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Authors
Kandhe, Carmel Gabha'bey
Kaba, Isaac Elaka
Mukanya, Jonathan Mpoyi
et al.

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Occurrence of aflatoxins in maize, groundnuts, and cassava from retail markets in Kinshasa, Democratic Republic of the Congo

Carmel Gabha'bey Kandhe [a,1], Isaac Elaka Kaba [b,*,1], Jonathan Mpoyi Mukanya [c,d,e], Dep Enoju Enofo [c], Merveille Ntumba Mbombo [c], Blaise-Pascal Ilunga Muntokole [c], Paulin Kapepula Mutwale [c,d], Mulunda Mwanza [f], Nadege Kabamba Ngombe [c,d,**]

[a] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC, 29208, United States
[b] Department of Chemistry, College of Chemistry, University of California, Berkeley, Berkeley, CA, 94720, United States
[c] Centre d'Etudes des Substances Naturelles d'Origine Végétale (CESNOV), Faculty of Pharmaceutical Sciences, University of Kinshasa, B.P. 212, Kinshasa, XI, Congo
[d] Centre de Recherche en Nanotechnologies Appliquées aux Produits Naturels (CReNAPN), Faculty of Pharmaceutical Sciences, University of Kinshasa, B.P. 212, Kinshasa, XI, Congo
[e] Center for Chemico- and Bio-Medicinal Research (CCBR), Department of Chemistry, Faculty of Sciences, Rhodes University, PO Box 94, Grahamstown, Eastern Cape, South Africa
[f] Department of Animal Health, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho, 2735, South Africa

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ABSTRACT

Aflatoxin contamination of staple food commodities is one of the most critical food safety threats in sub-Saharan Africa. Yet, systematic surveillance data from the Democratic Republic of the Congo (DRC) remain exceedingly scarce. In this study, we assessed the prevalence of aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) in 43 food samples comprising maize (*Zea mays* L.) raw grain (n = 18), cassava (*Manihot esculenta* Crantz) raw tuber (n = 11), and groundnut (*Arachis hypogaea* L.) raw grain (n = 14), collected from two retail markets in Kinshasa, DRC. Analyses performed by reverse-phase HPLC coupled with fluorescence detection and Kobra® electrochemical post-column derivatization revealed the presence of aflatoxins in 41 of 43 samples, with AFB_1 being the most prevalent aflatoxin (95.35%), followed by AFB_2 (55.81%), AFG_1 (44.19%), and AFG_2 (34.88%). Total aflatoxin concentrations ranged from below the detection limit to 191.20 μg/kg, with a mean of 16.40 μg/kg. Most samples exceeded the EU AFB_1 regulatory limit of 2 μg/kg and the EU total aflatoxin limit of 4 μg/kg. Temperature and relative humidity recorded across eight food storage depots at both retail markets ranged from 29.60 to 30.90 °C and 34.00 to 56.00%, respectively, and serve as indicative of the prevailing storage environment, which may contribute to *Aspergillus* proliferation and aflatoxin biosynthesis at retail markets in Kinshasa. Future work that combine dietary exposure assessment, quantitative risk modeling, and multi-mycotoxin profiling across Kinshasa and all provinces of the DRC is needed to fully characterize the public health burden and inform evidence-based intervention strategies.

1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* that contaminate agricultural commodities before harvest, during harvest, or throughout post-harvest storage and distribution (Cai et al., 2020; Sinkel et al., 2018). Their capacity to cause acute and chronic toxicity in humans and animals, combined with their remarkable thermal and chemical stability, makes them among the most dangerous food contaminants of global significance (Lee et al., 2015). Globally, mycotoxins are estimated to affect 25 to 40% of all agricultural products annually, imposing a burden that extends from field-level crop losses to population-level health consequences (Myers et al., 2017; Peles et al., 2021). The economic cost of mycotoxin contamination in sub-Saharan

* Corresponding author. Department of Chemistry, College of Chemistry, University of California, Berkeley, Berkeley, CA 94720, United States.
** Corresponding author. Centre d'Etudes des Substances Naturelles d'Origine Végétale (CESNOV), Faculty of Pharmaceutical Sciences, University of Kinshasa, B. P. 212, Kinshasa, XI, Congo.
E-mail addresses: isaac_kaba@berkeley.edu (I.E. Kaba), Nadegengk@gmail.com (N.K. Ngombe).
[1] Authors contributed equally.

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Africa alone has been estimated at several hundred million US dollars per year in lost agricultural trade and productivity (Jallow et al., 2021).

Among all known mycotoxins, aflatoxins represent the greatest public health and economic concern worldwide. Produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxins colonize a wide range of agricultural commodities, including maize, groundnuts, tree nuts, cottonseed, and spices, particularly in warm and humid environments such as sub-Saharan Africa and East Asia (Zhang et al., 2018). The four naturally occurring aflatoxins most commonly encountered in food, namely B_1, B_2, G_1, and G_2, are named for their blue (B) or green (G) fluorescence under ultraviolet light and their characteristic chromatographic separation by thin-layer chromatography (Ok et al., 2016). AFB_1 is the most biologically potent: it is classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (Jallow et al., 2021) and is the most prevalent aflatoxin detected in contaminated agricultural commodities worldwide. At the molecular level, AFB_1 is metabolically activated by hepatic cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4, to yield the AFB_1-8, 9-*exo*-epoxide. This highly reactive electrophile forms covalent adducts with guanine residues in genomic DNA, generating AFB_1-N7-guanine adducts that, if not repaired, lead to G→T transversions in the TP53 tumor suppressor gene, the molecular signature of aflatoxin-induced hepatocellular carcinoma (HCC) (Lee et al., 2015). The association between chronic dietary exposure to AFB_1 and HCC is especially pronounced in sub-Saharan Africa, where aflatoxin exposure co-occurs with a high prevalence of hepatitis B virus infection (Jallow et al., 2021).

