

Fungal and Aflatoxin Contamination of Smoke Dried Catfish and African Bush Mango Seeds (*Ogbono*) Sold in Markets in Selected Processing Zones in Benue State, Nigeria

Abstract

The aim of this research was to evaluate the fungal and aflatoxin content of smoke dried catfish and African bush mango seeds obtained from selected processing zones in Benue State, Nigeria. Thirty two smoke dried catfish samples and forty eight African bush mango seed samples were collected from different markets in Makurdi, Katsina-Ala, Ogbadibo, Kwande and Vandeikya. These were analyzed for fungal load, fungi species and aflatoxin contamination using standard microbiological methods and ELISA technique for aflatoxin determination. The results revealed the presence of *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium spp*, *Penicillium spp*, *Mucor spp* and *Fusarium spp* isolated from both smoke dried catfish and African bush mango seeds; also *Rhizopus spp* and *Saccharomyces spp* from bush mango seeds. Fungal count of smoke dried catfish from Makurdi ranged from 3.28 to 4.61 log CFU/ml while that of Katsina-Ala ranged from 4.26 to 4.98 log CFU/ml. The fungal count of African bush mango seeds ranged from 3.62 to 3.94 log CFU/ml (Kwande), 3.61 to 4.93 log CFU/ml (Makurdi), 3.61 to 4.85 log CFU/ml (Ogbadibo) and 4.08 to 4.89 log CFU/ml (Vandeikya). The aflatoxin concentration in the smoke dried catfish samples ranged from 4.10 to 15.00 ppb for samples from Makurdi and 2.05 to 7.45 ppb for samples from Katsina-Ala, while that of African bush mango seeds ranged from 1.75 to 3.25 ppb (Kwande), 0.00 to 1.50 ppb (Makurdi), 1.08 to 8.10 ppb (Ogbadibo) and 0.00 to 1.65 ppb (Vandeikya). Both the smoke dried catfish and African bush mango seeds had aflatoxin levels below the maximum acceptable limit of 20ppb by National Agency for Food and Drug Administration and Control which can be considered safe, but hygienic measures must be maintained in handling such foods and better drying and preservation techniques applied in order to lower the incidence of fungi growth and aflatoxin contamination.

Keywords: *Fungi, Aflatoxin, Smoked Catfish, Ogbono.*

1.0 INTRODUCTION

Fungi are ubiquitous plant pathogens that are major spoilage agents of foods and foodstuffs. Some of these fungi, under favorable environmental conditions, may introduce metabolites such as mycotoxins into food hence making it poisonous [1]. Mycotoxins are mainly produced by certain filamentous fungi belonging to *Aspergillus*, *Penicillium* and *Fusarium* genera. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Aflatoxins have been identified as the most toxic of these mycotoxins [2].

Aflatoxin exposure is an important aspect of food safety in developing countries as cases of aflatoxin poisoning have been a recurrent public health challenge [3]. It has been linked to several disease conditions including hepatitis, cirrhosis and kidney damage [4]. It is also associated with stunting, immune system suppression, reduced weight gain and rapid death. In Nigeria, several foods including nuts, cereals, dry fish, spices, and melon seeds among other food substances, are susceptible to contamination with aflatoxins due to the critical conditions of

high temperature and humidity known to favour the growth of aflatoxin-producing molds [2, 3, 5].

Aflatoxins are hepatotoxic and highly carcinogenic mycotoxins that are produced by *Aspergillus* species, specifically *Aspergillus flavus* and *Aspergillus parasiticus* [6, 7]. Enforcement of aflatoxin regulatory limits in foods and feeds results in loss of markets and reduced income [3, 8]. The four major naturally produced aflatoxins are known as B₁, B₂, G₁ and G₂, with the most toxic and carcinogenic member of this family being aflatoxin B₁ (AFB₁) [9]. In Nigeria, great attention has been given to aflatoxin contamination in cereals and peanuts but there is a need to increase research in other food stuff.

