



Storage Conditions, Proximate Composition, Fungal Load and Aflatoxin Contamination of Cassava Flour Sold in Local Markets of Jos Metropolis, Plateau State, Nigeria

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ABSTRACT

Among the most widely consumed staples in Nigeria is Cassava (*Manihot esculenta*) flour, but poor drying and storage practices leave it vulnerable to colonisation by aflatoxigenic fungi. This study assessed the storage conditions, proximate composition, fungal load and aflatoxin (B1, B2, G1 and G2) content of cassava flour sold in five major markets (Terminus, Katak, Bukuru, Farin-gada and Chobe) within Jos metropolis, Plateau State, Nigeria. Five samples were collected and analysed for moisture, ash, fat, fibre, protein and carbohydrate content using standard AOAC procedures; fungal load was determined by serial dilution and culture on potato dextrose agar; and aflatoxins were quantified by thin-layer chromatography (TLC). Relative humidity (13.0–14.3%) and temperature (30.0–32.5 °C) recorded at the point of sale exceeded FAO/FDA-recommended storage thresholds for dried cassava products in all samples. Moisture content ranged from 10.15 to 17.10%, with 60% of samples above the 12% safe-storage threshold, while total fungal counts ranged from 7.67×10^3 to 56.67×10^3 CFU/g. Sixty percent of the samples were contaminated with aflatoxins B1, B2, G1 and G2, with total aflatoxin levels ranging from below the detection limit to 1.0082 ± 0.09 µg/kg (sample CK08). Correlation analysis revealed weak and statistically non-significant relationships ($P > 0.05$) between total aflatoxin level and relative humidity/temperature, moisture content, and fungal load, indicating that pre-storage handling and processing hygiene, rather than storage conditions alone, are the stronger determinants of contamination. All samples fell within the European Union (4 µg/kg), Codex Alimentarius/NAFDAC (10–20 µg/kg) and ISIRI (15 µg/kg) permissible limits, indicating no immediate acute health risk from the cassava flour sold within Jos metropolis, although routine monitoring remains advisable in view of the cumulative health effects of chronic low-dose aflatoxin exposure.

Keywords: Cassava flour; Aflatoxin; Fungal load; Thin-layer chromatography; Food safety; Jos metropolis, Nigeria

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important staple food crops in the tropics, ranking among the leading sources of dietary carbohydrate for a large share of the population of sub-Saharan Africa, Asia and Latin America (Julie *et al.*, 2009). Global cassava production reached about 278 million metric tonnes in 2018, of which Africa accounted for roughly 61%, and Nigeria alone contributed about 21.5% of world output (FAO, 2020). The crop's tolerance of drought and poor soils, low labour requirement and year-round availability make it an important buffer against food insecurity (Parmar *et al.*, 2017). Despite this importance, industrial utilisation of cassava still accounts for less than 5% of total production, and the crop continues to be processed largely by traditional methods into gari, fufu, chips, pellets and flour (Shittu *et al.*, 2016).

Cassava roots are highly perishable, with a shelf life of about 48 hours after harvest, which necessitates rapid processing into more stable dried forms such as flour (Parmar *et al.*, 2017). Traditional processing typically involves peeling, chipping or grating, fermentation and sun-drying on open trays or bare ground before milling (Westby, 2002). This open-air drying exposes the product to soil, dust and airborne fungal spores, and inadequate drying or subsequent re-wetting during humid storage can support the growth of toxigenic moulds, majorly *Aspergillus flavus* and *A. parasiticus*, together with *Fusarium* and *Penicillium* species (Adjovi *et al.*, 2015). These fungi can produce aflatoxins which is a group of difuranocoumarin secondary metabolites comprising aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) and are classified as Group 1 human carcinogens by International Agency for Research on Cancer (IARC, 2002).

Aflatoxins are of major public-health concern, because chronic dietary exposure is associated with hepatocellular carcinoma, immune suppression, and growth impairment in children, while acute high-dose exposure can cause fatal hepatotoxicity (Bhat *et al.*, 2010; WHO, 2018). Nigeria has the second-highest burden of childhood stunting in the world, and dietary aflatoxin exposure has been proposed as a contributing factor (Ebenezer & Smith, 2022). The Food and Agriculture Organization has long estimated that about 25% of the world's food crops are significantly contaminated with mycotoxins, predominantly aflatoxins, although more recent systematic reviews suggest the true global prevalence may be considerably higher (Eskola *et al.*, 2020).