In the Democratic Republic of the Congo (DRC), food contamination with aflatoxins represents a context of particular concern requiring public health surveillance, given its combination of a high-risk food supply environment and a near-total absence of systematic food safety monitoring. With an estimated 33.4 million people facing acute food insecurity as of 2020 and 57.8% of the population experiencing poor or limited food consumption (Kandhe et al., 2026; Kasongo et al., 2024; Medina et al., 2017), the DRC depends critically on domestically produced and traded staple food commodities. Three main crops that form the nutritional backbone in Kinshasa, the capital of DRC, with an estimated metropolitan area population of 18,552,800 as of 2026 (Macrotrends, 2026), are maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), and groundnuts (*Arachis hypogaea* L.). These three commodities are cultivated across a geographically vast and climatically diverse supply catchment area, reaching the capital through supply chains that involve extended transit times of days to weeks under wholly uncontrolled temperature and humidity conditions (Bervis et al., 2021). Kinshasa, with mean daily temperatures typically in the range of 25 to 32 °C and a bimodal rainfall pattern, creates conditions that closely mirror those shown experimentally to maximize *Aspergillus* proliferation and aflatoxin biosynthesis (Liu et al., 2017; Yunes et al., 2020). Despite this high-risk environmental context, research on aflatoxin contamination of food commodities marketed in the DRC remains remarkably rare (Kasongo et al., 2024). For example, cassava, which is one of the most consumed staples throughout the country, has been mostly overlooked in the national and regional mycotoxin literature, despite being a potential vehicle of daily dietary aflatoxin exposure for consumers. We therefore conducted a systematic assessment of aflatoxin contamination in maize (*Zea mays* L.) raw grain (n = 18), cassava (*Manihot esculenta* Crantz) raw tuber (n = 11), and groundnut (*Arachis hypogaea* L.) raw grain (n = 14), collected from two retail markets in Kinshasa. Specifically, we determined the prevalence and quantitative distribution of AFB_1, AFB_2, AFG_1, and AFG_2 in the three commodities, determined the proportion of samples that exceeded the EU regulatory limits used as reference benchmarks in DRC food safety assessments for AFB1 (2 μg/kg) and total aflatoxins (4 μg/kg) in food though no domestically enacted statutory mycotoxin limits currently exist in the DRC. Lastly, we recorded mean daily temperature and relative humidity conditions in food storage depots at both retail markets over a 30-day monitoring period to assess their relevance to the risk of ongoing aflatoxin accumulation in stored commodities.

2. Materials and methods

2.1. Materials

2.1.1. Study commodities and study area

The study was conducted in Kinshasa, the capital city of the DRC, with an estimated metro area population of 18,552,800 as of 2026 (Macrotrends, 2026). Two high-volume retail food markets were selected as the sites of this study: the Liberté market, locally known as "Wenze Ya Bitabe" (GPS: 4° 23′ 33″ S, 15° 23′ 02″ E [−4.3925°, 15.3839°]), and the Ngaba market (GPS: 4° 22′ 32″ S, 15° 18′ 47″ E [−4.3756°, 15.3131°]), both among the most important commodity trading hubs supplying the general population of Kinshasa. Three commodity types studied were maize (*Zea mays* L.) raw grains, cassava (*Manihot esculenta* Crantz) raw tubers, and groundnut (*Arachis hypogaea* L.) raw grains (see Supporting Information Fig. S1), which are known as the three most widely consumed staple foods in Kinshasa and are recognized for their susceptibility to aflatoxin contamination (Kasongo et al., 2024; Okori et al., 2022).

2.1.2. Chemical reagents and reference standards

Certified aflatoxin reference standards for AFB_1, AFB_2, AFG_1, and AFG_2 were purchased from Trilogy Analytical Laboratory (Washington, DC, USA). Acetonitrile (HPLC-grade, LiChrosolv®, Merck KGaA, Darmstadt, Germany) was used for standard preparation. Sodium chloride (NaCl; analytical grade), methanol (HPLC-grade), and potassium bromide (KBr; analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.4) was prepared from PBS tablets (Sigma-Aldrich). Easi-Extract® aflatoxin immunoaffinity columns (R-Biopharm Rhône Ltd., Glasgow, UK) were used for sample clean-up. Nitric acid (65%, v/v; analytical grade) was purchased from Merck KGaA. All water used was ultrapure (18.2 MΩ/cm; Milli-Q, Millipore, Billerica, MA, USA).

2.2. Sample collection and study design

A total of 43 composite food samples were randomly collected from eight storage depots between 15 and 20 August 2023 at the two study markets (Liberté and Ngaba markets): 18 maize samples, 11 cassava samples, and 14 groundnut samples. Samples were collected from randomly selected stalls and storage depots at each market. Each composite sample was assembled from five individual sub-samples (each approximately 100 g) taken from different positions within a given lot (surface, middle depth, and base), then combined and homogenized by thorough manual mixing to yield a representative working sample of at least 500 g per composite. This approach is consistent with Codex Alimentarius guidance for aflatoxin sampling in cereals and oilseeds (Codex Alimentarius Commission, 2019). To facilitate market-level comparisons, samples were coded according to the market of origin (L: Liberté; N: Ngaba) and commodity (M: maize, A: groundnuts [from *arachide* in French], Ma: cassava [from *manioc*]). Immediately after collection, each sample was placed in a clean, labelled, sealed polyethylene bag, with information on the sample code, market of origin, declared place of origin, and collection date and time. Samples were transported on ice to the *Centre d'Etudes des Substances Naturelles d'Origine Végétale* (CESNOV), Faculty of Pharmaceutical Sciences, University of Kinshasa, and stored at 4 °C pending analysis. The study was conducted on commercially available food commodities only and did not involve human or animal subjects.

2.3. Extraction of aflatoxins

Aflatoxins were extracted from all samples using immunoaffinity chromatography (IAC), following the Easi-Extract® manufacturer

protocol with minor modifications as described by Adetunji et al. (2014). Briefly, 5 g of NaCl was thoroughly mixed with a 50 g portion of each finely ground, homogenized sample in 100 mL of 80% (v/v) aqueous methanol and extracted by overhead blending at high speed for 5 min. The resulting slurry was filtered through Whatman No. 1 fluted filter paper (Whatman, Maidstone, UK). A 2 mL aliquot of the clarified filtrate was transferred to a 50 mL polypropylene tube, diluted with 14 mL of phosphate-buffered saline (PBS), and passed through a pre-conditioned IAC column at a controlled flow rate of 1 drop/s. The column was then washed with two successive 10 mL aliquots of PBS to remove matrix interferences. Bound aflatoxins were eluted with 1 mL of HPLC-grade methanol (100%, v/v) into a glass vial. Eluates were stored at −20 °C in the dark until chromatographic analysis, performed within 48 h of extraction.

