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IS COMMERCIALLY AVAILABLE POWDERED OKRO IN KWARA STATE SAFE FOR CONSUMPTION?

***Wasiu AWOYALE[1], Olabanji I. ABODUNRIN[1], Lateefat A. OYAFAJO[2] & Lateef O. SANNI[3]**
[1]*Department of Food Science & Technology, Kwara State University Malete, PMB 1530, Ilorin Kwara State, Nigeria.*
[2]*Department of Food Science & Technology, Osun State University, PMB 4494, Osogbo, Osun State, Nigeria.*
[3]*Office of the Executive Director, Nigerian Stored Product Research Institute, PMB 1489, Ilorin, Kwara State, Nigeria.*
*Corresponding author: *wasiu.awoyale@kwasu.edu.ng*
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Abstract: *The production and commercialization of powdered okro is growing in the informal sector in Kwara state, with no documentation on consumer safety related to heavy metal and aflatoxin contamination. Therefore, this study aims to evaluate the heavy metal and aflatoxin contents of powdered okro sold in several Kwara state markets. Samples of powdered okro (200 g) were collected from markets in Offa, Osi, Ojatuntun, Ojaoba, Elemere, and Malete in a Ziplock bag, and analyzed for heavy metals (lead, cadmium, copper, and arsenic) and aflatoxins (B1, B2, G1, and G2) content using standard methods. As a control sample, powdered okro made in a laboratory was utilized. The findings show that the most common heavy metals and aflatoxin in the powdered okro were, on average, copper (0.20 mg/kg) and aflatoxin G2 (2.95 µg/kg), respectively. Okro from the Malete market had more copper (0.32 mg/kg), while okro from the Ojatuntun market had less copper (0.11 mg/kg). Aflatoxin G2 levels in powdered okro were highest (3.60 µg/kg) in the Malete market and lowest (1.96 µg/kg) in the Elemere market. Malete market's powdered okro and others may be safe for human consumption because their copper and aflatoxin G2 levels are within the FAO/WHO permitted range. However, to reduce the potential risks associated with long-term accumulation of copper and aflatoxin G2 in the human body, the use of healthy okro fruits, and the processing of the fruits in a controlled-free environment of vehicular movement, environmental pollution, and process them in a clean, dry place away from traffic, pollution, and moisture.*
Keywords: *powdered okro, markets in Kwara state, contamination, heavy metals, aflatoxin, food safety*

1. Introduction

In Nigeria and Sub-Saharan Africa, food contamination caused by careless handling and poor processing is a significant issue. Food contamination by heavy metals and aflatoxin is frequently caused by industrial expansion, agricultural chemicalization advancements, and human activity in metropolitan areas [1]. One of the main environmental problems that might affect plant production and jeopardise the safety of plant-based foods for human consumption is heavy metal contamination, which is one of the main pollutants found in the food supply [2]. Globally, this issue is only becoming worse, particularly in emerging nations. In general, heavy metals have lengthy biological half-lives, are not biodegradable, and can accumulate in many human organs, potentially causing unfavorable side effects [3]. Food contaminated with heavy metals can seriously harm a person's health since it can deplete the body of some vital minerals [4]. According to Galanis et al. [5], there is evidence linking exposure to toxic metals with a range of serious health issues, including renal difficulties, neurobehavioral and developmental abnormalities, high blood pressure, and possibly cancer. Thus, understanding the levels of heavy metals in food is important because some of these elements, like copper, iron, manganese, and zinc, are vital