Fish contains high protein and essential nutrients such as vitamins, fats and minerals, required in the diets of man which helps in the building and maintenance of the body [10]. In spite of these valuable nutrients derived from fish, it is highly susceptible to spoilage by a great number of microorganisms especially in the tropics, where environmental temperature is high enough to enhance proliferation of most decomposing microorganisms. Smoke drying has been the commonest method of preservation of fish, with wood as the main source of energy. Wood smoke contributes to the characteristic smoke smell and colour of smoke dried fish [11]. Smoke drying extends the shelf life of fish by combining lowered water activity, bactericidal and antioxidant components of the smoke [12, 13]. This method has been reported to be inadequate with regards to preservation of fish from contamination by aflatoxigenic fungi and aflatoxin has been reported in smoke dried fish [4, 14, 15]. During storage and distribution of smoke dried fish, the fish is usually stacked in cartons or boxes, creating a warm and humid environment which encourages fungi growth and probably mycotoxins production.

Irvingia gabonensis is a species of African tree that is sometimes called “African bush mango” or “Ogbono” [16]. African Bush mango is highly demanded due to its nutritional, economical and medicinal worth [17]. The seeds are highly regarded because it serves as soup thickener [16]. Sun-drying of the African bush mango seeds helps to preserve the shelf life of the seeds since it is high in moisture content. A well dried and preserved African bush mango seed can be used for more than a year [17]. These seeds have been reported to be prone to fungi attack. The presence of fungi in African bush mango seeds makes it lose its thickening ability, becomes tasteless, and loses its nutritional value [18, 19] and leads to production of mycotoxins such as aflatoxins. The growth of molds on these seeds is majorly as a result of poor post harvest handling, especially during the process of cracking, drying, storage and transportation [18]. An investigation into the aflatoxin content of African bush mango seeds in Nigeria revealed a 35% non compliance with the European Union standard [14].

Since aflatoxin contamination has continued to be a food safety issue of great concern, continuous examination of foods in our environments intended for human consumption is very needful. Although fungal and aflatoxin contamination of Ogbono and smoke dried catfish have been reported by previous researchers, limited information exists on the fungi species and aflatoxin levels in these foods sold in markets within the processing zones: Makurdi, Vandeikya, Ogbadibo, Kwande and Katsina-Ala in Benue State. This research therefore, evaluates fungal and aflatoxin contamination of smoke dried catfish and African bush mango seeds obtained from major markets in the selected processing zones in Benue State.

2. MATERIALS AND METHODS

2.1 Sample Procurement and Study Site

A total of 32 samples of smoke dried cat fish and 48 samples of African bush mango seeds (*Ogbono*) were purchased randomly from four major markets in the selected processing zones in Benue State. Smoke dried catfish were purchased from 3 points each within the same market, from four major markets in Makurdi and Katsina-Ala. African bush mango seeds were purchased from 4 different points each within the same market, from four major markets in Kwande, Makurdi, Ogbadibo and Vandeikya. Samples collected from the same market were grouped together to make a single sample. These were aseptically packaged and stored for analysis.

2.2 Enumeration, Isolation and Identification of Fungi

All homogenized samples were serially diluted with 0.1% peptone water. One ml aliquot from each dilution was transferred aseptically into sterile petri dishes. To each plate about 15ml of sterilized and cooled potato-dextrose agar acidified with lactic acid was added, swirled and allowed to solidify. The inverted plates were incubated for 5 days at 30°C after which the colonies were counted. The distinct colonies were isolated and sub-cultured to obtain pure cultures which were examined under the microscope after staining with lactophenol cotton blue [11, 20]. The isolates were identified using their morphological characteristics and microscopic structure [21, 22].

2.3 Aflatoxin detection and quantification

Analyses of total aflatoxin from all samples was carried out by Enzyme-Linked Immunosorbent Assay (ELISA) method. 10 g of each sample was extracted with 20 mL methanol: water (70:30). The residue was dissolved in 1 mL of methanol: water (3:1, v/v) and 200 ml of diluted extract was applied to the enzyme immuno-sorbent assay (ELISA) plate in order to determine the total aflatoxin content. Each one of the samples and standards were applied in duplicates. The ELISA procedure was performed using AgraQuant assay kit (Romer Labs) according to the manufacturer's instructions. The total aflatoxin concentration was read at 450 – 630 nm. The optical densities (ODs) were compared to those of the standards. Total aflatoxin concentration in each sample was expressed in parts per billion (ppb).

