Assessment of aflatoxins and heavy metals in Zingiber officinale (ginger) across the value chain in Kaduna and Oyo states, Nigeria and the use of probiotics for aflatoxin detoxification

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Abstract

Zingiber officinale (ginger) is a high-value crop with significant nutritional benefits, but it is susceptible to food hazards, including mycotoxins and heavy metals. Fifty-one ginger samples were collected along the value chain in Kaduna state (local government areas: Jaba, Kachia and Zango) and 44 samples obtained from markets in Oyo state (local government areas: Ibadan North, Ibadan North East, Ibadan North West, and Ibadan South West). The incidence of aflatoxins (B1, B2, G1, and G2) and heavy metals (mercury, cadmium, lead, copper, zinc, and chromium), as well as risk assessments, were determined. Half (50%) of the samples from Kaduna contained aflatoxins and all (100%) of the samples from Oyo State contained aflatoxins. On average, 18 to 60% of split dried samples and 17 to 83% of fresh from Kaduna exceeded 10 ng/g total aflatoxin concentration. In Oyo State 67 to 100% of the split dried samples exceeded 10 ng/g and 100% of both fresh and milled samples exceeded 10 ng/g total aflatoxins. The probable tolerable limit of mercury (0.572 mg/kg bw/day) exceeded the limit of 0.23 mg/kg bw/day from Kaduna samples, whereas others were within the tolerable limits. Lead, which has no defined tolerable daily intake, was not detected in Kaduna State samples. In Oyo State samples, lead was detectable with an estimated limit of 0.007 mg/kg bw/day whereas the others were within the limit. The estimated probable daily intake (PDI) of aflatoxin B1 was estimated at 4.1 µg/kg bw/day and 1.5 µg/kg bw/day from Oyo State and Kaduna state samples respectively. The provisional maximum tolerable daily intake (TDI) for aflatoxins is 2 µg/kg bw/day. The use of probiotics was explored for the detoxification of aflatoxin-spiked ginger samples (at 50 ng/g). *Lactiplantibacillus plantarum* ATCC 8014 (LP) and *Lactobacillus helveticus* ATCC 15009 (LH) reduced aflatoxin concentrations by 50% when used alone or together at the end of a 120-h incubation period. The study highlights the incidence and risk of exposure to multiple toxicants in ginger and the need for management of aflatoxins and heavy metals along the ginger value chain.

Introduction

Zingiber officinale Roscoe, commonly known as ginger, is a geocarpic monocotyledonous crop of the Zingiberaceae family. Its rhizome is valued globally and is used majorly to flavour foods and beverages, it is also valued for its medicinal properties e.g. anti-inflammatory, antioxidant properties and for prevention of neurodegenerative diseases (Ezzat et al., 2017a; Arcusa et al., 2022; Ballester et al., 2022). In West Africa fermented foods, drinks and cereal-based gruels (*zobo*, *kunun zaki*, *ogi* and *koko*) include ginger as an adjuvant for flavour (Ayofemi and Adeyeye, 2016; Nwankwo et al., 2023). Ginger is rich in vitamins (vitamins B6 and C), minerals (potassium, magnesium and manganese); phytochemicals (gingerol, shogaol) and; fibre (that aids digestion) (Akerele, Umar and Adetoye, 2017; Abara et al., 2021). These nutrients are important and contribute to normal body functioning, physiology and biochemical processes. Consequently, ginger is an important crop for healthy diets and for food security. Given its widespread use, the contributions of this crop to food security is critical.

In Africa, Nigeria is the largest producer and exporter of ginger. It is the second largest exporter of ginger globally after India, with 86,000 tonnes of ginger (valued at 475 million USD) (World Bank, 2023). However, losses from a disease outbreak in 2023 resulted in a decline in Nigeria's share of the export market (Arise News, 2023; Jaiyesimi, 2025). Nigerian ginger (yellow variety UG1 is referred to as *Tafingiwa* (elephant foot) and black variety UG2 is known as *Yatsunbiri* (monkey finger) respectively in Hausa (Abah, 2019a; Yoon, Jang and Cho, 2020). The ginger varieties are sourced globally for their unique flavour and taste, with 80% produced in Nigeria being exported (Abah, 2019a; Yoon, Jang and Cho, 2020; Donatus, 2025). However, the ginger value chain is faced with challenges that threaten the quality and safety of the product from the country. Mycotoxin contamination has been reported in ginger samples including the varieties from Nigeria, in multiple academic literature (Lippolis et al., 2017; Yang et al., 2017; Okereke and Godwin-Egein, 2018; Negedu et al., 2022). Dietary exposure to heavy metals can be hazardous to health, especially when levels exceed the tolerable/safe limits (Kuiper-Goodman, 1995; Praveena and Omar, 2017; Rehman et al., 2018). However, there is limited information about the exposure of ginger to multiple food hazards along the value chain. As Nigeria has a high ginger market share, the better-quality ginger likely meets the regulatory requirements of importing countries (majorly outside the African continent such as Asia, Europe and the United States of America) (World Bank, 2023), as sub-optimal quality products exported will be rejected (The Nation, 2017; Anagor-Ewuzie, 2023). Consequently, this increases the burden of unsafe food in the domestic markets where monitoring measures are sub-optimal.

Mycotoxins are fungal toxins produced as secondary metabolites under the ideal conditions of crop stress (pre-harvest) and in enabling environments of saturation deficits, warm temperatures, and insect damage (postharvest) (Manu et al., 2019; Casu et al., 2024; Ntwali, Latif and Müller, 2024). Dietary exposure to mycotoxins has negative health impacts especially when ingested at elevated concentrations and outbreaks have been recorded (CDC, 2004; Kamala et al., 2018). Although there are no 'safe' concentrations for class 1 carcinogens like aflatoxins (Kuiper-Goodman, 1995), regulatory limits are set at 10 ng/g for total aflatoxin concentration in ginger by the European Union Food Safety Authority (EFSA) (The European Commission, 2023). High exposures to aflatoxin contamination have been linked to liver cancers, growth deficiencies, immune suppression, liver and kidney diseases (Liu and Wu, 2010; Monson, Coulombe and Reed, 2015; PACA, 2020). It is therefore important to control the prevalence of this mycotoxin in crops and limit dietary exposure to consumers.