2.4. Calibration standards and method validation

Matrix-matched calibration curves were constructed by spiking certified aflatoxin-free blank maize extracts, whose freedom from aflatoxins was verified by prior HPLC analysis, with the four aflatoxin reference standards at four concentration levels (0, 0.002, 0.02, and 0.20 μg/kg for AFB_1 and AFG_1, and 0, 0.0005, 0.005, and 0.050 μg/kg for AFB_2 and AFG_2) (see Supporting Information Fig. S2). Linearity was assessed by linear regression; a coefficient of determination (R^2) of ≥0.99 was required for acceptance. The limit of detection (LOD) and limit of quantification (LOQ) for each analyte were determined individually as the concentration yielding signal-to-noise ratios of 3 and 10, respectively, using standard solutions injected at progressively decreasing concentrations (Adetunji et al., 2014). Samples with analyte responses above the upper limit of the calibration range were diluted 10- to 1000-fold with PBS and re-injected until analyte signals fell within the linear dynamic range. Method accuracy was evaluated by calculating mean recoveries from blank maize matrix spiked at two low fortification levels (0.05 and 0.10 μg/kg), performed in triplicate (n = 3 per level). These levels were selected to characterize recovery in the low-concentration region near the limit of detection and quantification, which is the range most relevant to the detection-frequency and prevalence estimates that constitute the primary aim of this occurrence survey. Samples with analyte concentrations exceeding the upper calibration limit were not measured directly at high concentration but were diluted into the validated linear range and re-injected, so that quantification of highly contaminated samples relied on the same validated in-range analytical response. Recovery at the EU regulatory maximum levels (AFB_1, 2 μg/kg; total aflatoxins, 4 μg/kg) was not assessed by direct fortification at those concentrations, which is a limitation of the validation. All recovery values were within acceptable ranges of 70-110% specified by AOAC Official Method 991.31 and consistent with the performance principles of Commission Implementing Regulation (EU) 2023/2782. The method LOD and LOQ, expressed at the matrix level in μg/kg of the original food sample, were 0.05 μg/kg and 0.17 μg/kg, respectively, for all four analytes (AFB_1, AFB_2, AFG_1, AFG_2) and all three commodity matrices (maize, groundnuts, and cassava). These values are consistent with the signal-to-noise ratios obtained from spiked matrix chromatograms and with the LOD of 0.05 μg/kg. A method performance summary, including matrix-specific LOD, LOQ, mean recovery (%), and linearity (R^2) for each analyte, is provided in Table S5 of the Supporting Information.

2.5. HPLC-FLD/Kobra® instrumentation and chromatographic conditions

All chromatographic analyses were performed on a Shimadzu Prominence HPLC system comprising an LC-20AT isocratic pump, a DGU-20A5R online degasser, and an RF-20A fluorescence detector (Shimadzu Corporation, Kyoto, Japan), operating in conjunction with a Kobra® electrochemical cell (KOBRA® CELL, 2022) (R-Biopharm Rhône Ltd., Glasgow, UK) set at 100 μA for post-column electrochemical derivatization. Analyte separation was achieved on a reverse-phase C_{18} analytical column (Intersil ODS-3V, 150 mm × 4.6 mm, 5 μm; GL Sciences Inc., Tokyo, Japan) protected by a matching guard cartridge (4 × 3 mm^2), maintained at 30 °C by a CTO-20A column oven. The mobile phase consisted of ultrapure water and methanol (55:45, v/v) supplemented with 119 mg/L KBr and 1 mL/L of 65% (v/v) nitric acid. The mobile phase was delivered at a flow rate of 1.0 mL/min. Fluorescence was detected at excitation wavelength $\lambda ex = 362$ nm with dual emission channels: $\lambda em = 425$ nm for AFB_1 and AFB_2, and $\lambda em = 455$ nm for AFG_1 and AFG_2. Each sample (100 μL) was injected using a SIL-20A autosampler. Under the above conditions, analytes eluted in the order AFG_2, AFG_1, AFB_2, AFB_1. Aflatoxin concentrations in samples were calculated by interpolation from the matrix-matched calibration curves based on measured peak areas.

2.6. Temperature and relative humidity monitoring in storage depots

Temperature and relative humidity (RH) were monitored continuously across eight food storage depots over 30 days from 15 December 2023 to 15 January 2024: four depots at Ngaba market (D1–D4) and four at Liberté market (D5–D8). Measurements were made using an HTC-1 digital hygrometer-thermometer (CLOCK/HUMIDITY, China) placed within each enclosure after a 30-min equilibration period. Readings were recorded three times daily (07:30, 12:30, and 16:30), and daily means were computed from these three readings. In addition to instrument-based measurements, each depot was systematically inspected and documented for physical storage conditions, including bag stacking practices, floor contact, evidence of moisture or mold, ventilation status, and pest pressure.

2.7. Statistical analysis

All aflatoxin concentration data were first assessed for normality using the Shapiro–Wilk test. Given the strongly non-normal, right-skewed distribution characteristic of mycotoxin concentration datasets, non-parametric tests were applied throughout. Differences in Total aflatoxin (AF*T*) concentrations across the three commodity types were evaluated by the Kruskal–Wallis one-way analysis of variance. Where the Kruskal–Wallis test indicated a significant overall group difference ($p < 0.05$), pairwise post-hoc comparisons were performed using Dunn's test with Bonferroni correction for multiple comparisons. Differences in AF*T* concentrations between markets (Liberté vs. Ngaba) within each commodity type were assessed using the Mann–Whitney *U* test. Comparisons of aflatoxin prevalence (proportion of positive samples) across commodity types were performed using Fisher's exact test. Spearman's rank correlation coefficient (ρ) was used to evaluate associations between individual aflatoxin analytes within each commodity. A significance level of $p < 0.05$ was adopted for all tests. For statistical calculations, concentrations recorded as < LOD were assigned the substituted value of LOD/2 (0.025 μg/kg), following the approach recommended by Codex Alimentarius (CAC/GL 59-2006) and widely applied in mycotoxin occurrence surveys (Adetunji et al., 2017; Jallow et al., 2021). Given the relatively small sample size used in this study, results should be interpreted with appropriate caution (Codex Alimentarius Commission, 2019). All statistical analyses were performed using IBM SPSS Statistics, version 25 (IBM Corp., Armonk, NY, USA) and Python (version 3.x) with the SciPy (v1.x) and scikit-learn (v1.x) libraries.