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to human health, while others like cadmium, lead, mercury, and arsenic can be hazardous even at low concentrations [6]. The two most vital resources for human survival, soil and water, are currently the main causes of heavy metal pollution. Unplanned urbanization, overuse of natural resources, mining, conflict, industrialization due to climate change, illegal sewage and effluent discharge, misuse of agrochemicals, atmospheric deposits, vehicle emissions, and fuel combustion are the primary causes of heavy metal contamination in food crops [7]. Nowadays, practically every type of water, including gulfs, seas, and all inland water bodies, is susceptible to heavy metal pollution [8]. Most of the irrigation water utilised in poor nations contains heavy metals [9]. Heavy metal levels in agricultural soil are raised by wastewater irrigation, a frequent practice in urban and peri-urban regions, which contaminates food crops and people [10]. One of the main causes of heavy metal pollution in food crops is the continuous use of wastewater for irrigation [11] and the overuse of agrochemicals [12]. Toxic secondary metabolites known as aflatoxins are mostly generated by the *Aspergillus fungus*. Aflatoxin B1 (AFB1), aflatoxin G1 (AFG1), aflatoxin B2 (AFB2), and aflatoxin G2 (AFG2) are the main types of aflatoxins [13]. According to Al-Ghouti et al. [14], 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 [15]. As a result, even at extremely low concentrations, aflatoxins were categorised as category 1 carcinogens by the International Agency for Research on Cancer [16]. Fungi may produce aflatoxin under a variety of growth conditions, 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 [13]. Dried Okro may not be left out of aflatoxin contamination.

Okro is one of the perishable commodities due to its high moisture content [17]. The main traditional method of post-harvest okro preservation is sun-drying in most African regions. After slicing the okro fruits into small chips, they are indiscriminately sun-dried on rooftops, concrete constructions, mats, along roadsides, and in courtyards for about three or more weeks, depending on the intensity of sunlight. This unhygienic art risks exposing the commodity to direct heavy metals and aflatoxin contamination or indirectly from dust, flies, rodents, and even human handlers [17]. There have been reports of heavy metal contamination of okro from various areas, including nickel, copper, zinc, and lead [18]. Kumar et al. [13] found that the amounts of heavy metals in the chosen okro were higher than the anticipated daily intake (<1 mg/kg). Also, the FAO/WHO [19] stipulated maximum permissible values of heavy metals in vegetables to be 0.2 mg/kg for cadmium, 0.3 mg/kg for lead, 67.90 mg/kg for nickel, 425.50 mg/kg for iron, 73.3 mg/kg for copper, and 99.40 mg/kg for zinc. *Aspergillus flavus, Aspergillus niger,* and *Aspergillus parasticus* were the isolated aflatoxins found in okro from certain markets in Ibadan, Oyo state [20]. The European Union Commission Regulation maximum acceptable level of aflatoxin in foods is 5 µg/kg [21], while that of the US Food and Drug Administration is 20 µg/kg contamination limit [22], There is a possibility of heavy metals and aflatoxin contamination of powdered okro, due to the introduction of environmental pollution and processing methods among others. However, no research on the safety of powdered okro to heavy metal and aflatoxin contamination has been published for Kwara State. Thus, this work aims to close this research gap.

2. Materials and methods

2.1 Materials

The commercial powdered okro samples were collected from nine marketplaces in Kwara State, whereas the fresh okro fruit used for producing the control sample was bought from the Ojaoba market in Ilorin, Kwara State. All the okro fruits prepared into powdered okro and sold in the different markets were grown in Kwara State, Nigeria.

2.2 Methods

2.2.1 Production of powdered okro and commercial sample collection.

The process of making powdered okro involved washing fresh okro fruits, cutting off the stem, calyx, and apex, and slicing the remaining portion transversely (10 mm thick). Then, the fruit was dried at a low temperature (50±2 °C) in a vacuum oven (WIPA, GEASS, Turin, Italy) [23]. The powdered okro was then sealed in a Ziplock bag and set aside for further analysis. About 200 g of powdered okro samples were purchased from different marketplaces. The markets are Offa market (8°9'32.22" N 4°43'28.22" E), Osi market (8°6'36.05" N 5°14'59.17" E), Ojatuntun market (8°25'9.36" N 4°36'27.09" E), Ojaoba market (8°29'49.15" N 4°32'57.16" E), Elemere market (8°43'0" N 4°30'0" E) and Malete market (8°42'39.81" N 4°27'48.32" E). The samples were collected and properly packaged and labeled in a Ziplock bag before laboratory analyses.