Within the production section of the value chain, in a temperate region, it has been reported that summer temperature (in contrast to winter) results in higher concentrations of aflatoxin (Omotayo et al., 2019). Being a geocarpic crop, the rhizomes are also in direct contact with the soil that harbours a myriad of fungi, including *Aspergillus* section *Flavi* that includes species and strains that are aflatoxigenic (Waliyar et al., 2015; Otto et al., 2020). Other sections of the value chain, including processing (e.g. drying and packaging) can have an impact on the incidence of aflatoxins in crops (Williams, Baributsa and Woloshuk, 2014; Falade et al., 2016; Muga, Marenya and Workneh, 2019a).

Another food safety attribute of importance in ginger is heavy metal contamination (Gaya and Ikechukwu, 2016). Also, information about heavy metals across the ginger value chain in Nigeria is sparse. Heavy metals occur naturally in soils and uptake into the crop, including the rhizome, is possible during production (Wang et al., 2003; Adah, Abah and Ubwa, 2013). Other routes of contamination could be practices which involve putting the crop in direct contact with contaminated soils, such as sun drying on bare surfaces, storage in contaminated containers, or during transportation, are avenues through which heavy metal contamination can occur. Heavy metals like mercury, species of arsenic, lead, chromium and copper are tightly regulated in food due to the negative

impact on human health causing neurological defects, allergic reactions (Rodrıguez, Jiménez-capdeville and Giordano, 2003; Ortega et al., 2021; Islam, Roy and Singha, 2025), association with cancer (lead, cadmium, chromium),(Ebrahimi et al., 2020; Parida and Patel, 2023) infantile diseases and mortalities (lead, mercury, arsenic, cadmium) (Parker et al., 2022; Haidar et al., 2023).

Due to natural circumstances, it may sometimes not be possible to exclude contaminants in food completely. For instance, warm, humid regions of the world are prone to mycotoxin contamination due to the enabling climate conditions. Consequently, management methods that include decontamination are important. Probiotics have been explored for decontamination, in addition to their benefits of improving gut microbiota, immune system boost, flavour, and aroma enhancement (Chlebicz and Śliżewska, 2020; Banwo et al., 2021; Banwo, Adebo and Falade, 2024). Lactic acid bacteria have been beneficial in aflatoxin reduction of up to 96.58% (Mosallaie and Jooyandeh, 2020)

The current study investigated the incidence of mycotoxins (aflatoxins) and selected heavy metals in ginger samples collected from along the value chain in Nigeria, including production, drying, storage, milled, and traded ginger in the domestic informal markets. Additionally, the potential of probiotic strains were examined for decontamination of contaminated fresh ginger aflatoxin-spiked samples.

Materials and Methods

Sampling sites and sample types

Samples were collected from ginger-producing areas in Kaduna state (Jaba, Kachia and Zango local governments (LGs): GPS coordinates 9.4747461, 8.0124917; 9.8570726, 7.6885857; and 9.8374975, 8.4065268, respectively) along the value chain. The ginger value chain comprises production, processing, marketing, and consumption (Abah, 2019a). Samples were collected from all stages except consumption. Samples were also obtained from Oyo State (Ibadan North, Ibadan North-East, Ibadan North-West, and Ibadan South-West LGs: GPS coordinates 7.4477245, 3.8967116; 7.3939066, 3.9273483; 7.403651, 3.871402; and 7.3733705, 3.8589668) at the market stage (Figure 1). Ginger samples were collected in fresh, split dried, and milled forms (Figure 2). Data on sampling point, drying method and the storage containers used were collected for Kaduna-sourced samples. In Oyo state, data was collected on sampling point and storage container used. Samples were transported to the Pathology/Mycotoxin laboratory of the International Institute of Tropical Agriculture (IITA) and stored in the cold room at 4 ^{o}C until time for mycotoxin analysis.

Fungal isolation and identification

Fungal isolates were retrieved from one gram of samples using the serial dilution. Briefly, one gram of sample was vortexed in 9 mL of sterile distilled water and serially diluted to the fifth dilution factor. Thereafter 2 mL of diluent was inoculated on Petri dishes containing solidified potato dextrose agar and incubated at 25 ^{o}C for 7 days. At the end of the incubation period, distinct non-coalescing colonies were transferred from the plates and pure colonies were obtained after several subculturing. The isolates were identified based on their morphological characteristics on differential culture media (PDA, CYA and V8 juice agar media) and under the microscope equipped with a micrograph (Omax light microscope, micrograph, China) at × 400 magnification after staining with lactophenol cotton blue dye.

Identification of aflatoxigenic species

In order to ascertain the aflatoxigenicity of identified *Aspergillus flavus* and *A. parasiticus* strains, pure isolates (500 µL of 1 × 10^6 spores/ml) were inoculated on sterile aflatoxin-free maize grains (previously analysed) and incubated at 25 ^{o}C for 7 days. At the end of the incubation period, the grains were analysed for aflatoxin concentration as described in the following section.

Aflatoxin Analyses of ginger and inoculated maize samples

Aflatoxins were extracted from ginger samples using the method described by others (Wen *et al.*, 2014; Omotayo *et al.*, 2019) with modifications. Briefly, ten grams (10 g) samples of each sample were blended with 100 mL of 80% methanol and 2 g of sodium chloride for 3 min and poured into Erlenmeyer flasks. Thereafter, the mixture in the covered Erlenmeyer flasks was agitated mechanically for 30 min on an orbit shaker at 400 rpm (Lab-line instrument, Inc., Melrose Park, IL, USA) to allow sufficient contact time between the extraction solvent and the substrate. Following this, the mixture was filtered through Whatman filter paper No 1. (Whatman Intl. Ltd., Maidstone, UK), and 20 mL of 10% sodium chloride solution and 12.5 mL of n-hexane were added to the filtrate and transferred to a separating funnel. Each separating funnel was shaken for one minute manually and left to separate. The lower phase was dispensed and combined in a separating flask with 10 mL of dichloromethane, it was allowed to separate, and the lower phase was dispensed into a bed of 20 anhydrous sodium sulphate. This was repeated with 7.5 mL of dichloromethane and the obtained extract was left to evaporate to dryness in a fume hood overnight. The extracted aflatoxins from the inoculated maize samples were used to ascertain the aflatoxigenicity of the isolates, as previously described by (Banwo *et al.*, 2023)

Analyses of heavy metals in sampled ginger

For fresh ginger samples, they were first sliced into small pieces using a rust-free stainless-steel knife and oven-dried at 80 ^{o}C for 24 h to obtain ginger with low moisture content (Precision Scientific Oven, Model 18EM, USA). On the other hand, split dried ginger and milled samples were not further dried. Un-milled samples were ground using a Warring Commercial blender (Torrington, CT, USA) before digestion and heavy metal analyses were carried out at the One Health Platform, IITA Cotonou, Benin.