3. Results and discussions

3.1. Aflatoxin prevalence and quantification

3.1.1. Aflatoxin contamination of maize

Aflatoxin contamination was detected in all 18 maize grain samples

(100%) (Table S1, Figs. 1 and 2). AFB_1 was the most prevalent analyte present in all 18 samples, followed by AFB_2 (15/18, 83.33%). AFG_1 and AFG_2 were detected in 10/18 (55.55%) and 7/18 samples (38.88%), respectively. Individual AFB_1 concentrations ranged from 0.56 to 150.30 μg/kg, and Total aflatoxin (AF*T*) from 0.56 to 173.00 μg/kg across the 18 samples. The mean AF*T* for the maize dataset was 20.40 μg/kg. Thirteen of 18 maize samples (72.22%) exceeded the EU AFB_1 limit of 2 μg/kg (Fig. 4A), and 10 of 18 samples (55.55%) exceeded the EU total aflatoxin limit of 4 μg/kg (Fig. 4B). The single most severely contaminated sample in the entire maize dataset, ML9 (Liberté market), contained 150.30 μg/kg AFB_1 and a total aflatoxin load of 173.00 μg/kg, representing approximately 87 times the EU AFB_1 limit. This level of contamination is deeply alarming in a commodity that is consumed daily in large quantities by adults, children, and infants throughout Kinshasa. The complete absence of any lot uncontaminated by AFB_1 among all 18 maize samples confirms that contamination is universal and not confined to isolated supply chains or market locations. Our findings are consistent with, and in certain respects more severe than, the only previous targeted assessment of aflatoxins in Kinshasa maize, conducted by Kamika et al. (2016), who reported 100% contamination of maize samples at concentrations reaching 300 times the WHO total aflatoxin guideline of 10 μg/kg (Kamika et al., 2016). Our maximum value of 173.00 μg/kg for total aflatoxins, while lower than the extreme values reported by Kamika et al. (2016), is nonetheless 17 times the WHO guideline. Studies conducted in other DRC provinces similarly report near-universal maize contamination: Udomkun et al. (2018) found that approximately 66% of maize grain and 88% of maize flour samples in North Kivu failed to meet EU thresholds, and Manizan et al. (2018) reported AFB_1 exceeding detection thresholds in more than 95% of North Kivu maize samples (Manizan et al., 2018; Udomkun et al., 2018). In Lubumbashi, Mulunda et al. (2013) documented contamination above 90% in both maize and groundnuts (Mulunda et al., 2013). The consistent picture emerging from all these studies is that maize marketed throughout the DRC is almost universally contaminated with aflatoxins at concentrations far exceeding acceptable limits, irrespective of geographic region.

3.1.2. *Aflatoxin contamination of groundnuts*

Of the 14 groundnut samples, 13 (92.85%) were positive for AFB_1 (Table S2, Figs. 1 and 2). AFB_2 was detected in 8 samples (57.14%), and both AFG_1 and AFG_2 in 10 samples (71.42%) and 7 samples (50.00%), respectively. Individual AFB_1 concentrations ranged from <LOD to 154.78 μg/kg and Total aflatoxin (AF*T*) from <LOD to 191.20 μg/kg, giving a mean AF*T* of 32.20 μg/kg, the highest mean contamination level

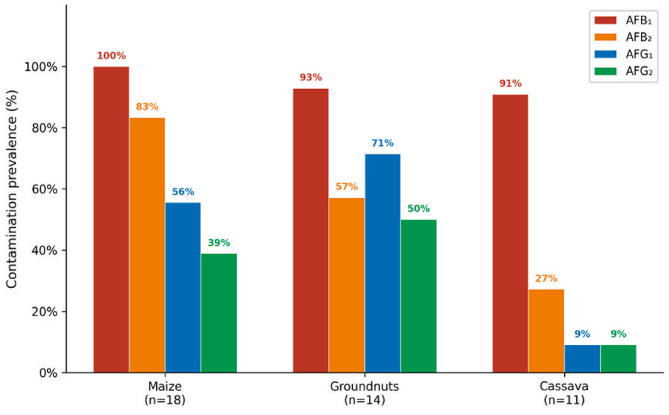

Fig. 1. Prevalence (%) of aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) in maize (n = 18), groundnuts (n = 14), and cassava (n = 11) collected from the Liberté and Ngaba retail markets in Kinshasa, DRC (August 2023). Values above bars indicate the percentage of samples in which each analyte was detected above the limit of detection (LOD = 0.05 μg/kg).

