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Research Article

Aflatoxin content and heavy metals composition of powdered pepper from selected markets in Kwara state, Nigeria

Wasiu Awoyale[1]*, Nwineh L. Zorbari[1], Lateef Oladimeji Sanni[2]

[1]*Department of Food Science & Technology, Kwara State University Malete, PMB 1530, Ilorin, Kwara State 241104, Nigeria*
[2]*Department of Durable Crop Research, Nigeria Stored Products Research Institute, Ilorin, PMB 1489, Kwara State 241104, Nigeria*

Abstract Aflatoxin and heavy metal concentrations may pose health risks to pepper consumers. No research has been published concerning aflatoxin and heavy metal contamination of powdered pepper in Kwara state, Nigeria, hence the need for this study. Powdered pepper samples from various Kwara state markets (Offa, Oja-Oba, Ganmo, Shao, Elemere, and Malete), were analyzed using standard methods for aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), and lead, cadmium, copper, and arsenic. A laboratory-prepared sample was used as the control. The samples' mean AFB1 was 2.86 μg/kg, AFG1 4.34 μg/kg, AFB2 2.03 μg/kg, and AFG2 1.88 μg/kg. In general, the levels of aflatoxin in the powdered pepper samples were found in the order of AFG1 > AFB1 > AFB2 > AFG2. The samples' aflatoxin concentration is less than the US Food and Drug Administration's (20 μg/kg) contamination threshold. The FDA's guideline for lead level in food, which is 0.1 mg/kg, was surpassed by the lead concentration, which varied from 1.84 to 3.45 mg/kg. The range of arsenic concentration was 10.18-22.68 mg/kg, which is higher than the World Health Organization's recommended limit of 0.0003 mg/kg. The copper concentration met the FDA's 10-mg/kg threshold, which ranged from 2.66 to 4.42 mg/kg. The study's findings demonstrate the need for a monitoring and education programme in Kwara state to ensure the public's safety while consuming powdered pepper.

Keywords food contamination, commercial pepper, aflatoxin concentration, heavy metals

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***Corresponding author**
Wasiu Awoyale
Tel: +234-8062146482
E-mail: wawoyale0101@gmail.com

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1. Introduction

In Nigeria and Sub-Saharan Africa, food contamination because of careless handling and inadequate processing is a significant issue. Food contamination by heavy metals and aflatoxin is frequently caused by industrial expansion, agricultural chemicalisation advancements, and human activity in metropolitan areas (Orisakwe et al., 2012). Aflatoxin and heavy metal contamination can result from fungal infection, growth, and colonization in peppers due to a variety of ecologies, weather conditions, agricultural and cultural practices, inadequate postharvest technologies, and storage systems used by workers throughout the pepper value chain (Farombi et al., 2020).

Toxic secondary metabolites known as aflatoxins are primarily produced by the Aspergillus fungus. Aflatoxin B1 (AFB1), aflatoxin G1 (AFG1), aflatoxin B2 (AFB2), and aflatoxin G2 (AFG2) are the main forms of aflatoxins (Kumar et al., 2021). According to Al-Ghouti et al. (2020), AFB1 is mostly found in food and is thought to be the most harmful to both people and animals. Aflatoxin-contaminated food can result in serious health hazards, including cancer in people and animals, as well as weakened immune systems and epidemics of type B viral hepatitis (Alamu et al., 2020). As a result, even at extremely low concentrations, aflatoxins were categorised as category 1 carcinogens by the International Agency for Research on Cancer (IARC, 2012). Fungi may produce aflatoxin under a range of growth circumstances, including temperature, humidity, water activity, light intensity, and pH. They can also produce aflatoxin under different growing, harvesting, transporting, processing, and storage conditions and times (Kumar et al., 2021).

On the other hand, heavy metals are one of the primary contaminants in our food supply and could be the biggest threat to the environment as they can lower plant production and jeopardise the safety of plant-based foods intended for human consumption (Zheljazkov et al., 2006). Globally, this issue is only becoming worse, particularly in emerging nations. Heavy metals generally have lengthy biological half-lives, are not biodegradable, and can accumulate in many human organs, potentially causing unfavourable side effects (Radwan and Salama, 2006). Eating food contaminated with heavy metals can seriously harm a person's health since it can deplete the body of some vital minerals (Arora et al., 2008). According to Galanis et al. (2009), there is evidence linking exposure to toxic metals to a range of serious health issues, including renal difficulties, neuro-behavioural and developmental abnormalities, high blood pressure, and maybe cancer.