Sample digestion and analyses for heavy metals

The oven-dried ginger samples were sieved through a 2-mm mesh sieve for the extraction of heavy metals as described by Maleki et al. (2014) with modifications. Briefly, one gram of ginger powder was dissolved into 18 mL of deionized water in a Pyrex beaker, then combined with 10 mL of HNO_3 and HCl (ratio 3:1). The mixture was left to stand overnight and then heated to 95°C until a transparent solution was obtained.

Heavy metals such as copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), mercury (Hg) and manganese (Mn) were quantified using a Metalyser HM 3000 ($Trace_2O$, Berkshire, UK). The quantification process consisted of three phases according to the manufacturer's instructions (1) the conditioning of electrode, which was specific for each metal, (2) sample analysis using 70 mL extract per sample and requiring 5-min incubation, and (3) display of the results including a corresponding graph on a multimedia tablet connected to the Metalyzer.

Health Risk Assessment of Toxicants in Ginger

The risk assessment of aflatoxins to Nigerian populations was calculated by estimating the risk of cancer for carcinogens (aflatoxins) and the risk of non-carcinogenic effects (for heavy metals). Information used to calculate these included the estimated probable daily intake and margin of exposure. The average body weight estimation was 60.7 kg, the average daily consumption of ginger used was 10 g, and the concentrations of the toxicants used was disaggregated by state, given that the toxicant concentrations from Oyo and Kaduna states were different. Estimations of cancer and non-cancer risk were calculated as described by (Kortei *et al.*, 2020; Falade *et al.*, 2022)

Briefly, the Estimated Daily Intake (EDI) is projected from the product of concentration of toxicants (aflatoxin B1 or heavy metals) and average daily consumption (estimated at 10 g), divided by the average body weight (estimated at 60.7 kg).

Hazard Quotient (HQ) that estimates the risk of non-carcinogenic effect is calculated as the ratio of exposure dose to reference dose, where a value below 1 indicates an exposure that is below the reference dose and unlikely negative health effect, whereas values above 1 indicate a likely negative health effect.

Target Hazard Quotient is calculated as the product of the exposure frequency 350 days/year), exposure duration (30 years), ingestion rate of ginger (10 g/day) and heavy metal concentration (mg/kg) divided by the product of oral reference dose (mg/kg/day), body weight (kg) and average exposure time for non-carcinogens 365 days/year × 30 years), then multiplied by the unit conversion factor of 10^{-3}.

Total THQ is the sum of the THQ for each of the toxicants

Decontamination of aflatoxin-spiked ginger

Extraction of aflatoxins from inoculated maize grains

Total aflatoxins were extracted from maize grains (five grams) inoculated with toxigenic *A. flavus* La3228, obtained from the International Institute of Tropical Agriculture Collection Centre. This strain, originally obtained from the Derived Savannah region in Nigeria, is a highly toxigenic *Aspergillus* strain (Atehnkeng *et al.*, 2008). This strain was inoculated on maize at a spore concentration of 500 µL of 1 x 10^6 spores/mL. Following a seven-day incubation period, the inoculated grains were extracted for aflatoxin and quantified as previously described (Agbetiameh *et al.*, 2018). To achieve the spiking concentration, aflatoxin extract was serially diluted with dichloromethane, confirmed by HPTLC, and then refrigerated at 4 ^oC until needed.

Obtaining spiked ginger at 50 ng/g

Spiked ginger was achieved by spiking to a mash concentration of 50 ng/g using extracted aflatoxins. The following formula was used to calculate the aflatoxin volume to be inoculated on ginger:

Weight of mash to be used (g) = (final sample weight (g)× final spiking level to be achieved (ng/g)/(concentration of extract (ng/g))

Source of lactobacilli and preparation for treatment

Two strains of lactobacilli were obtained from ATCC: *Lactiplantibacillus plantarum* ATCC 8014 (LP) and *Lactobacillus helveticus* ATCC 15009 (LH). The strains were cultured from glycerol suspensions on MRS broth, incubated at 30 °C for 48 h under anaerobic conditions, and then streaked on MRS agar plates and incubated microaerophilically at 30 °C for 48 h. To obtain the desired concentration of lactobacilli, for each of the strains, a loopful from the plates was transferred to 5 mL of sterile MRS broth, incubated for 48 h, then washed with PBS solution (pH 7.2) and centrifuged to obtain a clear cell suspension. Cell concentrations were determined using comparison with McFarland standards (Fonseca Moreria da Silva *et al.*, 2015), and a concentration of 10^8 live cells was used for decontamination experiments

Treatment groups for decontamination assessment

Treatment groups included triplicates of the following groups (i) unspiked ginger alone (control), (ii) spiked ginger, (iii) spiked ginger with one of the two lactobacilli (iv) spiked ginger with both of the lactobacilli, (v) unspiked ginger with lactobacilli. Milled ginger samples (10 g) previously quantified to establish background contamination closest to the undetectable limit were weighed into a sterile Falcon tube. Spiked treatment groups were spiked to 50 ng/g and lactobacilli cells of 10^8 live cells were used. Treatment groups were incubated at 30 °C for 24 h, 48 h, 72 h, 96 h and 120 h. Ginger used in this experiment was first autoclaved to remove extraneous organisms that could interfere with the assessment of the probiotic inclusion.

At the end of the incubation periods, experiments were terminated by extracting the samples as earlier described. The extent of lactic acid bacteria-mediated aflatoxin decontamination was determined via recovery of aflatoxins from the samples as follows.

Recovery = (final conc. – initial conc.)/ initial conc × 100% (Banwo *et al.*, 2023)

Descriptive Analysis and Analysis of Variance among Variables

Descriptive analyses of the samples were conducted using Excel, analysis of variance (ANOVA) among variables was determined and box plots were developed using the R software.

