: Yellowish

Table 2: Detection of aflatoxin from packaged and unpackaged milk samples

Sample (s)	DMS (cm)	S.F (cm)	R*f*	Aflatoxin	Detection
				Florescence	Aflatoxin
P_1	0.8	4.6	0.173	N. D	N. D
P_2	11.8	12.7	0.929	Blue green	Aflatoxin G
P_3	11.8	12.7	0.929	Blue green	Aflatoxin G
P_4	4.0	4.6	0.869	N. D	N. D
P_5	11.7	12.7	0.912	Blue-green	Aflatoxin G
P_6	11.4	12.7	0.897	Blue-green	Aflatoxin G
P_7	9.6	12.3	0.780	Blue-green	Aflatoxin G
P_8	9.7	11.8	0.822	Blue	Aflatoxin B
P_9	10.0	12.3	0.813	N. D	N. D
P_{10}	8.3	12.3	0.674	Blue green	Aflatoxin G
U_1	11.5	12.7	0.905	Blue-green	Aflatoxin G
U_2	11.7	12.7	0.921	Blue	Aflatoxin B
U_3	9.8	11.8	0.830	Blue	Aflatoxin B
U_4	10.0	11.8	0.847	Blue-green	Aflatoxin G
U_5	8.5	11.8	0.720	N. D	N. D
U_6	9.5	11.8	0.805	Blue-green	Aflatoxin G
U_7	9.5	11.8	0.805	Blue-green	Aflatoxin G
U_8	8.3	12.3	0.822	Blue	Aflatoxin B
U_9	10.6	12.3	0.861	Blue	Aflatoxin B
U_{10}	9.1	12.3	0.739	Blue	Aflatoxin B

Key- P_1 to P_{10}: packaged milk samples; U_1 to U_{10}: unpackaged milk samples; DMS: Distance Move by substance; S.F: solvent Front; N.D: Not detected; R*f*: retention factor

Specifically, aflatoxin was detected in seven packaged milk samples, whereas three samples showed no detectable levels. In contrast, nine unpackaged milk samples contained aflatoxins, with one sample exhibiting no detectable levels.

3.3. Morphological characteristics of isolated fungal biota

Morphological analysis of fungal colonies isolated from powdered milk samples revealed distinct features indicative of specific fungal species (Table 3). Macroscopically, four samples exhibited colonies with pin-like black growths, characteristic of *Aspergillus niger* (mold). Microscopic examination of sub-cultured isolates revealed non-branched conidiophores with bulbous ends, bearing conidia arranged in a radiating pattern resembling sun rays, thereby confirming the identification of *A. niger*. Additionally, two samples displayed colonies with pin-like green growth, consistent with *Aspergillus flavus*.

Aflatoxins were detected in a significant proportion of powdered milk samples, with 70% of packaged and

Table 3: Morphological characteristics of the fungal isolates

Sample location	Sample	Microscopic examination	Macroscopic examination	Organism
Kofar Durbi (P_1)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Black growth	Mold (*A. niger*)
Kofar Durbi (P_2)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Black growth	Mold (*A. niger*)
Kofar Durbi (P_3)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Black growth	Mold (*A. niger*)
Central market (U_1)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Green growth	Mold (*A. flavus*)
Central market (U_2)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Black growth	Mold (*A. niger*)
Central market (U_3)	Milk	Non-branched conidiophore with bulb end carries conidia like sun rays	Pin like Green growth	Mold (*A. flavus*)

90% of unpackaged samples testing positive. Among the contaminated samples, packaged milk was primarily contaminated with aflatoxin G (6/10), whereas unpackaged milk exhibited higher levels of aflatoxin B (5/10), which is a more toxic variant. However, chi-square analyses showed no statistically significant association between packaging type and aflatoxin detection (X^2=0.31, p=0.576) or between aflatoxin type and packaging ($X^2 \approx 2.78$, $p>0.20$). The retention factor (R*f*) values of aflatoxins ranged from 0.173 to 0.929, with mean R*f* values of 0.780 in packaged and 0.826 in unpackaged samples, but no significant differences were found between the two groups (t=-0.61, p=0.554). Additionally, there was no significant variation between aflatoxin B and G (t=-0.44, p=0.665).

The detection of aflatoxins in both packaged and unpackaged powdered milk emphasizes an ongoing food safety concern, with unpackaged products presenting a higher risk of contamination than packaged products. This finding is consistent with the broader challenges in developing countries, where poor storage conditions, weak regulatory enforcement, and inadequate handling practices contribute to mycotoxin exposure (Imade et al., 2021; Ojochenemi et al., 2016). The identification of *Aspergillus niger* and *A. flavus* further highlights the susceptibility

