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# Aflatoxins Investigation and Mycobiota of Selected Marketed Smoked - Dried Fish Samples in Ado-Ekiti, Nigeria and Their Environmental Health Implications

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author EDF designed the study, wrote the protocol and carried out the aflatoxin detection. Author OUL carried out the microbiological analysis, performed the statistical analysis, managed the literature search, and wrote the first draft of the manuscript. Authors EDF and OUL managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study was carried out to assess the mycobiota and aflatoxins contamination in selected common smoked-dried fish samples sold at Ojo Oba market in Ado Ekiti, Nigeria and their environmental health implications.
**Place and Duration of Study:** Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria, between March 2012 and February, 2013.
**Methodology:** Smoked dried fish (Bonga, Cat, Wet African Shad, Butter and Sole) were randomly sampled and purchased from five different marketing sites located at Ojo Oba main market in Ado

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Ekiti town, Ekiti State, Fifty samples, ten from each related species were analysed. Mycological analysis was done with Potato Dextrose agar using direct plating, washing and dilution methods while the fungi were identified using standard procedure. The moisture content of the fish samples were determined by oven drying at 105ºC for $4^1/_2$ h. The aflatoxin extraction, quantitative and qualitative determination was carried out as previously described while the results were analysed using Duncan multiple range test.

**Results:** Eleven different fungal species belonging to six genera were found to be associated with the smoked dried fish samples from the markets sites. The fungal species are *Aspergillus niger, A. fumigatus, A. tereus, A. flavus, Absidia* sp., *Rhizopus* sp., *Penicillium* sp., *Penicillium citrinum, Penicillium italicum, Mucor* sp and *Fusarium moniliformis. Aspergillus flavus* and *Penicillium* sp. had the highest rate of occurrence among the fungi isolated. Aflatoxin B1 and G1 was found in Cat fish (*Gymnallabes typhus*), West African Shad (*Ilisha africana*), Sole fish (*Cynoglossus browni*) while it was not detected in the rest. The aflatoxin B1 and G1 concentration ranged from 2.731 to 4.031 $\mu g.kg^{-1}$ and 2.015 to 3.528 $\mu g.kg^{-1}$ respectively while the fungal count ranged from $4.7x10^2$ to $9.1x10^4 cfu.g^{-1}$. The moisture content ranged from 21.1 to 28.8%.

**Conclusion:** This study showed that smoked dried fish displayed for sale at different market sites in Ojo Oba market in Ado Ekiti, Nigeria were contaminated with species of fungi and aflatoxin which pose a great threat on the health of the consumers. However, fish samples should be well smoked and dried to reduce the moisture content, the samples for sale should be kept in a covered container or show glass to reduce settling of droplets and spores, gloves should be worn by wholesalers and retailers to reduce direct inoculation and storage in a well ventilated environment to reduce contaminations.

*Keywords: Aflatoxins; mycobiota; smoked dried fish; moisture content; public health.*

## 1. INTRODUCTION

Fish and its products are considered as preferable source of high nutritional values and highly desirable food due to its high quality animal protein content, its exceptional richness in calcium and phosphorus and its generous supply of β-complex vitamins [1]. According to Adebayo-Tayo et al. [2] fish has the best sources of protein that is better digested than that of beef. It is a source of income to many people in developing countries. Fish is highly perishable due to its high moisture and fat content [3].

In Nigeria, fish is eaten fresh, preserved or processed. The conventional methods for preserving fish include drying, salting, pickling and smoking [4]. Processed fish either salted or smoked may be exposed to microbial contamination derived from either the air or subsequent handling of fish which results in microbial spoilage through utilization of protein and lipids [5]. Food manufacturers and processors usually enumerate these organisms only when a problem occurred, due to off flavors, sliminess, lipolysis and unpalatable taste that render the product of inferior quality, unmarketable or even unfit for human consumption [6].

Fungal contamination of fish is considered the main cause of signs of spoilage as rotten odour unpalatable taste and may constitute a public health hazard as well as many of economic losses [6]. Mycotoxins producing fungi such as some species of *Aspergillus*, *Fusarium* and *Penicillium* have been implicated to constitute a public health hazard due to the type of mycotoxins they produce in stored and preserved food samples such as aflatoxin, ochratoxin, patulin and zearalenone [4].

Adebayo-Tayo et al. [2] reported the isolation of twelve different fungi found to be associated with smoked dried fish samples sold in the three different markets. They also found that Aflatoxin B1 and G1 were associated with the samples. Hassan et al. [6] also reported the mycobiota and the detection aflatoxins contamination of randomly selected thirty smoked fish in shops and retail markets in Egypt, hence the need to carry out this study in our immediate environment.

This study was carried out to assess the mycobiota and aflatoxins contamination in selected common smoked-dried fish samples sold at Ojo Oba market in Ado Ekiti, Nigeria and their environmental health implications. This study will be useful for fish retailers and consumers as well as public health specialists.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Smoked dried fish (Bonga, Cat, Wet African Shad, Butter and Sole) were randomly sampled and purchased from five different marketing sites located at Ojo Oba main market in Ado Ekiti town, Ekiti State, Nigeria between March, 2012 and February, 2013. Fifty samples, ten from each related species were kept in sterile polyethylene bags and transported to the laboratory for analyses.