Results

Concentrations of aflatoxins and heavy metals between Oyo and Kaduna States

Fifty-one (51) samples were obtained from Kaduna State and 44 samples from Oyo State. As Kaduna State is a major ginger-producing state, samples were collected along the value chain from fresh (five samples each), drying (five samples each), storage (five samples each) and sales points (two samples each) at each of the local governments. There were 18 fresh ginger, 33 split dried samples collected in Kaduna State. In Oyo State samples (sample number in parenthesis) were collected from markets in Ibadan North (9), Ibadan North-East (9), Ibadan North-West (17) and Ibadan South-East (9) local governments. There were 15 fresh samples, 14 split dried and 15 milled samples collected from the sales points at the market.

Samples from Kaduna and Oyo States contained aflatoxins B1, B2, G1 and G2. Generally, concentrations of each of these aflatoxins were statistically higher ($p<0.05$) in Oyo state compared to concentrations in ginger sampled from Kaduna state (Table 1). Overall, fresh and split dried samples from Kaduna were less contaminated ($p<0.05$) than fresh and split dried samples from Oyo state. There were no milled samples collected from Kaduna state. In all the milled samples collected from Oyo state, concentrations of aflatoxins B1, B2 and G1 were higher in milled samples compared to fresh and split dried samples. However, the concentrations of the detected heavy metals: copper, mercury and zinc were statistically the same ($p>0.05$). (Table 1). There was no lead detected in ginger samples taken from Kaduna state.

In samples collected from Kaduna, 50% of the samples were contaminated with aflatoxins. Aflatoxin concentrations ranged from undetectable concentrations to 82.2 ng/g. Aflatoxin concentrations in fresh samples collected at harvest ranged from 0.0 to 37.6 ng/g in Jaba, and 0.0 to 82.2 ng/g in Zango and were below detection levels in Kachia local governments. Whereas in split dried samples the total aflatoxin concentrations were 0.0 – 19.0 ng/g in Jaba, 0.0 – 18.6 ng/g in Kachia, and 0.0 – 20.8 ng/g in Zango local governments.

Due to the low degrees of freedom for statistical analyses, descriptive analysis was conducted, for comparing samples among sampling locations, instead of ANOVA, as was done between states: In Jaba and Zango, more than 50% of the fresh samples contained above 10 ng/g samples (83% and 60% in Jaba and Zango respectively). However, whereas in Zango, 60% of the split dried samples contained above 10 ng/g total aflatoxins, in Jaba, and Kachia, less than 50% of the split dried samples (27% and 18% respectively). contained above 10 ng/g of total aflatoxins (Table 2).

In Oyo, 100% of the samples contained detectable concentrations of aflatoxins. Total aflatoxin concentrations ranged from 7.7 to 184.7 ng/g. Ranges of aflatoxin concentrations and means are reported in Table 2. In all cases, except in Ibadan North, milled samples contained higher concentrations of aflatoxins compared to fresh and split dried samples. The average total aflatoxin concentration in 100% of the samples exceeded 10 ng/g.

Table 1: Total aflatoxin concentrations of samples collected along the ginger value chain in Kaduna State, Nigeria

Local Govts.	Stage of sampling	N	Average total aflatoxin concentrations in ng/g				Percentage of samples above 10 ng/g
			Harvest	Drying	Storage	Market	
Jaba	Split dried	11	NA	7.1	0.6	14.6	27
	Fresh	6	23.5	NA	NA	42.4	83
Kachia	Split dried	11	NA	8.7	0.0	0.0	18
	Fresh	6	0.0	NA	NA	13.5	17
Zango	Split dried	11	NA	6.8	13.6	31.6	60
	Fresh	6	38.0	NA	NA	19.3	67

NA means Not Applicable

Table 2: Total aflatoxin concentrations of samples collected from markets in Oyo State, Nigeria

			Average total aflatoxin concentration (ng/g)		Samples above 10 ng/g (%)
Local Govts.	**Ginger form**	**N**	**Market**	**Range**	
Ibadan North	Split dried	3	14.9	7.7 – 20.2	67
	Fresh	3	45.2	42.5 – 50.2	100
	Milled	3	137.4	88.3 – 184.7	100
Ibadan North East	Split dried	3	94.8	85.0 – 106.2	100
	Fresh	3	52.8	34.5 – 85.2	100
	Milled	3	64.7	43.3 – 85.6	100
Ibadan North West	Split dried	5	82.3	20.1 – 123.7	100
	Fresh	6	79.6	39.4 – 109.3	100
	Milled	6	96.3	47.1 – 180.6	100
Ibadan South West	Split dried	3	38.7	21.0 – 52.8	100
	Fresh	3	46.6	34.5 – 55.4	100
	Milled	3	95.8	73.7 – 119.9	100

Cadmium and chromium were not detected in any of the sampled ginger from both states. Among the fresh ginger samples, 100% and 86% from Kaduna and Oyo states respectively, met the safety requirements for lead set by the EC of 0.8 mg/kg. However, nearly half (51%) of samples taken from Kaduna and Oyo states did not meet the 0.1 mg/kg regulatory limit for mercury (Hg) set for salt and supplements. There are no specific requirements for ginger, but as ginger is used as a dietary supplement (Schwertner, Rios and Pascoe, 2006; Nemati *et al*., 2021) this standard may apply. One hundred per cent (100%) and 93% of ginger samples from Kaduna and Oyo states met the safety requirement of cadmium set by the European Commission (0.05 mg/kg). The guidelines for copper (Cu) and zinc (Zn) in adults is estimated at 5 mg/day for copper and 7 to 10 mg/day for zinc (EFSA, 2014; EFSA Scientific Committee, More *et al*., 2023). The concentrations of copper and zinc detected were within the guidelines set for these heavy metals/ micronutrients. The maximum concentrations of copper in Kaduna and Oyo States were 2 mg/kg and 4 mg/kg and the maximum concentrations of zinc in Kaduna and Oyo states were 4 mg/kg and 3 mg/kg respectively.

Storage containers

In Kaduna, 100% of the fresh samples were stored in polythene sacks, whereas 31/33 (94%) of split dried samples were stored in polythene sacks and 2/33 (0.06%) were stored in bowls. Ginger actors (97%) dried the split-dried samples on uncovered bare surfaces, including bare cement or bare soil. Only one sample (0.03%) was dried using a solar dryer and the surface covered with tarpaulin.