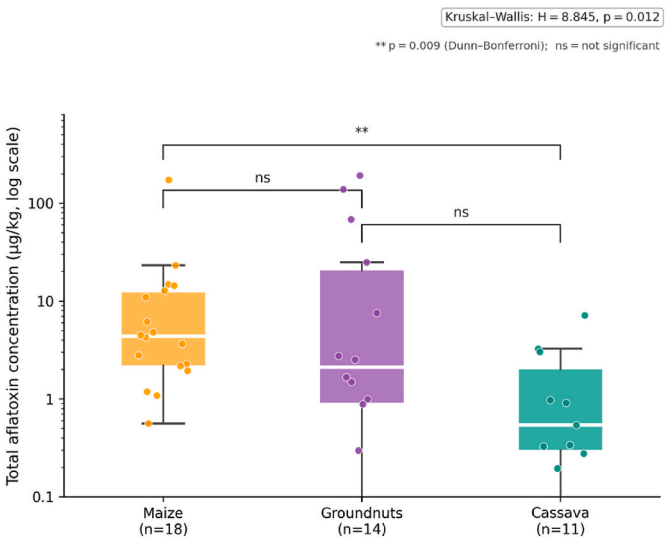

Fig. 2. Distribution of total aflatoxin (AF^T) concentrations (μg/kg, log scale) in maize (n = 18), groundnuts (n = 14), and cassava (n = 11) collected from the Liberté and Ngaba retail markets in Kinshasa, DRC (August 2023). Boxes represent the interquartile range (IQR); horizontal white lines indicate the median; individual sample values are overlaid as scatter points. Whiskers extend to the minimum and maximum observed values within 1.5 × IQR. Concentrations below the LOD (0.05 μg/kg) were assigned LOD/2 (0.025 μg/kg) for statistical purposes and are not displayed. A Kruskal–Wallis test revealed a statistically significant overall difference in AF^T concentrations across commodity types (H = 8.845, p = 0.012). Pairwise comparisons by Dunn–Bonferroni post-hoc analysis identified a significant difference between maize and cassava (**p = 0.009); differences between maize and groundnuts (p = 0.748) and between groundnuts and cassava (p = 0.213) were not statistically significant (ns).

of the three study commodities. Five of 14 samples (35.71%) exceeded the EU AFB_1 limit of 2 μg/kg (Fig. 3). Sample AN1, collected from the Ngaba market, was the most severely contaminated sample in our entire study, containing 154.78 μg/kg AFB_1 and a total aflatoxin load of 191.20 μg/kg, equivalent to approximately 96 times the EU total aflatoxin limit of 4 μg/kg for food commodities. The health implications of such concentrations, in a commodity that is consumed in multiple daily preparations, including groundnut paste, boiled groundnuts, and groundnut stew throughout Kinshasa, could have severe health implications. Only one groundnut sample (AN5, Ngaba market) was entirely free of detectable aflatoxins. The high contamination of groundnuts is consistent with the literature: groundnuts are widely recognized as the

Fig. 3. Storage conditions observed in food commodity depots at retail markets, Kinshasa, DRC (August 2023). (**A**) Sacks of cassava raw tubers and groundnut grains were stacked directly on the bare floor in high-density conditions with restricted airflow at Ngaba Market. (**B**) Sacks of maize are stored directly on the bare floor in high-density conditions with restricted airflow and no humidity management at Liberté Market.

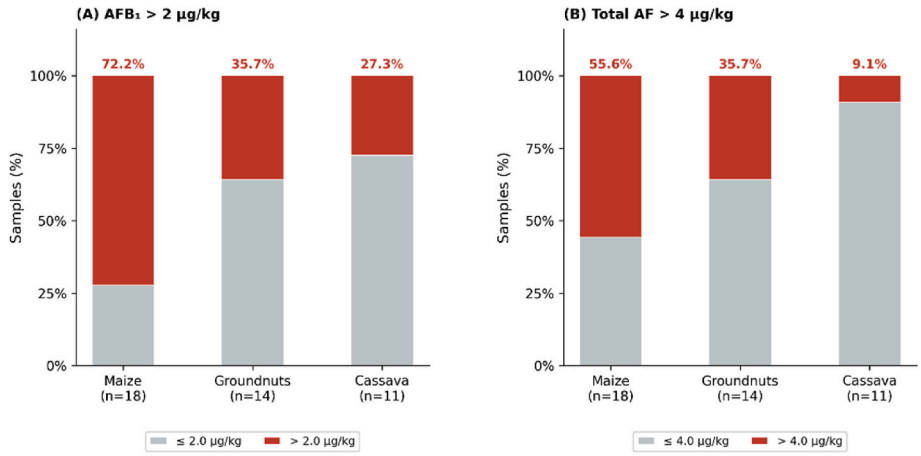

Fig. 4. Proportion of maize (n = 18), groundnut (n = 14), and cassava (n = 11) samples collected from Kinshasa retail markets exceeding EU regulatory limits for aflatoxins: (A) proportion of samples with AFB_1 concentrations exceeding the EU limit of 2 µg/kg; (B) proportion of samples with total aflatoxin (AF^T) concentrations exceeding the EU limit of 4 µg/kg. Grey bars represent samples within the regulatory limit; red bars represent exceedances. Percentage values above bars indicate the proportion of each commodity group that exceeded the relevant limit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

agricultural commodity most susceptible to *A. flavus* and *A. parasiticus* colonization, owing to their high lipid content, hypogeal cultivation, and vulnerability to pod damage during harvest (Jallow et al., 2021; Zhang et al., 2018). The higher prevalence of AFG_1 and AFG_2 in groundnut samples (71.42% and 50.00%, respectively), compared to maize (55.55% and 38.88%), is consistent with a greater contribution of *A. parasiticus* to groundnut contamination relative to maize, where *A. flavus* (which predominantly produces B-aflatoxins) tends to dominate (Zhang et al., 2018). This observation is further supported by the proportionally greater contribution of G-type aflatoxins to mean total aflatoxin in groundnuts compared to maize, as illustrated in Fig. 1.

3.1.3. *Aflatoxin contamination of cassava*

Ten of 11 cassava samples (90.90%) contained detectable AFB_1 (Table S3, Figs. 1 and 2). AFB_2 was detected in 3 of 11 samples (27.27%), while AFG_1 and AFG_2 were each detected in a single sample (9.09%), with AFG_1 detected in sample MaL6 and AFG_2 detected in sample MaL1. The near-exclusive occurrence of B-type aflatoxins in cassava, with virtual absence of G-type compounds, is consistent with, though not conclusive evidence of, a predominance of *A. flavus* as the predominant contaminating species in this commodity. Definitive attribution would require fungal isolation and precise molecular identification, and this interpretation remains speculative in the absence of direct mycotoxin isolation; rather than *A. parasiticus*, which typically co-produces both B and G aflatoxins (Zhang et al., 2018).