### 2.2 Mycobiota of Sample Fish

Three methods were used for the isolation of fungal species from the sample fish in this study.

#### 2.2.1 Direct plating method

The fish samples were examined for external mouldness. The surface of the twenty five randomly selected fish samples was scrapped using sterile dissecting forceps and plated aseptically on Potato Dextrose Agar (PDA) plate and incubated at 28ºC for 5 days as described by Lawal and Fagbohun [7].

#### 2.2.2 Washing method

This was carried out by transferring 10 g of randomly sampled fish in 90 ml of sterile distilled water in a beaker. After shaken thoroughly (5 min at 200 rpm), 1 ml of the suspension was plated on Potato Dextrose Agar plates and was spread evenly on the surface with sterile glass spreader. The plates were incubated at 28ºC for 5 days and were observe for visible fungi growth.

#### 2.2.3 Dilution method

Ten grams of the fish samples obtained from each of the markets were weighed aseptically and macerated in 90 ml sterile potato dextrose broth using a Warring blender. From this, subsequent tenfold dilution was made up to $10^{-6}$. One milliliter of each dilution was dispensed in triplicate in sterile Potato Dextrose agar plates to which 1 mg/l of penicillin and 0.2 g/l streptomycin had been incorporated. They were gently rotated to ensure even dispersion, allowed to solidify and were incubated at 28ºC for 5 days.

### 2.3 Identification of Fungal Species

The pure cultures obtained were identified according to Dungan [8] using morphological characteristics, spore formation, the production of fruiting body and biochemical reactions. They were also compared with already identified fungal species obtained from the Plant Pathology Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.

### 2.4 Moisture Content Determination

This was determined for all the sampled fish by oven drying at 105ºC for $4^1/_2$ h according to the method of Adebayo-Tayo et al. [2].

### 2.5 Aflatoxins Detection in Sampled Fish

#### 2.5.1 Extraction of aflatoxin

This was carried out according to the methods recommended by AOAC [9] and described by Alhussaini [10]. Ten grams each of the fishes samples obtained from the market sites were weighed aseptically and macerated using a Warring blender and were extracted with chloroform: water (10:1 v/v) mixture. The obtained crude extracts were purified by column chromatography containing anhydrous sodium sulphate (15 g) and silica gel (10 g). Extracts were air dried and kept in dark vials till chromatographic analysis.

#### 2.5.2 Qualitative estimation of aflatoxins

This was carried out according to the methods recommended by AOAC [9] and described by Alhussaini [10]. Precoated silica gel plates were used. Rectangular glass jar was used for developing chromatoplates. A suitable volume of solvent mixture (chloroform: methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be 1 cm above the upper surface of the solvent mixture. The chromatographic plates were activated by heating 1hr at 120ºC in a hot air oven, and removed immediately to a desiccator to cool. Parallel starting spots, 2 cm from each side of the plate and 1.5 cm apart, were made with micropipets from chloroform extracts with reference aflatoxins. Spots were left to air dry. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front

was marked and the plates were air dry. Spots were viewed under UV light (366 nm) and the outline of each fluorescent spots was marked by sharp pin. Retention factor (Rf) values, colors, and intensities of the spots were compared with reference mycotoxins [11].

#### 2.5.3 Quantitative determination of aflatoxins

The dilution-to-extinction [12] and comparison of standards [9] techniques were used for estimation of aflatoxins concentrations [10].

### 2.6 Statistical Analysis

Duncan multiple range test (DMRT) was used to compare significant differences between the means as described by Duncan [13] and Oloyo [14].

## 3. RESULTS AND DISCUSSION

### 3.1 Mycobiota of Smoked Dried Fish Samples

A total of eleven fungal species belonging to six different genera were isolated, including *Aspergillus flavus*, *Penicillium* sp., *Aspergillus niger, Rhizopus* sp., *Fusarium moniliformis*, *Aspergillus fumigatus*, *Aspergillus tereus, Absidia* sp., *Penicillium citrinum, Penicillium italicum,* and *Mucor* sp. from the smoked dried fish samples Fig. 1. The occurrence of the fungal species varied with the fish sample types. *Aspergillus niger* was found to be associated with bonga fish and West African shad while *Rhizopus* sp. was isolated from bonga fish and cat fish. *Aspergillus flavus* was isolated from Cat fish, West African shad and Sole fish while *Penicllium* sp. was isolated from West African shad, butter fish and sole fish. *Fusarium* sp. was isolated from Bonga fish and Sole fish samples while *Absidia* sp. and *Aspergillus tereus* were isolated from bonga fish and cat fish samples respectively. In addition, *Penicillium italicum* was isolated from cat fish only while *P. citrinum* and *Mucor* sp. were isolated from West African shad.