In Oyo state, 100% of the split-dried samples were stored in polythene bags. However, fresh samples were stored in varied containers: Four of the fifteen (26.67%) samples were stored in raffia baskets, 4/15 (26.67%) were stored in polythene bags, 4/15 (26.67%) were stored in polythene sacks, and 3/15 (20%) were stored in bowls.

Health Risk Assessment of Toxicants in Ginger

Risk associated with aflatoxin ingestion in contaminated ginger.

The average concentration of aflatoxin B1 in Oyo State and Kaduna States was 25.1 ng/g and 9.0 ng/g, respectively. These resulted in a probable daily intake of aflatoxin B1 of 4.1 ng/g bw/day and 1.5 µg/kg bw/day, respectively (at an estimated 10 g per day) (Table 4). The risk of liver cancer from exposure to aflatoxins in ginger in Oyo state from this margin of exposure (41.2) compared to 115.3 (in Kaduna state) was three times higher than in Kaduna State at an annual risk of 18.7 cases/100,000, compared to 6.7 cases/100,000 persons/ year in Kaduna.

Table 3: Estimated probable daily intake of heavy metals and aflatoxins in ginger

Food Hazard	Estimated probable daily intake (mg/kg bw/day)		Tolerable Intake/ (mg/kg bw/day)
	Oyo State	Kaduna State	
Mercury	0.180	0.572	0.23
Lead	0.007	0	no established TDI
Copper	0.037	0	0.04
Zinc	0.025	0.059	0.3
	Estimated probable daily intake µg/kg bw/day)		PMTDI* (µg/kg bw/day)
Aflatoxin B1	4.1 µg/kg bw/day	1.5 µg/kg bw/day	2

*PMTDI = provisional maximum tolerable daily intake (this applies to aflatoxin B1)

Risk associated with heavy metal ingestion in contaminated ginger

The estimated daily intake of mercury was within the limits for mercury in Oyo State, but exceeded the limits for samples collected from Kaduna State by 60%. However, the estimated probable daily intake for the other heavy metals and aflatoxin B1 was within tolerable limits. On the contrary, for exposure to hazards in samples taken from Oyo State, lead was detected (there is no tolerable daily intake for lead) as this is considered harmful even in very minute concentrations. The probable daily intake of aflatoxin B1 was also 51% higher in samples collected from Oyo State. However, the daily intake of mercury, copper and zinc were within the tolerable limits.

The likely adverse effects from the consumption of these heavy metals was greater than one for mercury and lead, indicating that these are risk factors in the ginger that require management. The total THQ was 8.694 for samples taken from Oyo state and 5.482 for samples taken from Kaduna state. These values being above one indicates a risk that non-carcinogenic effects could occur from the ingestion of ginger from these states. The THQ was above one for mercury (1.727 and 5.481 for samples collected from Oyo and Kaduna states respectively). THQ was 6.966 for lead for samples collected from Oyo state and below one for samples collected from Kaduna state. There was no risks estimated (values were below one) for exposure to cadmium, copper and zinc in both Oyo and Kaduna States.

Table 4: Detoxification of aflatoxin-spiked ginger samples treated with *Lactiplantibacillus plantarum* and *Lactobacillus helveticus*

Incubation period (h)	**Aflatoxin concentrations** (**ng/g**)		
	LP-treated spiked ginger	**LH-treated spiked ginger**	**LP and LH treated spiked ginger**
0	50.0	50.0	50.0
24	42.75	36.18	50.83
48	32.57	31.67	36.12
72	21.29	54.71	41.07
96	27.30	25.90	28.87
120	24.11	23.61	24.07
Reduction (%)	48.22	47.22	48.14

LP: *Lactiplantibacillus plantarum,* LH: *Lactobacillus helveticus*

Aflatoxin concentrations in probiotic study

Spiked ginger that was treated with LH or LP resulted in reduced aflatoxin concentration at the end of the 120 h incubation period by 49.5%. Total aflatoxin concentrations reduced similarly from 50 ng/g to 24 ng/g when both LP and LH were used combined to treat the spiked ginger samples at the end of the 120-h treatment period. However, there were difference between LH and LP treatments at the 72 h: whereas there was a steady reduction in aflatoxin concentrations when LP was used, when LH was used or combined with LP, at 72 h, the concentration returned to the original aflatoxin concentration before again reducing at 96 h and 120 h.

Discussion

Ginger is widely consumed globally and is used as a supplement and an adjuvant in foods (Jagetia, Baliga and Venkatesh, 2004; Schwertner, Rios and Pascoe, 2006; Wen et al., 2014; Nemati et al., 2021; Adejobi et al., 2024). Its safety is therefore paramount as it could serve as a source of contamination for foods meant for human consumption. With the growing demand for natural supplements and the use of nature-based products, the usage of ginger in diets has grown. This was particularly observed during the coronavirus disease pandemic (COVID-19) where the use of ginger was promoted for relieving symptoms and facilitating recovery, attributed to its antiviral, anti-inflammatory properties, among others (Ezzat et al., 2017b; Jafarzadeh, Jafarzadeh and Nemati, 2021)

The results of food safety attributes of ginger sourced from ginger-producing Kaduna state and ginger-consuming Oyo state show that ginger contamination is present in the ginger value chain from both states. From results of samples of ginger collected from harvest, during drying, in storage and in the markets, demonstrate that ginger contamination can begin from the field. This necessitates the need to control from production stage. Technologies such as the use of biological control have been utilised for pre-harvest aflatoxin control in cereals, legumes and spices (Ezekiel et al., 2019; Agbetiameh et al., 2020). Being a geocarpic crop, the ginger rhizomes can be easily associated with microorganisms present in the soil, such as *Aspergillus* species which includes mycotoxin-producing species and strains. Management of ginger at this stage using technologies like biological control products, such as Aflasafe and *Trichoderma* species, can offer protection to the crop (Atehnkeng et al., 2018; Yue et al., 2022). However, this has not been reported in efficacy trials.