According to Wikandari et al. (2020), the infected dried chili pepper in Indonesia's traditional and modern markets had levels of aflatoxins B1 and B2 between 39.3 μg/kg and 139.5 μg/kg, and 2.6 μg/kg and 33.3 μg/kg, respectively. The researchers noted that Indonesian dried chili peppers can become infected with fungus both in the field and in storage. Pepper sold at large markets in Osogbo, Osun state, Southwest Nigeria, has been shown to contain significant amounts of heavy metals (Farombi et al., 2020). When compared to market samples free of vehicle exhaust, the researchers found that the collected pepper samples were not contaminated with heavy metals (cadmium, iron, lead, zinc, cobalt, and chromium). However, the introduction of environmental pollution, primarily from vehicle exhaust and other gaseous air pollutants, may have increased the value of these heavy metals. No research concerning aflatoxin and heavy metal contamination has been published on the safety of powdered pepper in Kwara state, Nigeria. Thus, this work aims to close this research gap.

2. Materials and methods

2.1. Materials

The fresh pepper fruit used to produce the control sample was purchased from a local market in Ilorin, and the commercial powdered pepper samples were collected from six markets (Offa, Oja-Oba, Ganmo, Shao, Elemere, and Malete) in Kwara state.

2.2. Methods

2.2.1. Production of okra powder, and commercial sample collection.

Powdered pepper samples (200 g) were obtained from selected markets (Offa, Oja-Oba, Ganmo, Shao, Elemere, and Malete) in Kwara state. The samples were collected and properly packaged and labeled in a Ziplock bag before laboratory analyses.

2.2.2. Determination of aflatoxin

Analysis of aflatoxin (B1, B2, G1, and G2) in the powdered pepper samples was performed with high-performance liquid chromatography (HPLC) device (1100 series, Agilent, Newark, NJ, USA) with a fluorescent detector, after purification using an immunoaffinity column (IAK). As a method, the method of AOAC (2005) (999.07) was used.

2.2.3. Determination of heavy metals

A Perkin-Elmer ELAN DRC-e model ICP-MS system equipped with a Scott Spray Chamber (Norwalk, Conn., USA) was used for simultaneous multi-element detection of Pb, Cd, Hg, and Ar. Before the instrumental analysis, the instrument was tuned by 10 μg/L of a multi-element standard solution. The ICP-MS operational conditions were as follows: RF Power was set to 1,000, Nickel skimmer and sampler cone were used, and nebulizer gas flow, auxiliary gas flow, and plasma gas flow were set to 0.81, 1.20, and 19 L/min Ar, respectively. Standard analytical mass (amu) mode was used for the elements. The number of sweeps, readings, and replicates were 20, 1, and 3, respectively. Dwell time per amu was set to 50 ms (Kilic and Serpil, 2019).

2.2.4. Statistical analysis

Mean values of the determinations of all the analyses were

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subjected to a one-way analysis of variance (ANOVA) to determine the significant difference, and the means will be separated using the Duncan multiple range test at a 95% confidence level ($p<0.05$) using statistical package for social sciences (SPSS) version 20.

3. Results and discussion

3.1. Aflatoxins content in powdered pepper from selected markets in Kwara state

Metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* are known as aflatoxin, one of the essential classes of mycotoxins. The harvesting, production, processing, and storage of various kinds of food commodities result in the production of aflatoxin, which is carcinogenic, immunosuppressive, immunotoxic, hepatotoxic, and teratogenic (Al-Ghouti et al. 2020). In most African nations, sun-drying peppers is a typical postharvest technique used to minimise pepper postharvest losses. This technique includes spreading peppers on soil in a single layer, which might lead to fungal infection (Iqbal et al., 2021). Because of their prevalence in nature and toxicity, the four main aflatoxins—AFB1, AFB2, AFG1, and AFG2—are the most significant mycotoxins in foods and feeds (Ifeanacho et al., 2017). The aflatoxin concentration of powdered pepper from several Kwara state market-places is displayed in Table 1. AFB1 is 2.86 μg/kg on average, AFG1 is 4.34 μg/kg, AFB2 is 2.03 μg/kg, and AFG2 is 1.88 μg/kg among the samples. AFG1 > AFB1 > AFB2 > AFG2 was the overall sequence in which aflatoxin levels were observed in the powdered pepper samples. Aflatoxin concentration varies significantly ($p<0.01$) among all pepper samples (Table 1).