2.4 Statistical Analyses

The data collected were subjected to analysis of variance and means separated by Tukeys test at $P= 0.05$ using Graphpad Prism 5, version 5.01 (2007) for Windows, GraphPad Software, San Diego California USA.

3.0 RESULTS AND DISCUSSION

3.1 Fungal and aflatoxin contamination of smoke dried catfish

Fungi were enumerated from smoke dried catfish obtained from Makurdi and Katsina-Ala as shown on Table 1. The fungal count ranged from 3.279 to 4.613 log CFU/ml for Makurdi samples and 4.255 to 4.978 log CFU/ml for Katsina-Ala samples. The highest fungal count (4.978 log CFU/ml) was observed in smoke dried catfish from Katsina-Ala. Fafioye *et al.* [23] and Sani *et al.* [15] reported an average fungal load of 10^4 CFU/ml in smoke-dried catfish.

Based on colonial and microscopic characteristics, *Aspergillus flavus*, *Aspergillus niger*, *Clasdosporium spp*, *Penicillium spp*, *Mucor* and *Fusarium spp* were identified from the smoke dried catfish sold in major markets in Makurdi and Katsina-Ala (Table 2). *Aspergillus flavus*, *Aspergillus niger* and *Penicillium spp* were the dominant fungi isolated. Similar fungi were reported by [24] in Jos Metropolis, Nigeria. Fafioye *et al.* [23], Shanthini [25], Adebayo-Tayo *et al.* [14], Makun *et al.* [4], Fagbohun and Lawal [26], Sani *et al.* [15] and Osibona *et al.* [27], also made similar reports where *Aspergillus spp* *Fusarium spp*, *Penicillium spp* and *Mucor* were detected in smoke dried catfish. In tropical countries where most drying and smoking of fish occurs, *Aspergillus spp* and *Penicillium spp.* are the dominant fungi [27, 28, 29].

Table 1: Fungal count (Log CFU/ml) of Smoke dried catfish obtained from major markets in Makurdi and Katsina-Ala

MARKET	MAKURDI	KATSINA-ALA
1	4.46 ^b ±0.24	4.28 ^c ±0.14
2	4.61 ^a ±0.12	4.98 ^a ±0.28
3	4.38 ^c ±0.04	4.36 ^b ±0.08
4	3.28 ^d ±0.22	4.26 ^c ± 0.10

Values are means ±SD (Standard deviation) of duplicate determinations.

Values with different superscript within the same column are significantly different (p<0.05).

Key: Makurdi Markets : 1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Katsina-Ala: 1-Donga, 2-Abaji, 3-Tom Anyiin, 4-Gbor

According to Saliu [30] and Tersoo-Abiem and Igyor [3], aflatoxin producing molds are found in most dry foods but are predominant in Sub-saharan Africa where the warm environmental temperature and humidity are optimum for their growth. The presence of these pathogenic

Table 2: Fungal Isolates from Smoke dried catfish obtained from major markets in Makurdi and Katsina-Ala

Market	Fungal isolates	
	Makurdi	Katsina-Ala
1	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Clasdosporium spp.</i>	<i>Aspergillus flavus</i> , <i>Penicillium spp</i>
2	<i>Aspergillus flavus</i> , <i>Penicillium spp</i> , <i>Fusarium spp</i>	<i>Aspergillus niger</i> , <i>Penicillium spp</i> , <i>Mucor spp</i>
3	<i>Aspergillus flavus</i> , <i>Penicillium spp</i> ,	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Penicillium spp</i> <i>Clasdosporium spp.</i>
4	<i>Aspergillus niger</i> , <i>Penicillium spp</i>	<i>Aspergillus niger</i> , <i>Clasdosporium spp</i> , <i>Mucor spp</i>