Total aflatoxin (AF^T) concentrations in Cassava ranged from < LOD to 7.16 µg/kg, with a mean of 1.55 µg/kg, the lowest among the three commodities. Three of 11 cassava samples (27.27%) exceeded the EU AFB_1 limit of 2 µg/kg (Fig. 4A). Only one cassava sample (9.09%, MaL6, 7.16 µg/kg) exceeded the EU total aflatoxin limit of 4 µg/kg (Fig. 4B); the remaining two samples that exceeded the AFB_1 limit had total aflatoxin concentrations of 3.26 and 3.03 µg/kg, both below the 4 µg/kg threshold. Although cassava exhibited the lowest absolute contamination levels of the three commodities studied, a finding consistent with its lower lipid content and less favorable composition as a substrate for aflatoxin-producing *Aspergillus* species relative to oilseeds and cereals (Jallow et al., 2021), the data present an important and often overlooked dimension of the Kinshasa aflatoxin burden. Cassava is the staple food of the DRC: it is consumed daily, in multiple meal forms (including *chikwangue*, *fufu ya manioc*, and boiled or fermented preparations), by virtually all socioeconomic groups. Its daily consumption frequency, combined with the fact that 27.27% of our samples exceeded EU regulatory limits, means that cassava may contribute meaningfully to the total dietary aflatoxin exposure of the Kinshasa population, particularly among children and pregnant women, for whom even moderate chronic aflatoxin exposure carries clinically meaningful risks (Jallow et al., 2021). We are not aware of any other published study reporting quantitative aflatoxin data for cassava raw tubers from Kinshasa markets. This critical surveillance gap should be urgently addressed: cassava must be integrated into any future DRC national food safety monitoring program.

3.2. *Temperature and relative humidity conditions in food storage depots*

All eight storage depots recorded mean daily temperatures in the range of 29.60 °C to 30.90 °C over the 30-day monitoring period (Table 1). These values are entirely consistent with the published optimal temperature for aflatoxin production by *Aspergillus* species, which is broadly defined as 25–35 °C with maximum biosynthesis near 30 °C (Yunes et al., 2020). Six of eight depots maintained mean relative humidity at or above 45%, with depot D8 recording the highest mean RH of 55.50%, approaching the 60% threshold associated with elevated water activity. Studies by Perrone et al. (2020) have demonstrated that a water activity of approximately 0.99 a*w* at 29–30 °C activates transcription of the key aflatoxin-biosynthetic genes *aflD*, *aflM*, and *aflP* in *A. flavus*, providing a mechanistic basis for the high contamination levels observed in our study. The two Ngaba depots with notably lower mean RH values (D3: 34.10%; D4: 34.60%) represent the only departing

Table 1
Mean daily temperature (°C) and relative humidity (%) measured in eight food storage depots at Ngaba market (D1–D4) and Liberté market (D5–D8) in Kinshasa, DRC, over 30 days (15 December 2023 to 15 January 2024). Values are expressed as mean ± SD from three daily readings over 30 days. Relative humidity was categorized as low (<40%), moderate (40–60%), and high (>60%) to facilitate interpretation of environmental conditions associated with fungal growth and aflatoxin contamination.

Depot	Market	Mean RH (%) ± SD	Mean T (°C) ± SD	Humidity category
D1	Ngaba	53.10 ± 3.0	30.30 ± 1.0	Moderate
D2	Ngaba	53.20 ± 2.1	30.90 ± 1.1	Moderate
D3	Ngaba	34.10 ± 2.4	30.10 ± 0.9	Low
D4	Ngaba	34.61 ± 2.0	30.82 ± 0.9	Low
D5	Liberté	52.80 ± 3.2	30.90 ± 1.0	Moderate
D6	Liberté	54.71 ± 3.1	30.72 ± 0.9	Moderate
D7	Liberté	45.32 ± 1.4	29.61 ± 1.0	Moderate
D8	Liberté	55.50 ± 1.8	30.50 ± 0.7	Moderate

observations in an otherwise uniformly high-risk storage environment; their slightly drier conditions may partly explain the comparatively lower contamination in some Ngaba samples, particularly for maize (Fig. 5).

Beyond the measured parameters, direct depot inspections confirmed that all eight storage facilities shared structurally similar risk conditions: food sacks stacked directly on bare concrete floors without palletization, stored at high density with restricted airflow, with no evidence of systematic pest exclusion or RH management. In most depots, sacks from older stock were buried beneath more recent arrivals, preventing first-in/first-out stock rotation and increasing the duration of exposure to contaminating fungi for older lots. Similar conditions were observed by Mulunda et al. (2013) and Okori et al. (2022) in other large cities of DRC, such as Lubumbashi. These storage conditions may constitute a risk factor for aflatoxin accumulation across both markets and possibly throughout Kinshasa's food distribution system. The storage temperature and relative humidity recorded across the eight market depots of Kinshasa (29.61–30.90 °C and 34.61–55.50%, respectively) are also consistent with ambient environmental conditions reported for Kinshasa and the broader DRC, where mean temperatures typically range from 22 to 32 °C year-round and relative humidity commonly exceeds 70% during the rainy seasons (September - May) (Cotty & Jaime-Garcia, 2007; Medina et al., 2017). Pre-harvest contamination driven by drought stress and elevated temperatures during grain filling is a well-documented pathway for aflatoxin accumulation in maize and groundnuts in sub-Saharan Africa (Medina et al., 2017), and high contamination levels consistent with multi-stage supply chain exposure have been reported in flour samples of cassava, maize, and groundnuts from Eastern DRC and neighboring Burundi (Udomkun et al., 2018; Udomkun et al., 2018). The contamination levels documented in the present study may be interpreted as an outcome of exposure at either pre-harvest, harvest, transit, retail storage stages or across several stages, rather than the product of retail storage conditions alone. It should be noted that moisture content and water activity of the sampled commodities were not directly measured in this study, and the temperature and RH measurements are presented here as indicative of the prevailing storage environment at these market locations and are conditions reported to support *Aspergillus* proliferation and aflatoxin biosynthesis (Liu et al., 2017; Yunes et al., 2020). Future work should measure commodity water activity directly to better characterize the relationship between depot storage conditions and contamination levels.