2.2.2 Determination of heavy metals

The okro sample (0.2 g) was weighed and transferred to the digestion vessel of a Milestone microwave digestion system. About 6 mL of concentrated HNO_3 and 2 mL of H_2O_2 were added to the pots and were closed and placed in the microwave. The microwave oven (MDS 2000 CEM, Indian Trail, NC, US) heating programme was carried out in three running steps. The microwave oven was at specific power and pressure programmed (from 80 to 150 °C ramp time 5 min; linearly increased again 225 °C hold time 15 min; 70 °C cooling time 10 min). After, the digested samples were diluted to a final volume of 25 mL with ultrapure water. Reagent blanks were tested for possible interferences in each set of samples [6]. A Perkin-Elmer ELAN DRC-e model ICP-MS system, which was outfitted with a Scott Spray Chamber (Norwalk, Conn., U.S.A.), was used to detect lead, cadmium, copper, and arsenic simultaneously with multiple elements. Before the instrumental analysis, the equipment was calibrated using 10 μg/L of a multi-element standard solution. The following were the ICP-MS operating conditions: RF The nebulizer gas flow, auxiliary gas flow, and plasma gas flow were adjusted to 0.81, 1.20, and 19 L/min Ar, respectively. The nickel skimmer and sampler cone were employed, and the power was set to 1000. For the elements, the standard analytical mass (amu) mode was applied. The number of sweeps was 20, readings 1, and replicates 3 [6].

2.2.3 Determination of aflatoxin content

The aflatoxin content was determined using the method of AOAC [24]. A Waring blender was used to completely mix 20 g of the homogenised powdered okro sample (in two replications) with 100 mL of 70% methanol for three minutes.

To facilitate the extraction of aflatoxins, the composite sample was placed in a 250 mL Pyrex ® conical flask, sealed with parafilm, and homogenised on an orbit shaker (Lab-line Instruments, Inc.) at 4 × 100 rpm for 30 min. A qualitative Whatman filter paper (No.1, 185 mm, cat No. 1001 185) was used to filter the mixture into a clean conical flask; 40 mL of the filtrate was then collected and transferred into a separating flask. Next, 25 mL of dichloromethane and 20 mL of distilled water were added, agitated gently by hand, and allowed to separate for a few minutes. A 20 g anhydrous sodium sulphate bed was used to drain the bottom phase into a 100 mL white tri-pour plastic beaker. For full extraction, 10 mL of dichloromethane was added to the remaining extract in the separating flask, repeating the separation procedure. In a fume hood, the mixed extract was dried for the whole night. After dissolving the dried extract in 1.5 mL of dichloromethane, it was moved into a sterile 1.5 mL Eppendorf tube and once more allowed to dry overnight in the hood. About 4 µL of each sample extract and the aflatoxin standard B1, B2, G1, and G2 solutions were added to the high-performance liquid chromatography (HPLC-Agilent, 1100 series, U.S.A.) after the dried extract had been redissolved in 1 mL of dichloromethane. Analysis of aflatoxin (B1, B2, G1, and G2) in the powdered okro samples was performed with the HPLC device with a fluorescent detector, after purification using an immunoaffinity column (IAK) [24].

2.2.4 Statistical Analysis

Mean values of the determinations of all the analyses were subjected to a one-way analysis of variance (ANOVA) to determine the significant difference, and the means were 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 Heavy metal content of powdered okro