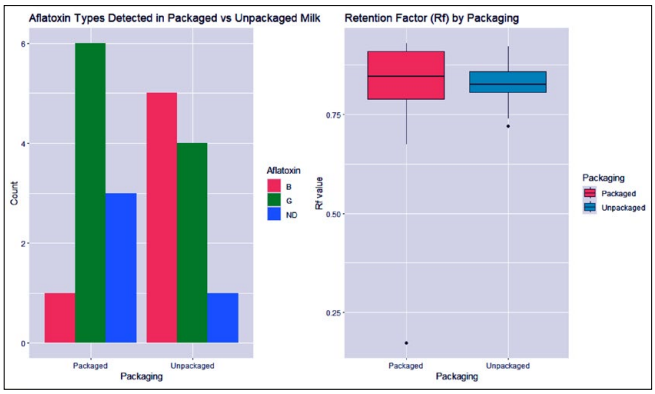

Figure 3: Aflatoxin distribution by packaging and R*f* values comparison

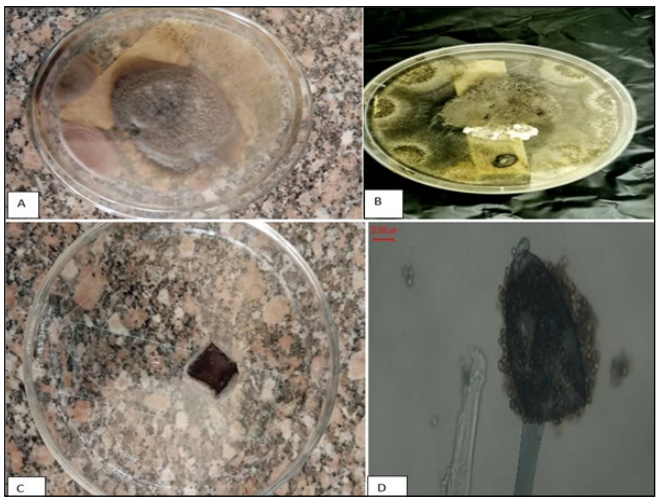

Figure 2: Morphological Characteristics of Fungal Isolates. (A&B) fungal culture plates; (C) slide culture; (D) microscopic view at 40X power

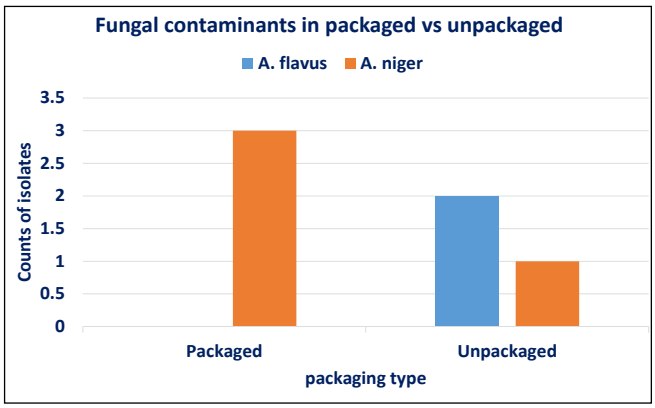

Figure 4: Fungal contaminants in packaged versus unpackaged milk

Table 4: Statistical analysis of aflatoxin detection and R*f* values in packaged and unpackaged powdered milk

Analysis	Comparison	Test statistic	d*f*	*p*-value	Mean/ proportion (Group 1)	Mean/ proportion (Group 2)	Interpretation
Chi-square test (Detection vs packaging)	Packaged vs unpackaged	X^2=0.31	1	0.5762	70% detected (Packaged)	90% detected (Unpackaged)	No significant association
T-test (Rf by packaging)	Packaged vs unpackaged	t=-0.61	10.41	0.554	0.780 (Packaged)	0.826 (Unpackaged)	No significant difference
T-test (Rf by aflatoxin type)	Aflatoxin B vs G	t=-0.44	13.36	0.665	0.833 (B)	0.848 (G)	No significant difference

of dairy products to fungal contamination, corroborating previous reports that identified these species as dominant producers of aflatoxins (Alkuwari et al., 2022).

The public health implications of aflatoxin exposure are considerable, with long-term effects such as hepatocellular carcinoma, immunosuppression, and stunted growth in children (Ullah et al., 2023). In this context, the widespread consumption of powdered milk, particularly by children, raises serious concerns. In line with global findings that report AFM1 contamination in dairy products at prevalence rates nearing 80% (Jahromi et al., 2025; Salari et al., 2020), the results of this study underscore the urgent need for improved local surveillance systems. Notably, unpackaged milk presents a higher public health risk, highlighting the critical need for stricter quality control and improved storage practices in this area.

From a regulatory standpoint, it is imperative to enforce the maximum allowable limits for aflatoxins in dairy products. Current food safety practices in Nigeria often lack systematic enforcement, undermining both consumer protection and compliance with international trade standards (Orina et al., 2021). The establishment of robust monitoring systems, the adoption of advanced detection methods such as HPLC or LC-MS/MS, and the implementation of rigorous quality assurance protocols are crucial for minimizing contamination risks.

Table 5: Chi-square test of association result for fungal isolates by packaging

Comparison	X^2 statistic	d*f*	*p*-value	Interpretation
Packaging×Fungal species (*A. flavus*, *A. niger*)	0.75	1	0.387	No significant association; *A. Niger* is more common in packaged, *A. flavus* only in unpackaged

4. CONCLUSION

This study revealed that both packaged and unpackaged powdered milk samples in Katsina State were contaminated with aflatoxins, with unpackaged samples showing higher prevalence. The detection of aflatoxins B and G, alongside *Aspergillus niger* and *A. flavus*, indicated significant public health risks. The findings highlighted the need for strengthened regulatory surveillance, improved storage practices, and public awareness to reduce exposure risks and enhance food safety in dairy products.

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