Aflatoxin concentrations were higher in Oyo State compared to Kaduna State. In Kaduna, elevated levels of ginger were recorded particularly at the sales points, indicating that accumulation of the toxin occurs between the time of harvest and its point of sale in the markets. It is therefore critical for aflatoxin management of ginger to continue post-harvest, even where strategies have been implemented during pre-harvest to limit the contamination. Establishing pre-harvest protection can also proffer post-harvest benefits when the associated strains are non-toxin producers (Bandyopadhyay et al., 2019)

The differences in cancer risk could be indicative of differences in the handling of ginger across both locations and differences in weather conditions. Logistics and storage play crucial roles in post-harvest management, particularly in regions that do not produce food commodities. Ginger production occurs primarily in Kaduna, therefore samples in Oyo likely originated from Southern Kaduna and were transported to Ibadan, about 800 km away. Given the deficits in road infrastructure in Nigeria, the movement of food commodities leads to losses in quality and quantity. These can result in biosynthesis of aflatoxins in transit and storage by associated aflatoxigenic fungi. Additionally, Ibadan is generally more humid than Kaduna, providing more ideal conditions for aflatoxin biosynthesis.

Additionally, whereas Kaduna samples did not have detectable lead concentrations, lead was present in samples taken from Oyo State. Lead exposure to food could be from the industrial pollution to the soils, from holding containers used for packaging and handling of ginger along the value chain, leaching into water from lead pipes or lead containing equipment. It is not clear the specific or primary routes of exposure through which lead has contaminated the ginger samples in this case. Consequently, this requires further investigation.(Codex Alimentarius Commission, 2004)

Based on the results obtained from this current study, open air sun drying particularly on bare soil or on cemented surfaces was the dominant form of drying used. Only four (7.8%) of the samples collected were from covered surfaces in Kaduna state (all from Zango local government). This indicates that sun-drying on uncovered surfaces is the common means of drying split ginger samples. Alternative solar drying innovations such as the parabolic solar dryers, offer superior results to open-air dryers offering 65% less drying time (Chiewchan, Mujumdar and Devahastin, 2015; Kamaldeen O. S and Okedokun O. W, 2022). Open-air drying on uncovered surfaces would predispose the ginger slides to direct exposure to environmental microbes in the soil and from the environment. As *Aspergillus* species are ubiquitous, it can be a route of exposure to contamination by the species and consequently lead to the accumulation of aflatoxins in the crop. Rapid drying is preferable as it would limit exposure to ubiquitous mycotoxins and reduce the rate of biosynthesis of aflatoxins encouraged by high moisture content in the crops and the humidity within containers holding the produce in storage (Muga, Marenya and Workneh, 2019b)

Co-occurrence of heavy metals with aflatoxins is reported in these samples.Umar and Salihu, (2014) reported the presence of non-essential and essential heavy metals in ginger, with higher concentrations in processed samples compared to the fresh samples. Among the samples obtained from Kaduna, all samples but one (a split-dried sample from Zango local government) contained detectable concentrations of at least one of the heavy metals investigated. The copper and zinc concentrations were below the toxicity levels for these metals, indicating that they were within the safe limits. However, as these are micronutrients needed for food, these low levels suggest that alternative food sources and supplements are required for the daily consumption requirements for these micronutrients to be met. On the other hand, 14% of the samples taken from markets in Oyo state contained unsafe concentrations of lead, and 51% of the samples from both Kaduna and Oyo states contained concentrations of mercury that exceeded the safe limits. These metals are naturally occurring and could have contaminated the rhizomes during production and postharvest, as they are detectable in both fresh and processed samples. The incidence of these heavy metals is a subject of concern.

Due to the limited infrastructure, including poor road organization, sub-optimal processing methods used along the ginger value chain by small holders, micro, small, and medium scale enterprises (MSMEs), the exposure of ginger to contamination is likely via the practices utilised in ginger processing (Abah, 2019b). It is therefore important for interventions to be in place to reduce the incidence of mycotoxins and ameliorate the risks associated with dietary exposure to aflatoxins. Although the milled ginger is reported to have a higher economic value than dried and fresh ginger (Abah, 2019b); in the current study, milled ginger from Oyo State was reported as the most contaminated with aflatoxins. For this reason, there have been fora, including initiatives by Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, a German development agency, to improve the ginger value chain deficits in Nigeria (Jonne Bruecher, Vinambres and Omoware, 2019). Export rejections and reparations harm the livelihoods of farmers and value chain players about income loss, but even more importantly impact the health and well-being of domestic consumers who are exposed to contaminated ginger from the repatriations or the domestically traded ginger that is not tightly monitored and regulated for safety and quality in domestic value chains.

Efforts to control mycotoxins and other toxicants are therefore important for the food security (including food safety) of consumers. Consequently, in the current study, samples were taken from a major ginger producing state in Nigeria (Kaduna State) and a major ginger consuming state in Nigeria (Oyo State) to assess the levels of incidence of aflatoxins and heavy metals in the samples along the value chain, from primary production to processing and marketing in the informal markets.

The use of probiotics provided some value in the reduction of total aflatoxin concentration over the 120-h treatment period. Within the first 24-h, the aflatoxin concentration was the same as the starting concentration or with only marginal reduction. Reduction was more marked and consisten till the 120-h time point with *L. plantarum*, but not so when *L. helveticus* was present. Rather in the presence of *L. helevticus* (either alone or in combination with *L. plantarum*), concentrations reverted to their starting concentration at 72-h time point. The authors surmise that the binding of the aflatoxins to the cells was temporarily

reversed at 72-h due to a saturation of the binding sites of *L. heleveticus* and possible crosstalk between the two LAB when *L. plantarum* was present. Aflatoxin decontamination by lactobacilli has been described to occur by binding via the cell wall constituents, comprising mainly the peptidoglycan layer in addition to polysaccharides, proteins, and teichoic acids (Shetty, Hald and Jespersen, 2007; Liew et al., 2018). The parity between *L. helveticus* and *L. plantarum* at the 72-h incubation may occur because of differences in the architecture or biochemical processes during aflatoxin binding or saturation on the cell wall constituents. However, this has not been described and requires further investigation. Whereas the structural architecture of *L. helveticus* has been reported to include a cell wall thickness of 20 to 80 nm(Firtel, Henderson and Sokolov, 2004) but that for *L. plantarum* has not been reported.