Because AFB1 may cause liver cancer in humans, it is the most dominant and common type of aflatoxin and is categorised as a Group 1 carcinogen. B1 aflatoxins are the most dangerous of all because they prevent animals from forming proteins and nucleic acids (Al-Ghouti et al., 2020). The powdered pepper samples' AFB1 level varied between the Elemere and Oja-Oba markets, ranging from 1.39 μg/kg to 7.64 μg/kg (Table 1). All these AFB1 levels are below the FDA's 20 μg/kg contamination limit (2021). However, the European Union Commission Regulation's (2010) maximum acceptable level for AFB1 (5 μg/kg) was exceeded by the AFB1 value of powdered pepper that was collected from the Oja-Oba market. All the samples' AFB1 values—aside from those from the Oja-Oba and Ganmo markets—are lower than the range of values (2.51-63.20 μg/kg) reported for ground red pepper that was collected from particular regions in Ethiopia's Amhara Region (Adugna et al., 2022). Poor transportation and storage practices, variations in temperature and humidity, and other factors might be responsible for these contamination differences (Adugna et al., 2022).

Hepatocellular carcinoma, a very aggressive type of cancer, is known to be caused by AFB2, a genotoxic carcinogen, and food contaminants (Gündüz et al., 2021). While research on AFB2's harmful effects on human health is limited, Santini and Ritieni (2013) noted that the compound has been shown

Table 1. Aflatoxins content (μg/kg) in powdered pepper from selected markets in Kwara state, Nigeria

Samples	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
Control	1.46 ± 0.04^{d}	1.96 ± 0.04^{b}	7.54 ± 0.04^{a}	1.80 ± 0.04^{d}
Offa market	$1.86\pm0.04^{1)c2)}$	2.24 ± 0.06^{a}	2.01 ± 0.06^{d}	2.55 ± 0.06^{a}
Oja-oba market	7.64 ± 0.05^{a}	1.91 ± 0.05^{b}	1.52 ± 0.05^{e}	1.91 ± 0.05^{cd}
Ganmo market	3.96 ± 0.07^{b}	1.93 ± 0.06^{b}	7.47 ± 0.06^{ab}	2.24 ± 0.06^{b}
Shao market	1.84 ± 0.04^{c}	1.92 ± 0.04^{b}	2.31 ± 0.04^{c}	1.38 ± 0.03^{e}
Elemere market	1.39 ± 0.04^{d}	1.88 ± 0.04^{b}	2.22 ± 0.04^{c}	2.00 ± 0.00^{c}
Malete market	1.87 ± 0.06^{c}	2.33 ± 0.06^{a}	7.36 ± 0.06^{b}	1.33 ± 0.06^{e}
Mean	2.86	2.03	4.34	1.88
p-level	***	*	***	***

1)All values are mean±SD (n=3).
2)Means with the same superscripts (a-e) within the same column are not significantly different ($p>0.05$).
*$p<0.05$, ***$p<0.001$.

to have harmful hepatotoxic, teratogenic, and carcinogenic effects on different kinds of farm animals. According to Table 1, the powdered pepper's AFB2 in this investigation varied from 1.88 μg/kg in the Elemere market to 2.33 μg/kg in the Malate market. It is essential to note that when compared to the control sample, the AFB2 concentration of all the powdered pepper samples collected from some Kwara state markets (except the Offa and Malete markets) did not differ significantly (p>0.05). The levels of AFB2 in the powdered pepper in this investigation were lower than the values (0.04-1.28 μg/kg) published in Turkey (Ozbey and Kabak, 2012), but they were still within the range of values (0.96-5.29 μg/kg) recorded for red pepper in Ethiopia (Adugna et al., 2022). However, none of the powdered pepper samples had an AFB2 value higher than 20 μg/kg (FDA, 2021).