Although cassava is comparatively resistant to pre-harvest aflatoxin contamination due its high moisture content and cyanogenic glucosides are thought to inhibit fungal colonisation while the root is fresh (Adjovi *et al.*, 2015) but processing into dried flour removes this natural protection and creates conditions conducive to fungal growth and toxin production during drying, transport and storage. Several studies across sub-Saharan Africa have reported aflatoxin contamination of cassava-derived products at varying levels including; Malawi and Zambia (Chiona *et al.*, 2014), Rwanda (Matsiko *et al.*, 2017), coastal and inland Kenya (Gacheru *et al.*, 2016), and Tanzania and the Republic of Congo (Manjula *et al.*, 2009). In Nigeria, recent surveys of dried cassava products and cassava-based flours have similarly detected regulated mycotoxins, including aflatoxins, although generally at levels considered low compared with maize and groundnut (Ekpakpale *et al.*, 2021; Oyesigye, *et al.*, 2024). However, some authors have questioned whether cassava supports natural aflatoxin formation at all (Wheatley, 1984; Bankole *et al.*, 2006), reflecting persistent uncertainty in the literature.

Despite this body of work, data on the fungal load and aflatoxin status of cassava flour marketed within north-central Nigeria, where the product is both dominantly produced and consumed in several forms, remain scarce. This study therefore assessed the storage conditions, proximate composition, fungal load and aflatoxin (B1, B2, G1, G2) contamination levels of cassava flour sold in local markets within Jos metropolis, Plateau State, Nigeria, and examined the relationships between storage/compositional parameters and aflatoxin contamination, to generate baseline data to inform food-safety monitoring and risk-management decisions.

2. Materials and Methods

2.1 Study Area and Sample Collection

Sampling was carried out in five major markets within Jos metropolis, Plateau State, Nigeria. These include Terminus, Katako, Bukuru, Farin-gada and Chobe markets selected for their high patronage by both domestic and industrial consumers of cassava flour. A total of ten samples (100 g each), comprising two representative samples per market, were purchased directly from vendors. Sampling followed European Commission Regulation (EC) No 401/2006 (EC, 2006) to ensure that each analytical sample represented the lot from which it was drawn. Samples were placed in sterile, tightly capped plastic containers and transported to the Chemistry Laboratory, University of Jos, where they were stored in a desiccator pending analysis.

2.2 Environmental Storage Conditions

The relative humidity and ambient temperature at each sampling point were measured and recorded at the time of purchase using a digital thermometer/humidity meter (DT-625, CEM, China), following the method of Gerelmaa *et al.* (2016).

2.3 Proximate Analysis

Moisture, ash, crude fat, crude fibre and crude protein contents were determined in triplicate following the Association of Official Analytical Chemists (AOAC, 2015) methods. Moisture content was determined by oven-drying at 105 °C to constant weight; ash content by incineration in a muffle furnace at 550 °C; crude fat by Soxhlet extraction with n-hexane; crude fibre by sequential acid to alkali digestion and ashing; and crude protein by the micro-Kjeldahl method (digestion, distillation and titration), applying the nitrogen-to-protein conversion factor of 6.25. Carbohydrate content was estimated by difference, subtracting the summed percentages of protein, fat, fibre and ash from 100 (AOAC, 2015).

2.4 Determination of Fungal Load

Total fungal load was determined by serial ten-fold dilution of 1 g of each sample in sterile distilled water (dilutions 10^{-1} to 10^{-10}). Aliquots (1 mL) from the 10^{-3} dilution were plated in duplicate on potato dextrose agar and incubated for 72 hours. Colony-forming units were enumerated and expressed as CFU/g, and pure isolates obtained by repeated sub-culturing were identified from their cultural and microscopic morphology with reference to a standard atlas for microbial identification (Barnett *et al.*, 2000).