3.3. Comparative analysis of commodities and markets

Data summarized in Table S4 revealed a pattern of near-universal aflatoxin contamination across all three commodities, with overall contamination ranked as groundnuts > maize > cassava in terms of mean AF*T* concentration, and maize > groundnuts > cassava in terms of the proportion of samples exceeding EU regulatory limits (Fig. 4). These rankings reflect differences in commodity-specific vulnerability to aflatoxin-producing fungi as well as differences in the supply chain histories of individual lots sampled in this study.

Kruskal-Wallis analysis of AF*T* concentrations across commodity types returned a statistically significant result ($H = 8.845$, $p = 0.012$), confirming that the observed inter-commodity differences in total aflatoxin load are unlikely to have arisen by chance. Post-hoc Dunn–Bonferroni pairwise comparisons identified a statistically significant difference between maize and cassava ($z = 2.973$, $p = 0.009$, **), while differences between maize and groundnuts ($z = 1.152$, $p = 0.748$, ns) and between groundnuts and cassava ($z = 1.805$, $p = 0.213$, ns) were not statistically significant. Market-level comparisons using the Mann–Whitney *U* test did not reveal statistically significant differences in AF*T* concentrations between the Liberté and Ngaba markets within any individual commodity group ($p > 0.05$ in all cases) (Fig. 5), consistent with both markets drawing from the same regional supply chains and being subject to structurally similar storage conditions. However, directional trends are discernible: the most severely contaminated maize and cassava samples originated from the Liberté market, while the most contaminated groundnut sample (AN1, 191.20 μg/kg) came from Ngaba. The absence of statistically significant market differences, combined with near-universal contamination across both markets, reinforces the conclusion that the contamination problem could be systemic and supply-chain-wide, rather than specific to individual market locations.

To contextualize our findings against reports from other African countries, contamination levels observed in this study are broadly consistent with the regional literature. For example, in Nigeria, aflatoxin contamination of maize exceeding EU limits has been documented in over 60% of market samples, with mean total aflatoxin concentrations commonly in the range of 10-50 μg/kg (Adetunji et al., 2017). In Côte d'Ivoire, Manizan et al. (2018) reported AFB_1 exceeding detection thresholds in over 90% of maize and groundnut samples, with maximum AFB_1 concentrations in groundnuts exceeding 100 μg/kg, comparable to the maximum of 154.78 μg/kg recorded in the present study. In

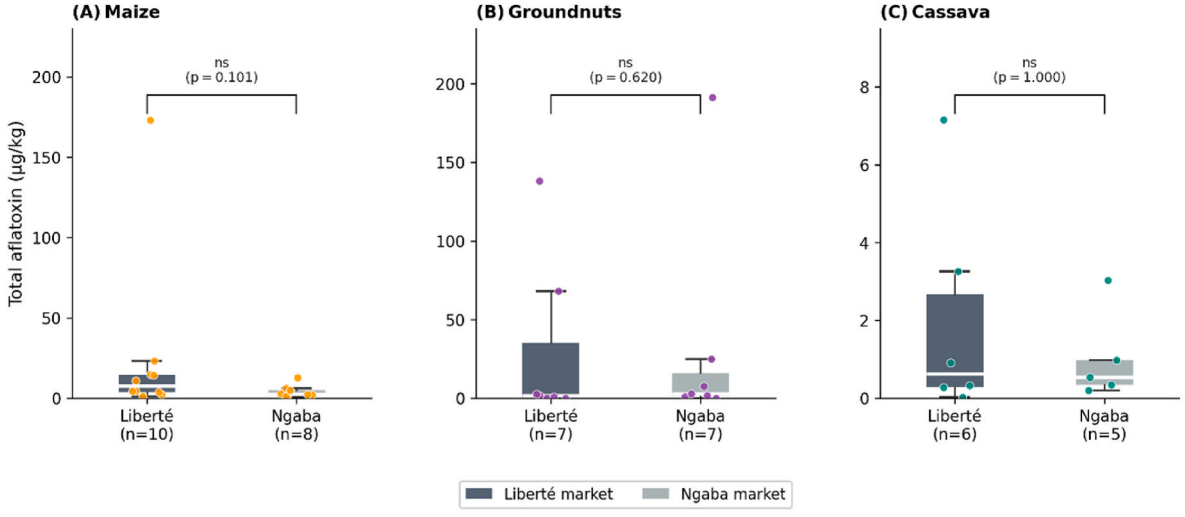

Fig. 5. Total aflatoxin (AF^T) concentrations (μg/kg) in maize (A), groundnuts (B), and cassava (C) samples stratified by market of origin (Liberté vs. Ngaba) in Kinshasa, DRC (August 2023). Boxes represent the interquartile range (IQR); horizontal white lines indicate the median; individual sample values are overlaid as scatter points. Whiskers extend to the minimum and maximum observed values within 1.5 × IQR. Significance brackets indicate the result of Mann–Whitney U pairwise comparisons between markets; ns = not significant ($p > 0.05$) for all three commodities.

sub-Saharan Africa broadly, groundnut contamination levels of 20-100 μg/kg total aflatoxins have been documented across multiple countries (Jallow et al., 2021). Importantly, methodological differences (sampling strategy, analytical approach, matrix type, study period) preclude direct numerical comparisons, but the consistent picture of near-universal contamination at levels well above regulatory thresholds across multiple African nations may indicate a region-wide food safety challenge that demands coordinated, cross-national monitoring and intervention frameworks.