Some fungal species, particularly those in the Aspergillus genus, contain a mycotoxin called AFG1, which causes chronic liver cancer, or hepatocellular carcinoma (Zamir-Nasta et al., 2021). AFG1 was lowest in powdered pepper from the Oja-oba market (1.52 μg/kg) and highest in the control sample (7.54 μg/kg) (Table 1). In terms of the AFG1 content, there was no statistically significant difference (p>0.05) between the powdered pepper samples obtained from the markets in Ganmo and Malete and the control sample. This suggests that the soil may be the source of the powdered pepper contamination rather than the individuals or the storage environment. The powdered pepper samples' range of AFG1 levels was like the values documented in the literature (Jalili, 2016; Karaaslan and Arslangray, 2015; Rosas-Contreras et al., 2016). While Adugna et al. (2022) found values ranging from 1.71 to 32.79 μg/kg for red peppers in Ethiopia, the current study's findings correspond with some of the higher values. Nonetheless, the AFG1 concentration in the powdered pepper samples is lower than the FDA's declared limit of contamination (FDA, 2021).

Mycotoxin AFG2 is produced by specific mold species, namely *Aspergillus flavus* and *Aspergillus parasiticus*. Although it is regarded as less dangerous than AFB1, this toxin is a member of the aflatoxin family, which is well-known for its strong carcinogenic and poisonous effects on both people and animals (Popescu et al., 2022). It may contaminate many types of food crops, especially in warm, humid climates where mold development is more common. When consumed or breathed, it offers serious health hazards since it can weaken the immune system and harm the liver (Popescu et al., 2022). According to Table 1, the powdered pepper's AFG2 level varied between 1.33 μg/kg in the Malete market sample and 2.55 μg/kg in the Offa market sample. Adugna et al. (2022) for red pepper in Ethiopia (0.53-2.04 μg/kg) and Rosas-Contreras et al. (2016) for pepper in Mexico (0.05-3.35 μg/kg) reported results that are comparable to the range of values found in this investigation. The AFG2 concentration of the powdered pepper in this investigation was lower than the aflatoxin contamination threshold published by the FDA (2021).

3.2. Heavy metals composition in powdered pepper from selected markets in Kwara state

The earth's crust naturally contains heavy metals. When it comes to contamination and pollution of the environment, they are steady and enduring. According to Upadhyay et al. (2019), heavy metals comprise both hazardous and essential elements, such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg). Essential elements include iron (Fe), zinc (Zn), copper (Cu), etc. The average Pb amount in the pepper samples is 3.47 mg/kg, whereas the average As and Cu contents are 12.01 mg/kg and 4.46 mg/kg, respectively. No Cd was found in any of the samples. The samples' Pb, Ar, and Cu contents varied significantly (p<0.01) (Table 2).

High levels of lead exposure have been linked to elevated blood pressure, neurological abnormalities, muscle and joint discomfort, and problems with reproduction (EPA, 2007). According to Sridevi-Sangeetha and Umamaheswari (2020), Pb has been linked to aberrant movement and reflexes, peripheral neurological effects, peripheral neuropathy, inhibition of ham biosynthesis, mild anaemia, and ulcers. According to the study's findings, the powdered pepper from the Ganmo market had the highest Pb level (3.45 mg/kg), while the powdered pepper from the Offa market had the lowest Pb value (1.84 mg/kg) (Table 2). All the pepper samples had Pb concentrations higher than the 0.1 mg/kg threshold for Pb in foods established by the FDA (2021). This might be attributed to the introduction of metallic contaminants during production and storage from commercial and industrial operations as well as vehicle exhaust emissions. A tentative maximum tolerated daily Pb consumption of 0.025 mg/kg body weight has been defined by the WHO (2006). This indicates that with time, eating powdered pepper from each market in food once may not cause lead poisoning. According to Upadhyay et al.

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Table 2. Heavy metals (mg/kg) composition in powdered pepper from selected markets in Kwara state, Nigeria

Samples	Lead	Arsenic	Copper	Cadmium
Control	3.45±0.01[1)c2)]	21.75±0.18[b]	3.05±0.10[f]	ND[4)]
Offa market	1.84±0.02[e]	22.68±0.11[a]	3.64±0.03[e]	ND
Oja-oba market	3.37±0.22[c]	14.53±0.13[e]	4.07±0.09[d]	ND
Ganmo market	6.25±0.04[a]	10.18±0.14[g]	2.64±0.03[g]	ND
Shao market	1.85±0.01[e]	20.22±0.14[c]	6.42±0.02[a]	ND
Elemere market	2.88±0.06[d]	18.46±0.01[d]	6.14±0.06[b]	ND
Malete market	4.64±0.04[b]	12.01±0.15[f]	5.27±0.09[c]	ND
Mean	3.47	17.12	4.46	
p-level	***3)	***	***	

1)All values are mean±SD (n=3).
2)Means with the same superscripts (a-g) within the same column are not significantly different (p>0.05).
3)***p<0.001.
4)ND, not detected.