2.5 Extraction and Determination of Aflatoxins by Thin-Layer Chromatography

Aflatoxins B1, B2, G1 and G2 were extracted and quantified following the methods of Ferreira, Reyes, and Andrade (2005), Atehnkeng *et al.*, (2008) and Odoemelam and Osu (2009), with minor modification. Twenty grams of homogenised flour was blended with 100 mL of 70% methanol for 3 minutes, transferred to a Pyrex conical flask and agitated on an orbital shaker at 400 rpm for 30 minutes. The extract was filtered through Whatman No. 1 filter paper, and 40 mL of the filtrate was partitioned with dichloromethane and distilled water; the organic (lower) phase was collected through anhydrous sodium sulfate and dried overnight. The dried residue was re-dissolved in dichloromethane and spotted (4 μ L) alongside aflatoxin B1, B2, G1 and G2 standards on calibrated thin-layer chromatography (TLC) plates, developed in diethyl ether–methanol–distilled water (96:3:1). Plates were scanned with a scanning densitometer at 360 nm; aflatoxins B1 and G1 were derivatised through a photochemical reactor to enable fluorescence detection (excitation 365 nm, emission 440 nm). Results were interpreted against the worldwide FAO/WHO regulatory benchmarks for total aflatoxin (4 ng/g) and aflatoxin B1 (2 ng/g), the Institute of Standards and Industrial Research of Iran limit (15 ng/g total; 5 ng/g B1), and applicable FAO, NAFDAC and WHO guidance values (Büyükcinal & Vural, 2007).

2.6 Statistical Analysis

All analyses were performed in duplicate or triplicate and results are expressed as mean $\pm$ standard deviation. Data were subjected to one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test for mean separation using GraphPad Prism (v.18.2), with significance accepted at $P \leq 0.05$. Pearson's correlation coefficient was used to examine relationships between storage conditions, moisture content, fungal load and total aflatoxin level using SPSS (v.13).

3. Results and Discussion

3.1 Storage Conditions of the Cassava Flour Samples

The relative humidity and temperature recorded at the point of sampling are presented in Table 1. Mean relative humidity ranged from 13.0 to 14.3%, and temperature from 30.0 to 32.5 °C. All five samples exceeded the storage relative humidity (<13%) and temperature (<24 °C) thresholds recommended by the FAO and the U.S. Food and Drug Administration for dried cassava flour, indicating storage conditions generally favourable to the growth of aflatoxin-producing fungi.

Table 1. Storage conditions of the cassava flour sample

Sample ID	Sampling Site	Relative Humidity (aw, %)	Temperature (°C)
CF12	Farin-gada Market	14.3	32.5
CB14	Bukuru Market	14.1	31.0
CC08	Chobe Market	13.4	32.5
CT08	Terminus Market	14.2	32.5
CK08	Katako Market	13.0	30.0

Recommended storage thresholds: relative humidity <13%, temperature <24 °C (FAO/FDA).

3.2 Proximate Composition of the Cassava Flour Samples

The proximate composition of the samples is presented in Table 2. Moisture content ranged from $10.15 \pm 0.01\%$ (CC08) to $17.10 \pm 0.02\%$ (CF12); 60% of the samples exceeded the FAO/FDA safe-storage threshold of 12% moisture, and none fell within the 12.0–13.0% range recommended for optimal flour stability (WHO/FAO, 2020). These values are broadly consistent with, though slightly higher than, moisture contents reported for cassava flour in South Africa 9.2–11.4% (Uchekukwu-Agua, 2015) and Lagos, Nigeria (10.38–11.58%) (Elemo *et al.*, 2014), and reflect the influence of drying method, season and storage conditions on final product moisture (Amadi & Adebola, 2008).

Crude protein ranged from $1.32 \pm 0.03\%$ (CC08) to $2.79 \pm 0.02\%$ (CK08), values that are low relative to some previously reported cassava flour (3.42–11.16%; Ogbonnaya & Hamza, 2015) and consistent with the generally poor protein quality of cassava-based products (Obboh & Akindahunsi, 2003). Crude fat (0.55–1.05%) and crude fibre (1.92–2.78%) were likewise low, while ash content (1.04–2.15%) and carbohydrate content (76.38–83.67%) fell within ranges previously reported for Nigerian cassava flour and were consistent with FAO/WHO (1998) recommended values for food carbohydrate content (65.0–80.5%). The relatively high moisture recorded in three of the five samples (CF12, CB14, CK08) is consistent with inadequate drying during processing and constitutes the principal compositional risk factor for downstream fungal and aflatoxin contamination.