Key: Makurdi Markets : 1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Katsina-Ala Markets: 1-Donga, 2-Abaji, 3-Tom Anyiin, 4-Gbor

fungi in food indicate food safety risks [25]. Fungi contamination in the smoke dried catfish samples evaluated in this study may have occurred due to the environment where they were stored, poor/unhygienic handling or improper processing which makes it vulnerable to mold contamination; the environment in which smoke dried fish are displayed in the market for sale is

another avenue for microbial contamination [27]. Fagbohun and Lawal [26] and Adesokan *et al.* [11] also made similar reports that most of the post processing microbial contaminants originate from poor handling practices while some could be from the air, the source of fish or from other degrading substances. Although fish with fungal load of less than 10^6 CFU/ml is acceptable from the microbiological point of view [31], the fungal load recorded in this study calls for attention. Therefore, proper hygiene in handling, and safe techniques of smoking should be applied in preservation and storage of smoke dried catfish. Also fish processors, retailers and consumers should be educated on the implications of fungi on human health.

Total aflatoxin concentration of smoke dried catfish samples from Makurdi ranged from 4.10 to 15.00ppb, and 2.05 to 7.45ppb for samples from Katsina-Ala. Aflatoxins were detected in all smoke dried catfish samples from both Makurdi and Katsina-Ala (Table 3). Samples from Wurukum market Makurdi had the highest aflatoxin concentration of 15ppb and samples from Abaji in Katsina-Ala had the lowest aflatoxin concentration (2.05ppb). Various levels of aflatoxin contamination in different species of smoke dried fish have been reported in different parts of the world [8, 26, 32, 33]. Aflatoxins have been reported in smoke dried catfish in Ekiti, Ogun, Uyo and Ibadan, Nigeria [11, 27, 33]. The aflatoxin concentrations reported in smoke dried catfish in this study were higher than those reported in dried fish samples collected in Ibadan [16]. This may be due to the environmental conditions in Benue state and the storage condition of the smoked fish, which may have favoured higher aflatoxin production. The detection of aflatoxins in smoke dried catfish is an indication that the fish has been contaminated with aflatoxin producing fungi especially *A. flavus*. The aflatoxin levels observed in this study may pose health hazards to its consumers with continuous intake, even though they are below the maximum recommended limit of 20ppb. Aflatoxin contamination in food is known to cause serious systemic dysfunctions and public health hazards: they exert carcinogenic, teratogenic, mutagenic and immunosuppressive effects on humans and animals [3]. Therefore extra efforts must be made to reduce fungi contamination of foods which will also reduce aflatoxin levels in foods since most mycotoxins are relatively stable to heat and are not destroyed during food processing or home cooking [3].

Table 3: Total Aflatoxin contamination (ppb) of Smoke dried catfish obtained from major markets in Makurdi and Katsina-Ala

SAMPLE	MAKURDI	KATSINA-ALA
1	15.00 ^a ±0.42	5.10 ^e ±0.14
2	12.45 ^b ±0.21	2.05 ^g ±0.21
3	8.95 ^c ±0.21	5.30 ^e ±0.14
4	4.10 ^f ±0.28	7.45 ^d ±0.35

Values are means ±SD (Standard deviation) of duplicate determinations.

Values with different superscript within the same column are significantly different ($p < 0.05$).

Key: Makurdi Markets :1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Katsina-Ala Markets: 1-Donga, 2-Abaji, 3-Tom Anyiin, 4-Gbor

3.2 Fungal and aflatoxin contamination of African bush mango seeds

Table 4 shows the fungal count of African bush mango seeds obtained from Kwande, Makurdi, Ogbadibo and Vandeikya markets. The fungal count ranged from 3.623 to 3.939 log CFU/ml for

samples from Kwande, 3.613 to 4.934 log CFU/ml for samples from Makurdi, 3.613 to 4.845 log CFU/ml for samples from Ogbadibo and 4.079 to 4.892 log CFU/ml for samples from Vandeikya, with a sample from Northbank market, Makurdi having the highest fungal count (4.934 log CFU/ml). All samples from Kwande markets had low fungal load compared to samples from other markets. Adebayo-Tayo *et al.* [32] reported lower fungal load in stored African bush mango seeds in Uyo.