(2019), arsenic is a common hazardous metal that is part of the periodic table's metalloid group. It may be found naturally in the lithosphere, hydrosphere, atmosphere, and biosphere. In nature, As may be found in both organic and inorganic forms (primarily in the form of complexes). Numerous pathways for movement within the environment have been established, and reports of quite high concentrations (mostly in water sources) have been reported in several parts of the world (Medunic et al., 2020). A general, preliminary maximum tolerated daily consumption of 0.0003 mg/kg body weight has been defined by the WHO (2006). Arsenic content was found to be higher (22.68 mg/kg) in powdered pepper from the Offa market and lower (10.18 mg/kg) in that from the Ganmo market (Table 2). The presence of As compounds in the powdered pepper samples may be related to the irrigation of pepper with contaminated water and growing in contaminated soil, since the control sample obtained in a sterile environment contained as much as 21.75 mg/kg of As (Medunic et al., 2020). This is in line with the findings of Shahid et al. (2018), who said that the As pollution of water—particularly groundwater, given its extreme toxicity—is seen as a serious health concern in many parts of the world. As a result, there's a chance that some of the pepper samples have arsenic poisoning.

In trace levels, copper, a naturally occurring heavy metal, is vital to human health. However, excessive Cu concentrations can be harmful. Cu may enter food as a contaminant from the environment, such as polluted soil or industrial pollutants. The clinical importance of Cu poisoning has been examined, and various illnesses can be caused by high exposure to Cu (Taylor et al., 2020). According to Table 2, the powdered pepper sample from the Shao market had the highest Cu level (6.42 mg/kg), whereas the powdered pepper sample from the Ganmo market (2.64 mg/kg) had the lowest Cu value. The fact that even the control sample, which was produced in a sterile environment, had a Cu content of up to 3.05 mg/kg is significant because it suggests that the root cause of the high Cu content in the powdered pepper samples may be soil contamination during plant cultivation rather than improper processing techniques. For all food additives, including spices, the Food and Drug Administration has set a maximum permissible quantity of Cu at 10 mg/kg (FDA, 2021). Consequently, the pepper samples' total Cu concentration is below the upper limit allowed, indicating that they do not contain any Cu toxicity. However, mechanical abrasion and wear and tear of vehicles may be the source of the changes in the Cu content of the powdered pepper samples, as well as the release of Cu into the roadside environment (Harrison et al., 1981).

4. Conclusions

AFG1 > AFB1 > AFB2 > AFG2 was the sequence in which aflatoxin levels were discovered in the powdered pepper samples used in this investigation. The aflatoxin levels in the samples are below the 20 μg/kg contamination standard set by the FDA. The lead levels in the powdered pepper samples exceeded the FDA's recommended amount of 0.1 mg/kg. The concentration range of arsenic was also above the 0.0003 mg/kg recommended limit by the WHO. The control sample has significant levels of lead and arsenic, which may be related to the farmed soil. The order of the heavy metal occurrence in the powdered peeper is As > Cu > Pb. This study emphasize the need for a monitoring and education program in Kwara state to ensure the safety of powdered pepper consumed by the public and the type of soil where pepper should be planted.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Awoyale W, Zorbari NL, Sanni LO. Methodology: Awoyale W, Zorbari NL. Formal analysis: Awoyale W, Zorbari NL. Validation: Awoyale W, Sanni LO. Writing - original draft: Awoyale W, Zorbari NL. Writing - review & editing: Awoyale W, Zorbari NL, Sanni LO.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Wasiu Awoyale (First & Corresponding author)
https://orcid.org/0000-0002-3635-1414
Nwineh L. Zorbari
https://orcid.org/0009-0007-6973-4562
Lateef Oladimeji Sanni
https://orcid.org/0000-0003-3363-6091

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