Conclusion

In this study, we find that multiple toxicants including aflatoxins and heavy metals co-occur in samples obtained from ginger and this pose both liver cancer risks (for aflatoxins) and non-carcinogenic risks (for lead and mercury). There are location variations with respect to the extent of contamination and the risks of population exposure, requiring further investigation and context-specific management measure for consumer health protection. Probiotics can offer a means of decontamination of aflatoxin-contaminated ginger. However, more insights are needed to understand binding mechanisms and potentials for intermittent release of the toxins.

Declarations

Author Contribution

T.F. conceptualised the idea, supervised the research sampling and laboratory analyses, managed the project, sought funding for the associated study costs, wrote the first draft of the manuscript and revised the manuscript. I.I. collected research samples, conducted research analyses, wrote parts of the manuscript and edited the draft manuscript. K.B. conceptualised the idea, supervised research activities and edited the draft manuscript. V.S. conducted laboratory analyses, wrote parts of the manuscript and edited the draft manuscript. R.D. sought funding for associated study costs, contributed to experimental design and edited the draft manuscript.

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Data Availability

Data has been submitted to the repository of the International Institute of Tropical Agriculture CKAN (Open Data Portal)

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Figures

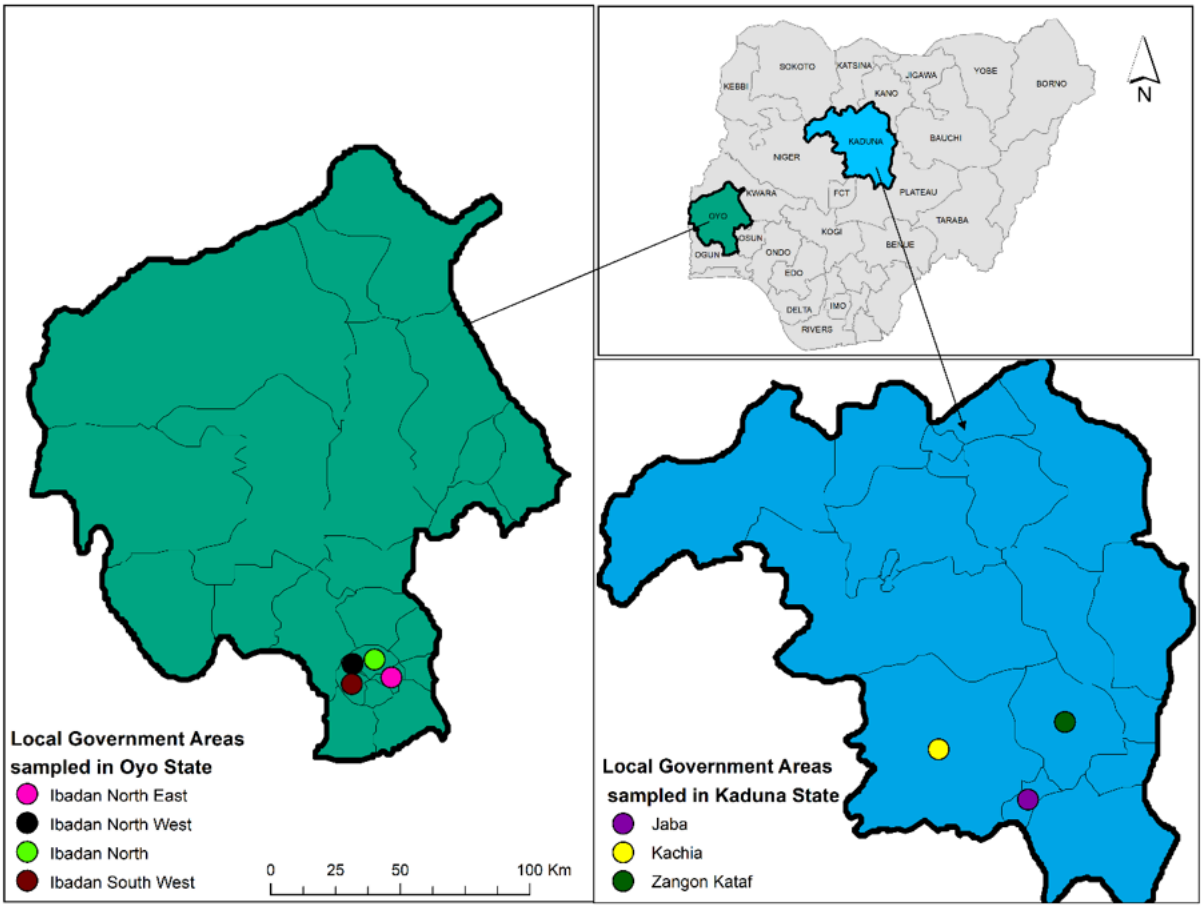

Figure 1

Map of the Nigeria showing locations in Kaduna and Oyo states where samples were collected.

Figure 2

Ginger samples obtained in (a) fresh (b) split dried (c) milled forms.