Plant species, metal concentrations in the soil and air, soil pH and chemical exchange capability, vegetation, season, and a few other factors significantly impact the accumulation and distribution of heavy metals in plants [25]. Understanding the levels of heavy metals in food is important because some of these elements, like copper, iron, manganese, and zinc are vital to human health, while others like cadmium, lead, mercury, and arsenic can be hazardous even at low concentrations [6]. The order of occurrence of heavy metals in the powdered okro is copper >lead> cadmium, as shown in Table 1. On average, copper content (0.20 mg/kg) was higher in the powdered okro, cadmium content (0.01 mg/kg) was the lowest, and arsenic was not detected in any of the okro samples. Every okro sample tested had led, except the sample from the Ojaoba market, which had no lead detected. The sample from the Malete market had the highest lead concentration at 0.06 mg/kg (Table 1). Anukwuorji et al. [26] revealed that *Citrullus colocynthis* obtained from markets in Southeastern Nigeria has a lead concentration ranging from 8 to 39 mg/kg, which is a larger range than this study. Omokaro et al. [27] reported that lead uptake in okro at the various locations studied was given market dumpsites (0.15 mg/kg), farmland dumpsites (0.10 mg/kg), residential land dumpsites (0.12 mg/kg), and faculty of Agriculture dumpsites (0.06 mg/kg). This means the possible source of lead contamination in powdered okro from the Malete market may be the product's exposure to environmental pollution in the market. The high lead content in the Malete market powdered okro may be attributed to high traffic volume with a simultaneous release of vehicular emission in the market [26]. All the powdered okro samples had lead concentrations less than the value stated for the maximum amount of lead in food that is allowed (0.30 mg/kg) [19]. However, to reduce the impact of lead accumulation in humans that can cause brain damage, kidney damage, gastrointestinal diseases, and adverse effects on blood and the central nervous system [19], it is very important to properly package the powdered okro after processing before sales [27].

Table 1.

Heavy metal content of powdered okro samples from different markets in Kwara state

Powdered okro samples	Lead (mg/kg)	Cadmium (mg/kg)	Copper (mg/kg)	Arsenic (mg/kg)
Offa market	$0.03±0.01^{b}$	$0.01±0.00^{a}$	$0.24±0.01^{b}$	ND
Osi market	$0.03±0.01^{b}$	$0.01±0.00^{a}$	$0.15±0.01^{d}$	ND
Ojatuntun	$0.02±0.01^{bc}$	$0.00±0.00^{b}$	$0.11±0.01^{e}$	ND
Ojaoba	$0.00±0.00^{c}$	$0.01±0.00^{a}$	$0.21±0.01^{c}$	ND
Elemere market	$0.02±0.01^{bc}$	$0.00±0.00^{b}$	$0.22±0.01^{b}$	ND
Malete market	$0.06±0.01^{a}$	$0.02±0.00^{a}$	$0.32±0.01^{a}$	ND
Control	$0.04±0.01^{b}$	$0.00±0.00^{b}$	$0.19±0.01^{c}$	ND
Mean	0.03	0.01	0.20	
p level	**	**	***	

**$p<0.01$, ***$p<0.001$, ND-Not detected,
Means with different superscripts within the same column are significantly different ($p<0.05$)

Powdered okro from the Malete market had higher levels of cadmium at 0.02 mg/kg, whereas samples from the Ojatuntun and Elemere markets showed no detectable levels of cadmium. Our results are less than the cadmium content (0.2-4.5 mg/kg) reported for several Nigerian vegetables and spices [28]. According to Abou-Arab et al. [29], the body's exposure to hazardous levels of heavy metals, such as cadmium, in food has been linked to developmental defects, particularly in young children. The limits for cadmium concentration in food set by Codex (0.2 mg/kg) [19] were greater than those found in this study. Therefore, the powdered okro samples from all the locations may not be a potential source of cadmium poisoning. The exposure of the powdered okro in the Malete market to the environment may be a possible source of cadmium contamination. To prevent injury to the gastrointestinal tract, pulmonary, hepatic, renal, pancreatic, and thyroid cancer caused by cadmium poisoning, it is imperative to properly package the powdered okro before displaying it in the market for sale [30]. The Malete market sample had a greater copper concentration (0.32 mg/kg) in the powdered okro than the sample from the Ojatuntun market (0.11 mg/kg). The high copper content in the Malete market sample may be attributed to mechanical abrasion and normal wear and tear of vehicular components such as tires and alloy rims [30]. However, the results of this investigation are consistent with reports of copper concentrations ranging from 0.01 to 2.12 mg/kg for several Nigerian vegetables and spices [28]. Since all the powdered okro samples' readings were lower than the 73.30 mg/kg published by Codex [19], it's possible that none of them had copper poisoning. Research shows that the copper concentration in okro fruits varies, and the highest uptake is in residential land with a value of 36 mg/kg while the lowest is in farmland with a value of 23 mg/kg [27]. This implies that the possible source of copper contamination of the powdered okro from the Malete market may be from okro fruits planted in residential land that might have been used as a dumping site for vehicular components such as tires and alloy rims [30]. This needs to be taken into consideration in sourcing fresh okro fruits to prevent nausea, vomiting, stomach cramps, or diarrhea caused by a high dose of copper in foods [19].