The fungal isolates identified from African bush mango seeds obtained from Kwande, Makurdi, Ogbadibo and Vandeikya were *Aspergillus niger*, *Clasdosporium spp*, *Penicillium spp*, *Aspergillus flavus*, *Mucor*, and *Saccharomyces spp*. The dominant microflora in the market samples were *Aspergillus flavus* in Kwande, *Aspergillus niger* in Makurdi and Ogbadibo, and *Penicillium spp* in vandeikya (Table 5). Adebayo-Tayo *et al.* [14] reported similar organisms in marketed bush mango seeds (*Ogbono*) stored for sale in Uyo. Aboloma and Ogunbusola [34] isolated *Rhizopus nigricans*, *Mucor mucedo*, *Trichoderma viride* and *Aspergillus flavus* while Ekundayo *et al.* [35] reported *Penicillium chrysogenum*, *Aspergillus flavus*, *A. niger*, *Trichoderma viride* and *Rhizopus stolonifer* in African bush mango seeds. Chuku and Aggrey [19] also reported similar fungi and more in African bush mango seeds. Sanyaolu *et al.* [18] had reported that *Aspergillus spp* and *Penicillium spp* were amongst the predominant fungi that reduce the nutritional and storage stability of African bush mango seeds.

Mold contamination of bush mango seeds may occur due to non-adherence to hygienic practices during the process of cracking to extract the cotyledons (kernels), during drying, storage (in moist and humid environments which promote optimum mold growth, in contaminated areas or containers) and distribution [36], since the seeds are also kept in open markets until sold, thereby exposing them to microbial infection [14]. The seeds of *Irvingia* contain high moisture and as a result they are easily covered with molds if not properly dried. High moisture and relative humidity lead to greater fungal growth and thus low storability of the seeds [34].

Table 4: Fungal count (Log CFU/ml) of African bush mango seeds obtained from major markets in the selected processing zones in Benue State

SAMPLE	KWANDE	MAKURDI	OGBADIBO	VANDEIKYA
1	3.92 ^a ±0.25	3.69 ^b ±0.23	3.61 ^d ±0.37	4.15 ^d ±0.22
2	3.94 ^a ±0.41	4.28 ^d ±0.21	4.11 ^c ±0.19	4.08 ^c ±0.24
3	3.62 ^b ±0.21	4.93 ^a ±0.44	4.18 ^b ±0.10	4.53 ^b ±0.14
4	3.93 ^a ±0.18	3.61 ^c ±0.28	4.85 ^a ±0.22	4.89 ^a ±0.08

Values are means ±SD (Standard deviation) of duplicate determinations.

Values with different superscript within the same column are significantly different (p<0.05).

Key:

Kwande Markets :1- Kwande, 2-Iyon, 3-Ikyogen, 4-Aga

Makurdi Markets :1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Ogbadibo Markets :1- Ahor, 2-Eke, 3-Ede, 4-Ukwo

Vandeikya Markets :1- Ihugh, 2-Tsar, 3-Agbo, 4-Agu

Table 5: Morphological characteristics of fungal isolates from African bush mango seeds obtained from major markets in the selected processing zones in Benue State

Fungal isolates

Market	Kwande	Makurdi	Ogbadibo	Vandeikya
1	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i>	Mucor spp	<i>Aspergillus flavus</i> <i>Aspergillus niger</i>	<i>Aspergillus niger</i>
2	<i>Aspergillus flavus</i> Mucor spp	<i>Aspergillus niger</i> <i>Rhizopus spp</i>	<i>Clasdosporium spp</i>	<i>Penicillium spp</i>
3	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Rhizopus spp</i> , <i>Saccharomyces spp</i>
4	<i>Aspergillus flavus</i> Saccharomyces <i>spp</i>	<i>Saccharomyces spp</i> , <i>Clasdosporium spp</i>	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>	<i>Penicillium spp</i>