3.4. Preliminary dietary exposure and risk assessment

The aflatoxin concentrations documented in this study may have severe implications for the health of the population of Kinshasa. AFB_1 is the most potent known naturally occurring hepatocarcinogen, and chronic dietary exposure at even moderate levels carries measurable risk of HCC development, particularly in populations with co-prevalent hepatitis B virus infection, which is endemic throughout the DRC (Jallow et al., 2021). Because aflatoxins are genotoxic carcinogens for which no safe threshold can be established, the Joint Expert Committee on Food Additives (JECFA) applies the ALARA (As Low As Reasonably Achievable) principle and has not set a tolerable daily intake; instead, cancer potency estimates of 0.01 per μg/kg body weight per day (HBsAg-negative) and 0.03 per μg/kg body weight per day (HBsAg-positive) are used for dietary risk assessment (JECFA, 2016; FAO/WHO, 2018). A preliminary dietary exposure estimate based on the mean AF*T* concentrations documented in this study, combined with typical per-capita daily consumption estimates for maize (200–400 g/day) and groundnuts (30–100 g/day) in Kinshasa (FAO, 2020a, 2020b), yields estimated aflatoxin intakes of 84–190 ng/kg body weight per day for a 60-kg adult from these two commodities alone. These values are 84 to 190 times higher than the 1 ng/kg body weight per day reference level proposed in the dietary exposure literature (Al-Jaal et al., 2019; Kuiper-Goodman, 1998) and correspond, using JECFA cancer potency estimates (FAO/WHO, 2018), to an excess hepatocellular carcinoma risk of approximately 1.4 cases per 100,000 per year in HBsAg-negative individuals and up to 41 cases per 100,000 per year in HBsAg-positive individuals, a population in which hepatitis B co-infection is endemic throughout the DRC (Muipata et al., 2025). When contributions from cassava (mean AF*T* 1.55 μg/kg; estimated intake 5–10 ng/kg bw/day at 200–400 g/day consumption) are included, total estimated aflatoxin exposure rises to 89–200 ng/kg body weight per day from these three staple commodities combined, before accounting for contributions from other contaminated foods or processed products derived from these raw commodities.

We should note that a key limitation of the present study is the sample size of 43 composite samples collected from two markets in Kinshasa over a period of five days. This constrains the statistical representativeness of these findings, which may not be directly extrapolated to the broader food supply chain of Kinshasa or the DRC as a whole. These results should be interpreted as an initial surveillance signal that requires follow-up studies through larger longitudinal surveys spanning multiple markets, seasons, supply chain nodes, and geographic regions. Additionally, the study period covered a single season, and aflatoxin contamination levels in staple commodities are known to fluctuate substantially across harvest cycles and storage durations. Nonetheless, the permanent structural storage deficiencies observed in these two markets (Fig. 3), such as food stacks stored directly in contact with bare floors and in high-density conditions, extremely restricted airflow, and absence of stock rotation, may suggest favorable conditions for mycotoxin accumulation. Interventions that address these practices, even in the absence of climate control, have been shown to meaningfully reduce the possibility of aflatoxin accumulation in depot storage in low-income tropical regions (Okori et al., 2022). The deployment of hermetic storage bags (e.g., PICS bags), solar drying before storage, and the mandatory use of raised pallets in market depots represent low-cost, contextually feasible options that should be evaluated in the DRC setting.

At the regulatory level, the DRC currently has no national mycotoxin monitoring program and no domestically enacted enforceable aflatoxin limits; the EU limits adopted as reference standards (Kamika et al., 2016) carry no legal force in the absence of a domestic regulatory framework. Establishing a functioning national monitoring system, with statutory limits, regular market sampling, and public reporting, is a prerequisite for consumer protection and for the DRC's participation in regional and international food trade. The existing One Health Coordination Commission (CCUS) and its provincial counterparts provide an institutional platform through which food safety surveillance could be coordinated in a multi-sectoral manner, engaging agricultural extension services, food trade regulators, public health authorities, and the research community (Yambayamba et al., 2024). Awareness campaigns targeted at food handlers, market operators, and consumers, particularly regarding the dangers of mold-contaminated commodities and the importance of dry, ventilated, palletized storage, are complementary measures that can be implemented in the near term.

4. Conclusion

In this study, we provide a comprehensive assessment of aflatoxin contamination across three major staple food commodities, maize, cassava, and groundnuts, collected at two retail markets in Kinshasa, DRC. Aflatoxins were detected in 41 of 43 samples analyzed (95.35%), with AFB_1 the dominant analyte and its prevalence reaching 100% in maize. The most severely contaminated samples in our study, ML9 (maize, 173.00 μg/kg total aflatoxins) and AN1 (groundnuts, 191.20 μg/kg), represent concentrations that are approximately 40-50 times the WHO total aflatoxin guideline for food safety. Seventy-two per cent of maize samples and 35.71% of groundnut samples exceeded the EU AFB_1 regulatory limit of 2 μg/kg; 55.55% of maize samples and 35.71% of groundnut samples exceeded the EU total aflatoxin limit of 4 μg/kg. To the best of our knowledge, this study is the first to establish that cassava raw tuber could be contaminated with aflatoxins, with 27.27% of samples exceeding the EU AFB1 limit of 2 μg/kg and 9.09% exceeding the EU total aflatoxin limit of 4 μg/kg. This finding is of public health significance given the central role of cassava in the daily diet of people living in Kinshasa. Temperature and humidity monitoring across food storage depots of the two retail markets suggests the persistence of conditions favorable to *Aspergillus* proliferation and aflatoxin biosynthesis throughout the post-harvest storage phase at retail markets. Future research combining dietary exposure assessment, quantitative risk modeling, and multi-mycotoxin profiling across all provinces of the DRC is needed to fully characterize the public health burden and inform evidence-based intervention strategies.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used Gemini in order to generate images for visual illustrations of the graphical abstract. The authors also used Claude in order to curate Python codes for statistical analysis. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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CRediT authorship contribution statement

Carmel Gabha'bey Kandhe: Conceptualization, Data curation,

Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Isaac Elaka Kaba:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jonathan Mpoyi Mukanya:** Data curation, Investigation, Resources. **Dep Enoju Enofo:** Data curation, Validation, Writing – review & editing. **Merveille Ntumba Mbombo:** Data curation, Resources, Validation, Writing – review & editing. **Blaise-Pascal Ilunga Muntokole:** Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Paulin Kapepula Mutwale:** Investigation, Methodology, Resources, Supervision. **Mulunda Mwanza:** Conceptualization, Investigation, Methodology, Resources, Supervision. **Nadege Kabamba Ngombe:** Conceptualization, Formal analysis, Methodology, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2026.112469.

Data availability

Data will be made available on request.

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