3.2. Aflatoxin contents of powdered okro

Food contamination by aflatoxin is considered a severe issue because it binds to DNA and stops genetic information from being transcribed, which can have negative effects on humans and other animals in the long run [31]. Furthermore, they are toxic both acutely and chronically, which has resulted in liver cirrhosis, acute liver injury, tumour induction, and teratogenic effects [12]. Table 2 shows the aflatoxin concentrations of powdered okro from several markets in Kwara state, with AFG2 being more prevalent (2.95 µg/kg) and AFB1 being less dominating. This finding contradicts the findings of Okigbo and Anene [20], who claimed that the high percentage incidence of *Aspergillus flavus* and *Aspergillus parasiticus* in dried okro makes AFB1 more common in dried okro. Aflatoxin B1, B2, G1, and G2 had average concentrations of 1.64 µg/kg, 2.26 µg/kg, 1.98 µg/kg, and 2.95 µg/kg, respectively, in the powdered okro. AFG1 and AFG2 of the powdered okro showed a significant difference ($p<0.05$), but AFB1 and AFB2 showed no significant difference ($p>0.05$) (Table 2). As the powdered okro's aflatoxin level is all within the legal limit of 20 µg/kg, there may not be a risk of an aflatoxin-related illness outbreak, making the samples safe to eat [22]. However, to reduce the possible effect of aflatoxin accumulation in the powdered okro, there is a need to control the risk factors such as agricultural practices, post-harvest handling, and environmental pollution, among others [32].

B1 aflatoxins are the most dangerous of all aflatoxins because they prevent animals from forming proteins and nucleic acids [14]. AFB1 levels in powdered okro ranged from 1.53 to 1.76 µg/kg, with powdered okro from the Ojaoba market having the highest level and the control sample having the lowest (1.54 µg/kg) (Table 2). The high percentage prevalence of *Aspergillus flavus* and *Aspergillus parasiticus* in the powdered okro sample from the Ojaoba market may be the cause of the elevated AFB1 levels [20]. Poor harvesting techniques, handling, processing, storing, and market sanitary procedures can all be blamed for the high AFB1 levels of the powdered okro from the Ojaoba market [20]. Contrary to the result of this study, the AFB1 levels in dried okro samples taken from several Ibadan marketplaces ranged from 26.69 to 33.49 µg/kg, which was greater than what was found in this investigation [20]. All the AFB1 levels in the powdered okro are below the European Union Commission Regulation [21] maximum acceptable level (5 µg/kg), and the FDA's 20 µg/kg contamination limit [22].

Hepatocellular carcinoma, a very aggressive type of cancer, is known to be caused by AFB2, a genotoxic carcinogen, and food contaminants [33]. While research on AFB2's harmful effects on human health is limited, Santini and Ritieni [34] noted that the compound has been shown to have harmful hepatotoxic, teratogenic, and carcinogenic effects on different kinds of farm animals. The samples of powdered okro had AFB2 levels ranging from 2.21 to 2.31 µg/kg. AFB2 levels in powdered okro from Ojatuntun were the highest, whereas powdered okro from Offa and Osi markets had the lowest levels (Table 2). For dried okro samples collected from several Ibadan marketplaces, a range of values (0.70 – 3.74 µg/kg) comparable to this study was observed [20]. There may not be an issue with AFB2 contamination in the powdered okro since the values in this study are below the FDA's 20 µg/kg contamination limit [22]. However, to prevent AFB2 accumulation in the Ojatuntun market powdered okro, the product should be packaged in a suitable packaging material and stored in an environment of low temperature and moisture, and the okro fruits must not be damaged before processing.