Key:

Kwande Markets :1- Kwande, 2-Iyon, 3-Ikyogen, 4-Aga

Makurdi Markets :1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Ogbadibo Markets :1- Ahor, 2-Eke, 3-Ede, 4-Ukwo

Vandeikya Markets :1- Ihugh, 2-Tsar, 3-Agbo, 4-Agu

Table 6 shows the level of total aflatoxin contamination of African bush mango seeds obtained from Kwande, Makurdi, Ogbadibo, and Vandeikya. Total aflatoxin concentration in the market samples from Kwande ranged 1.75 to 3.25ppb, 1.25 to 1.50ppb for samples from Makurdi, 1.08 to 8.10ppb for samples from Ogbadibo and 0.00 to 1.65ppb for samples from Vandeikya. Aflatoxin was detected in bush mango seeds from most of the processing zones surveyed, with the highest concentration (3.25 ppb) recorded in the sample from Adikpo market in Kwande. Aflatoxin was not detected in the samples from Highlevel and Wadata markets in Makurdi and samples from Tsar, Agbo and Agu markets in Vandeikya. The lowest aflatoxin concentration (1.25 ppb) was recorded in the sample from Northbank market in Makurdi. Higher total aflatoxin levels of 63.4 ppb and 61.7ppb in ogbono seeds were observed by Ezekiel *et al.* [37] respectively. The detection of aflatoxins in the marketed bush mango seeds from most of the samples evaluated in this study indicates that *A. flavus* associated with the bush mango seeds produced toxins. *Aspergillus* species such as *A. flavus* and *A. parasiticus* are the most notorious of the common isolates from ogbono due to their high potential for producing aflatoxins [27]. The production of aflatoxins is affected by several factors which influence mold growth such as moisture contents, high relative humidity (RH) (>70%) and temperature (>25⁰C), substrate composition and the presence of competing microorganisms [14, 38]. It is worthy to note that the environmental conditions in Nigeria favour growth of fungi and aflatoxin production in foods. It is therefore important to take precautions when handling and processing dry foods especially. The growth of molds on African bush mango seeds is a pointer to the potential health risk associated with its consumption [38].

Table 6: Total Aflatoxin contamination (ppb) of African bush mango seeds obtained from major markets in the selected processing zones in Benue State.

SAMPLE	KWANDE	MAKURDI	OGBADIBO	VANDEIKYA
1	3.25 ^b ±0.07	1.50 ^{ef} ±0.14	1.08 ^{cde} ±0.28	1.65 ^{de} ±0.21
2	2.05 ^c ±0.21	0.00 ^g ±0.00	1.80 ^a ±0.14	0.00 ^g ±0.00
3	1.75 ^{cde} ±0.21	1.25 ^f ±0.07	2.00 ^c ±0.00	0.00 ^g ±0.00
4	1.85 ^{cd} ±0.07	0.00 ^g ±0.00	8.10 ^a ±0.28	0.00 ^g ±0.00

Values are means ±SD (Standard deviation) of duplicate determinations.

Values with different superscript within the same column are significantly different (p<0.05).

Key:

Kwande Markets :1- Kwande, 2-Iyon, 3-Ikyogen, 4-Aga

Makurdi Markets :1- Wurukum, 2-High level, 3-North Bank, 4-Wadata

Ogbadibo Markets :1- Ahor, 2-Eke, 3-Ede, 4-Ukwo

Vandeikya Markets :1- Ihugh, 2-Tsar, 3-Agbo, 4-Agu

4. CONCLUSION

This study has revealed that a good number of different fungi are associated with smoke dried catfish and African bush mango seeds sold in different markets in Benue state. Although the total aflatoxin levels in the samples analysed were below the maximum acceptable limits (20ppb) specified by international regulatory agencies in food and agricultural products, prolonged intake of these quantities may constitute a health hazard to consumers; this will also reduce the economic value of the food. Therefore, better drying and storage techniques should be applied to reduce the incidence of fungi and aflatoxins in these foods.

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