Table 2. Proximate composition (%) of the cassava flour samples (mean $\pm$ SD, n = 2)

Parameter (%)	CF12	CB14	CC08	CT08	CK08
Moisture content	17.10 ± 0.02	15.71 ± 0.03	10.15 ± 0.01	11.18 ± 0.05	15.17 ± 0.01
Ash content	2.15 ± 0.07	1.25 ± 0.02	1.04 ± 0.02	2.03 ± 0.01	1.32 ± 0.03
Crude fat	0.55 ± 0.01	0.84 ± 0.08	1.05 ± 0.01	0.73 ± 0.01	0.58 ± 0.01
Crude fibre	2.14 ± 0.05	1.92 ± 0.02	2.78 ± 0.02	2.17 ± 0.01	2.68 ± 0.03

Parameter (%)	CF12	CB14	CC08	CT08	CK08
Crude protein	1.68 ± 0.02	1.76 ± 0.07	1.32 ± 0.03	1.56 ± 0.02	2.79 ± 0.02
Carbohydrate	76.38 ± 0.08	78.51 ± 0.04	83.67 ± 0.04	82.33 ± 0.05	77.44 ± 0.09

SD = standard deviation of duplicate analysis (n = 2). Method: AOAC (2015).

3.3 Fungal Load of the Cassava Flour Samples

Total viable fungal counts (Table 3) ranged from $7.67 \pm 0.88 \times 10^3$ CFU/g (CC08) to $56.67 \pm 4.33 \times 10^3$ CFU/g (CT08), a highly significant difference across samples (LSD = 8.64; $P < 0.0001$). The variation in fungal load across samples is consistent with differences in initial moisture content, drying efficiency, and permeability of the packaging materials used by vendors to atmospheric oxygen, carbon dioxide and water vapour (Uchechukwu-Agua, 2015; Ogbonnaya & Hamza, 2015). Notably, high total fungal counts did not necessarily translate into high aflatoxin levels (see Section 3.5), underscoring that total fungal load is a poor proxy for aflatoxigenic risk since most contaminating fungi including many *Aspergillus*, *Penicillium* and *Fusarium* species are non-toxigenic.

Table 3. Total fungal counts in the cassava flour samples

Sample ID	Total Fungal Count (CFU/g × 10 ³)
CF12	53.33 ± 2.60
CB14	15.33 ± 2.33
CC08	7.67 ± 0.88
CT08	56.67 ± 4.33
CK08	37.33 ± 3.48

LSD = 8.64; $P < 0.0001$ (one-way ANOVA, Duncan's Multiple Range Test).

3.4 Aflatoxin Levels in the Cassava Flour Samples

Aflatoxins B1, B2, G1 and G2 were quantified by TLC (Table 4; Figure 2) and Sixty percent of the samples (CF12, CB14 and CK08) tested positive for all four aflatoxin types. Sample CC08 and CT08 aflatoxin levels were below the instrument detection limit, consistent with their comparatively low moisture content and, by inference, better drying and storage hygiene. Total aflatoxin levels among the positive samples ranged from 0.5527 ± 0.06 µg/kg (CF12) to 1.0082 ± 0.09 µg/kg (CK08), which had the highest contamination of all samples analysed. Aflatoxin G2 was consistently the dominant type detected, followed by B2, with B1 and G1 present only at trace levels which is in consistent with co-occurring activity of both *Aspergillus flavus* (B-type aflatoxins) and *A. parasiticus* (both B- and G-type aflatoxins) reported for cassava products in some similar studies (Adjovi *et al.*, 2015).

Table 4. Levels of aflatoxins B1, B2, G1, G2 and total aflatoxin in the cassava flour samples (µg/kg)

Sample	AFB1	AFB2	AFG1	AFG2	Total AF
CF12	0.0233 ± 0.001	0.1367 ± 0.01	0.0468 ± 0.01	0.3459 ± 0.04	0.5527 ± 0.06
CB14	0.0154 ± 0.001	0.1866 ± 0.01	0.0216 ± 0.03	0.3461 ± 0.03	0.5697 ± 0.07
CC08	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
CT08	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
CK08	0.0213 ± 0.001	0.1147 ± 0.04	0.0363 ± 0.01	0.8359 ± 0.04	1.0082 ± 0.09

LSD = 0.01; $P < 0.0001$. Method: thin-layer chromatography (TLC).