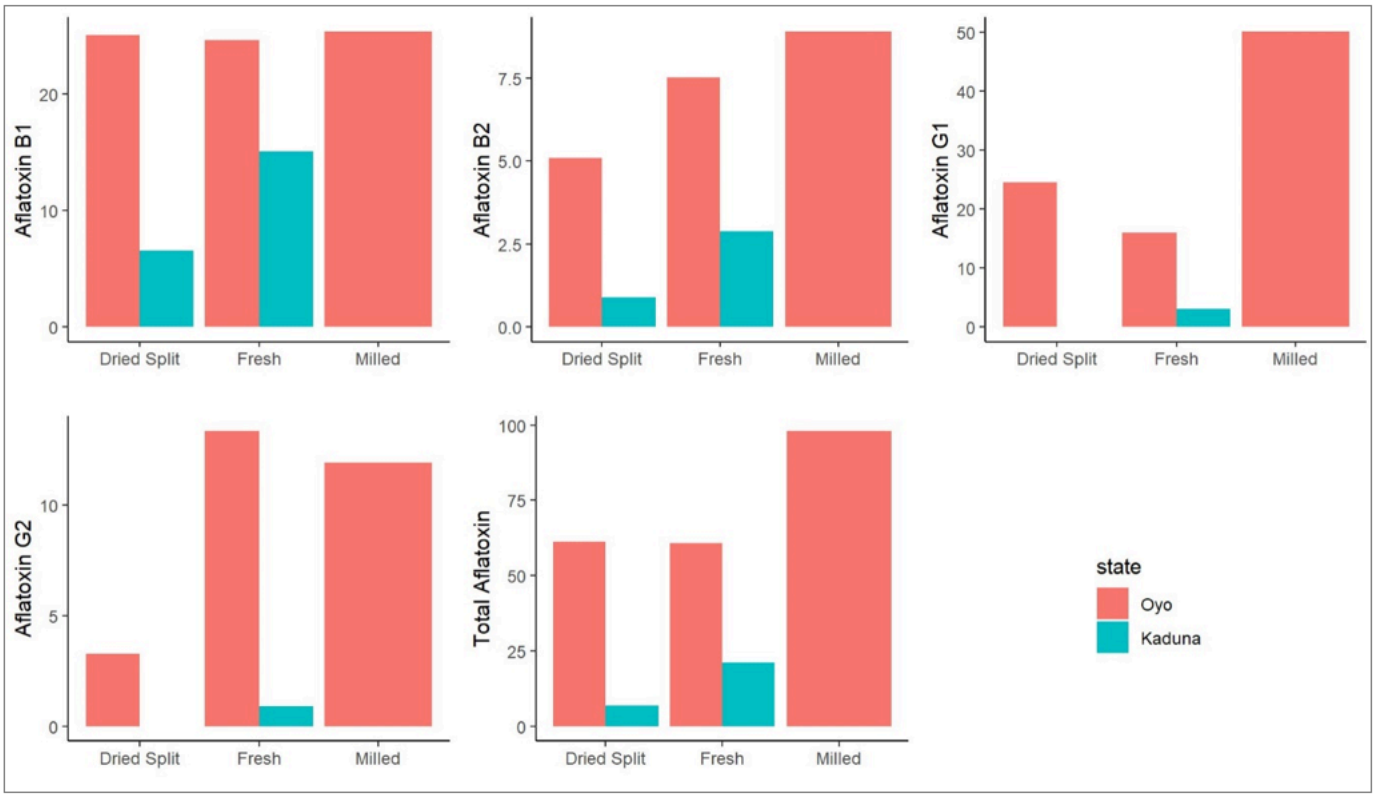

Figure 3

Aflatoxin concentrations in ginger sampled from Kaduna and Oyo States in different form

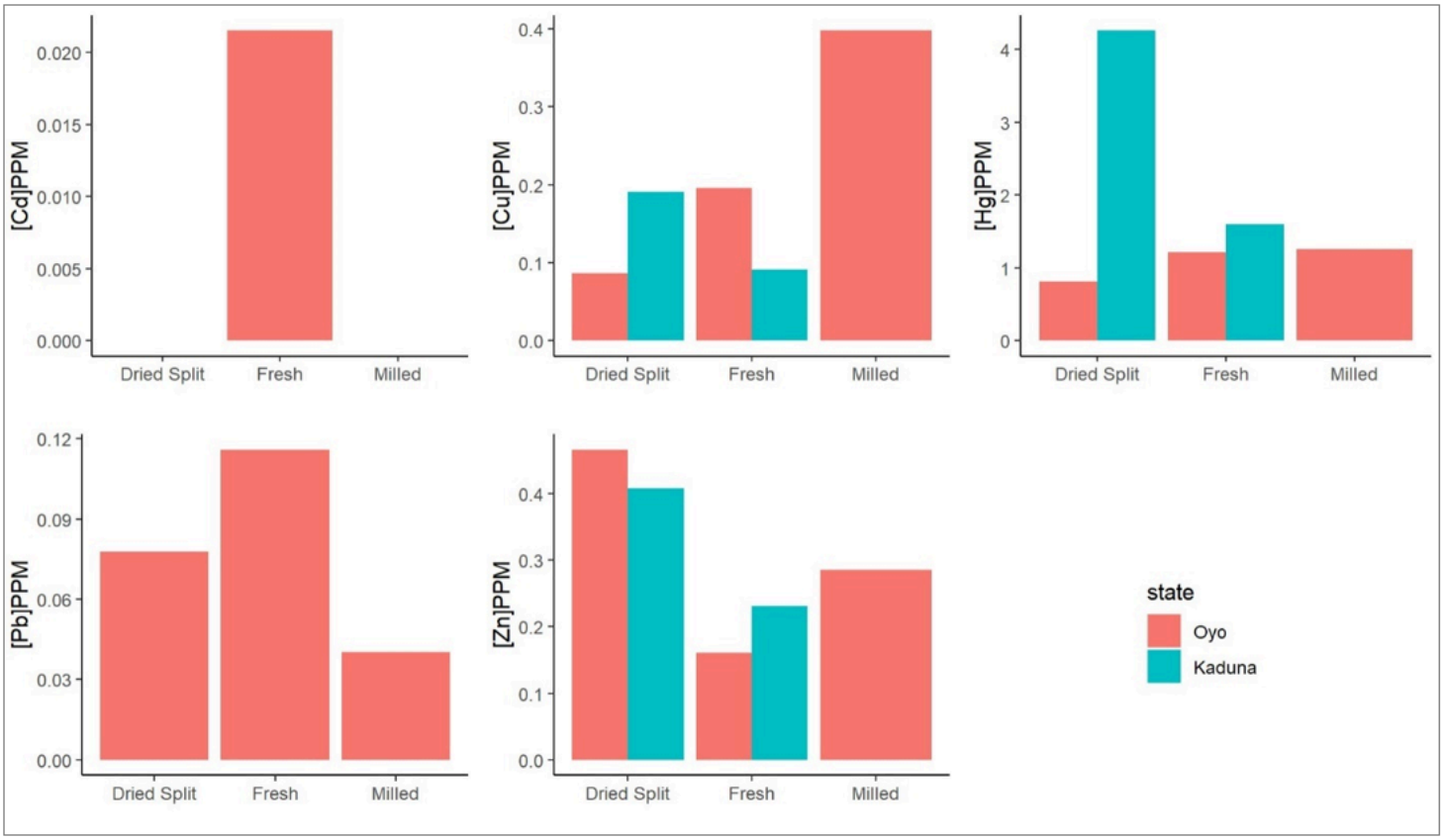

Figure 4

Heavy metal concentrations in ginger sampled from Kaduna and Oyo States in different forms