Some fungal species, particularly those in the *Aspergillus* genus, contain a mycotoxin called AFG1, which causes chronic liver cancer or hepatocellular carcinoma [29]. Table 2 shows that samples taken from the Ojatuntun market had higher AFG1 concentrations (2.13 µg/kg) than samples from the Elemere market (1.52 µg/kg). According to this investigation, the AFG1 level of powdered okro is within the range (1.36 to 22.58 µg/kg) reported by Akigbo and Anene [20] and below the limit of contamination reported by the FDA [22]. The use of suitable packaging materials, storage conditions, and healthy okro fruits may reduce the accumulation of AFG1 in the powdered okro from the Ojatuntun market.

AFG2 is produced by specific mould species, namely *Aspergillus flavus* and *Aspergillus parasiticus* [35]. 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 [35]. It may contaminate many types of food crops, especially in warm, humid climates where mould development is more common. When consumed or breathed, it offers serious health hazards since it can weaken the immune system and harm the liver [35]. The powdered okro samples had varying levels of AFG2 concentration, ranging from 1.96 to 3.60 µg/kg. AFG2 levels in powdered okro from the Malete market (3.60 µg/kg) were greater than the 1.96 µg/kg found in powdered okro from the Elemere market (Table 2). Similar results for the AFG2 of several dried okro samples from various Ibadan marketplaces were reported by Okigbo and Anene [20]. The AFG2 concentration of the powdered okro in this investigation was lower than the aflatoxin contamination threshold published by the FDA [22]. The accumulation of AFG2 in the powdered okro from Malete market could be reduced by storing the product in a dry environment that will not support the growth of moulds [35].

Table 2.
Aflatoxin content of powdered okro samples from different markets in Kwara state

Powdered okro samples	Aflatoxin B_1 (µg/kg)	Aflatoxin B_2 (µg/kg)	Aflatoxin G_1 (µg/kg)	Aflatoxin G_2 (µg/kg)
Offa market	$1.63±0.02^a$	$2.21±0.00^a$	$1.98±0.01^a$	$2.89±0.03^c$
Osi market	$1.64±0.02^a$	$2.21±0.01^a$	$1.97±0.01^a$	$2.90±0.04^c$
Ojatuntun	$1.65±0.07^a$	$2.31±0.00^a$	$2.13±0.13^a$	$3.28±0.04^b$
Ojaoba	$1.76±0.22^a$	$2.27±0.16^a$	$2.11±0.04^a$	$3.20±0.01^b$
Elemere market	$1.61±0.06^a$	$2.28±0.04^a$	$1.52±0.35^b$	$1.96±0.02^d$
Malete market	$1.63±0.03^a$	$2.28±0.06^a$	$2.06±0.08^a$	$3.60±0.23^a$
Control	$1.54±0.01^a$	$2.24±0.02^a$	$2.09±0.07^a$	$2.82±0.01^c$
Mean	1.64	2.26	1.98	2.95
p level	NS	NS	*	***

**$p<0.01$, ***$p<0.001$, NS-Not significant
Means with different superscripts within the same column are significantly different ($p<0.05$)

4. Conclusion

The study shows that the most common heavy metal and aflatoxin in powdered okro in Kwara State were, on average, copper and AFG2, respectively. The occurrence of heavy metals in the powdered okro samples from the different markets in Kwara state is in the order of copper > lead > cadmium, and with no arsenic. Also, the order of occurrence of the aflatoxin in the powdered okro is AFG2 > AFB2 > AFG1 > AFB1. The powdered okro samples from all the markets may be safe for human consumption because their levels of copper and AFG2 are within the FAO/WHO permitted range. However, to reduce the potential risks associated with long-term exposure to and accumulation of copper and aflatoxin G2 in the human body, the use of healthy okro fruits, and the processing of the fruits in a controlled environment free of vehicular movement, environmental pollution, and storage in a cold dry place free of moisture buildup should be considered in the production of the Malete market powdered okro.

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