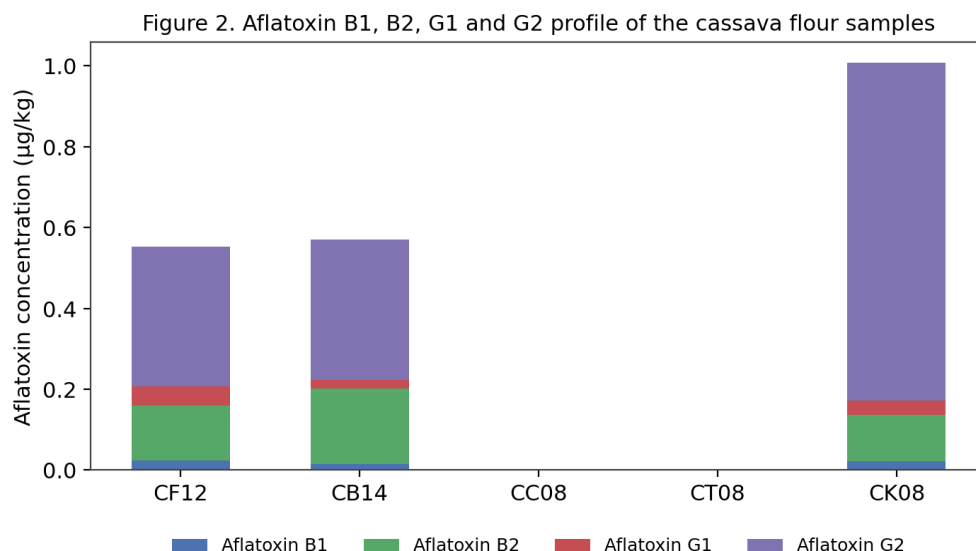


Figure 2 shows the relative contribution of each aflatoxin congener to total contamination across the samples.

None of the samples exceeded the WHO/FDA regulatory benchmark of 20 µg/kg (Kaaya & Kyamuhangire, 2006), the European Union limit of 4 µg/kg total aflatoxin for ready-to-eat food, the Codex Alimentarius/NAFDAC limit of 10 µg/kg for processed foods (Atanda, 2005), or the ISIRI limit of 15 µg/kg total aflatoxin (5 µg/kg for AFB1). The overall 60% contamination incidence recorded in this study is lower than the 80% incidence reported for cassava flour by Osuret *et al.*, (2015) in Kampala, Uganda, and lower than levels reported in parts of Malawi, Zambia and the Democratic Republic of Congo (Chiona *et al.*, 2014; Mupunga *et al.*, 2014; Kamika *et al.*, 2014), but higher than the non-detectable-to-low incidence reported for gari and high-quality cassava flour some other regions in Nigeria (Oyesigye *et al.*, 2024). It is also broadly consistent with reports that fresh cassava roots are intrinsically resistant to aflatoxin formation due to their high moisture content and cyanogenic glucosides, with contamination arising primarily during post-harvest drying and storage rather than in the field (Adjovi *et al.*, 2015; Guchi, 2015).

3.5 Relationships Between Storage Conditions, Moisture Content, Fungal Load and Total Aflatoxin Level

The relationships between relative humidity, temperature and total aflatoxin level are illustrated in Figure 1 and summarised in Table 5. Contrary to expectation, sample CK08 which recorded the highest total aflatoxin level (1.0082 µg/kg) had the lowest relative humidity (13.0%) and temperature (30.0 °C) among the five samples, while CF12 and CB14, sampled under comparatively higher humidity and temperature, showed lower aflatoxin levels. A simple linear regression of total aflatoxin against relative humidity across the samples was weak and non-predictive ($AFL = 4.2402 - 0.2764 \times \text{humidity}$; $R^2 = 0.134$), and Pearson correlation analysis of fungal load against total aflatoxin level likewise showed only a weak, statistically non-significant positive association ($r = 0.241$; $P = 0.680$).

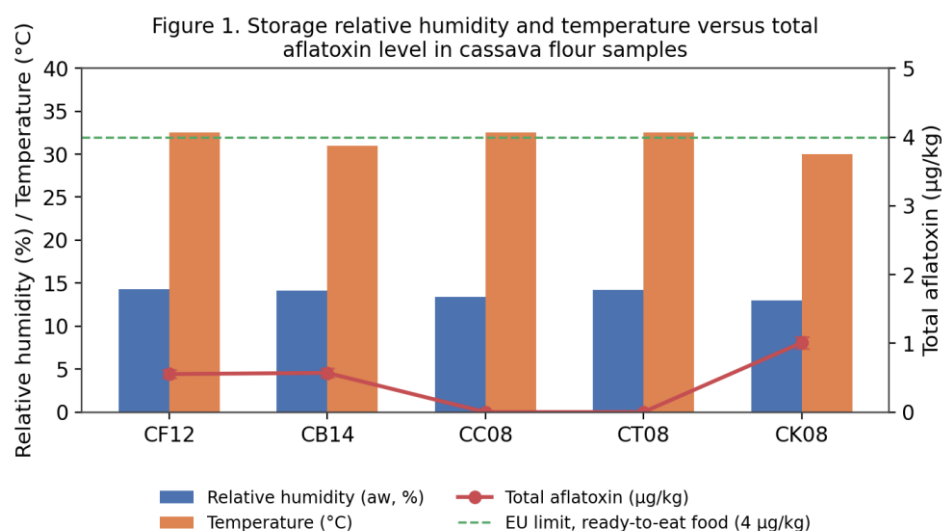


Figure 1. Storage relative humidity and temperature versus total aflatoxin level in the cassava flour samples, with the EU regulatory limit (4 µg/kg) shown for reference.

Table 5. Summary of correlation indicators between storage/compositional parameters and total aflatoxin level

Relationship	Statistic	Interpretation
Relative humidity vs total aflatoxin	$R^2 = 0.134$	Weak, non-predictive linear relationship
Total fungal count vs total aflatoxin	$r = 0.241$; $P = 0.680$	Weak positive correlation; not significant ($P > 0.05$)
Moisture content vs total aflatoxin	Threshold effect near 12% MC	No aflatoxin detected below $\approx 12\%$ moisture; above this, the relationship was non-linear

This absence of a strong, consistent linear relationship suggests that storage humidity and temperature alone do not fully explain the contamination pattern observed, and that pre-storage factors including the extent of initial fungal contamination during field drying, the specific proportion of toxigenic (versus non-toxicogenic) *Aspergillus* strains present, and handling hygiene during processing are likely stronger determinants of aflatoxin outcome than storage conditions measured at the point of sale (Hell *et al.*, 2000). This is consistent with the general understanding that aflatoxin biosynthesis, favoured by water activity above about 0.82 and temperatures of 25–35 °C, ultimately depends on the presence of aflatoxigenic strains and substrate suitability, not moisture and temperature alone (Bhatnagar, *et al.*, 2006). A comparable threshold effect was observed for moisture content and no aflatoxin was detected in the two samples with moisture below approximately 12% (CC08, CT08), while the three samples above this threshold all tested positive, although the relationship above the threshold was not strictly proportional (Table 5).

Therefore, while the total aflatoxin levels recorded in this study remained below all applicable regulatory limits, the finding that the most heavily contaminated sample (CK08) does not correspond to the sample with the most extreme storage conditions reinforces the need for aflatoxin testing as a direct food-safety measure, since indirect indicators such as ambient humidity, temperature or total fungal counts cannot reliably predict contamination risk (Osuret *et al.*, 2015). Because aflatoxins bioaccumulate and there is no established safe threshold of human exposure (Liu & Wu, 2010), continuous monitoring of cassava flour in Jos metropolis markets remains warranted, particularly given the potential contribution of chronic low-dose exposure to child growth impairment in a population already carrying a high national burden of stunting (Ebenezer & Smith, 2022; Gong, Hounsa, *et al.*, 2004).

4. Conclusion

This study assessed the storage conditions, proximate composition, fungal load and aflatoxin contamination of cassava flour sold in five major markets within Jos metropolis, Plateau State, Nigeria. Storage, relative humidity and temperature at the point of sale exceeded FAO/FDA recommended thresholds in all samples, and 60% of samples exceeded the recommended safe moisture threshold for dried cassava flour. Total fungal counts varied widely ($7.67\text{--}56.67 \times 10^3$ CFU/g) without a consistent relationship to aflatoxin contamination. Sixty percent of the samples were contaminated with aflatoxins B1, B2, G1 and G2, with total aflatoxin levels up to 1.0082 ± 0.09 µg/kg, all of which remained below the European Union, Codex Alimentarius/NAFDAC and ISIRI regulatory limits. Correlation analysis showed only weak and statistically non-significant relationships between total aflatoxin level and storage humidity/temperature, moisture content and fungal load, suggesting that pre-storage handling and processing hygiene are more influential determinants of contamination than storage conditions alone. On the balance of evidence, the cassava flour sold within Jos metropolis markets at the time of sampling did not pose an immediate acute health risk, but the detection of aflatoxins in the majority of samples, together with the known cumulative toxicity of chronic low-dose exposure, justifies continued surveillance, public sensitization, strict regulatory enforcement, proper agricultural and processing practices, aflatoxin management plans and warrant further research for in depth risk characterization analysis.

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