This is similar to the work of Laciakova [15] who reported the isolation of *Aspergillus*, *Penicillium* and *Fusarium* from heat processed meat in summer months in some countries. Adebayo-Tayo et al. [2] reported the isolation of *Aspergillus flavus, Aspergillus tereus, A. fumigatus, Absidia* sp., *Rhizopus* sp., *Aspergillus niger, Mucor* sp., *Cladosporum* sp., *Penicillium italicum, Penicilium viridatus, Candida tropical*is and *Fusarium moniliformis* from selected smoked fish from different markets sites in Uyo, Akwalbom state. Hassan [6] also reported the isolation of several species of fungi belonging to the genera *Penicillium*, *Aspergillus, Fusarium, Rhizopus* and *Mucor* from smoked dried fish sold at market place in Giza Governorate, Egypt.

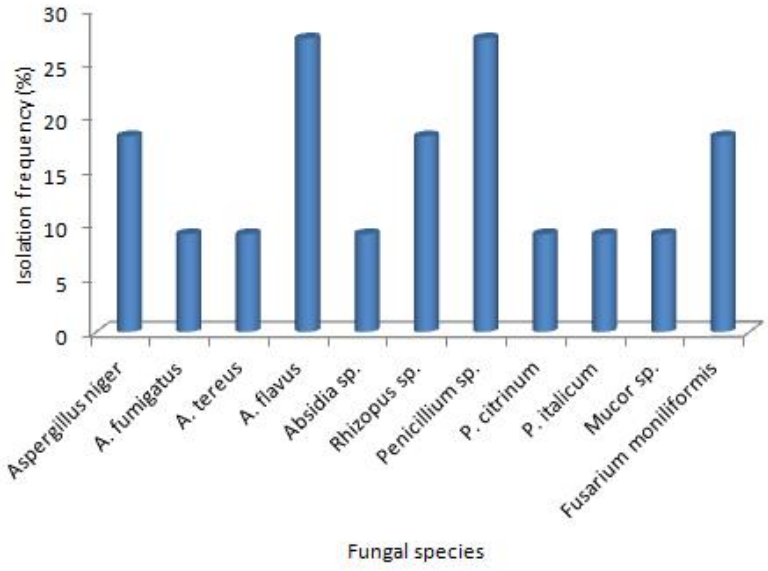


**Fig. 1. Percentage occurrence of fungi associated with marketed smoked dried fish samples fungi associated with smoked dried fish samples**

The occurrence of these fungal contaminations in smoked dried fish samples using the three methods of isolation as shown in Table 1 could be attributed to improper sanitation from the catching stage to the smoking stage. Moreover, there is ever increasing demand for smoked dried fish and in the quest of the retailers to meet this need the fish are overloaded on the smoking kiln during processing as a result, they are exposed to a reduced intensity of heat for short period of time. This leads to improper processing and vulnerability of the fish to fungal contamination [4]. In addition, during storage of smoked dried fish products, wholesalers and retailers store the products in poorly ventilated stores where pest can gain access and may directly inoculate them with fungi. The market place where the fish products are displayed for sale most times are not clean or hygienic, such as in open trays without coverage [6]. This in tune allows the dusts and fungal spores to settle on the product and lead to fungal invasion, production of toxins and spoilage.

## 3.2 Fungal Count and Moisture Content of the Smoked Dried Fish Samples

The result of the fungal count and moisture content of the smoked dried fish samples are shown on Table 2. The average fungal count ranged from $4.7 \times 10^2$ in Butter fish to $9.1 \times 10^4$ in West African Shad. This microbial level is considerable high and could detrimental to the health of consumers especially the immunocompromised individuals because of the possibility of causing opportunistic infections. The moisture content of the smoked dried fish samples ranged from 21.1 in Butter fish to 28.8 in West African Shad fish. The moisture content however correlated with the fungal count. Moisture is an important factor for growth of fungi. The West African Shad had the highest fungal count and fungal species. This may be as a result of the high moisture content of the products [5].

## 3.3 Aflatoxin Detection in Smoked Dried Fish Samples

The result of the aflatoxin detection in smoked dried fish samples is shown in Table 3. Aflatoxin was detected in three of the five species of smoked dried fish sampled. The concentration of aflatoxin B1 ranged from 2.731 $\mu gkg^{-1}$ in Sole fish to 4.031 $\mu g.kg^{-1}$ in West African Shad while that of aflatoxin G1 ranged from 2.015 $\mu g.kg^{-1}$ in Sole fish to 3.528 $\mu g.kg^{-1}$ in West African Shad. From this study, West African shad had the highest concentration of aflatoxin B1 and G1 while Sole fish had the lowest. Aflatoxin was not detected in bonga fish and butter fish. This might be because *Aspergillus flavus* which is known to produce aflatoxin was not isolated from the two fish samples.

**Table 1. Fungal species isolated from smoked dried fish sampled using three methods of isolation**

| Methods of isolation | Fungal species |
|---|---|
| Washing method | *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Fusarium* sp., *Penicillium* sp., *Penicillium citrinum, Penicillium italicum, Mucor* sp. |
| Direct plating | *Aspergillus niger, Aspergillus fumigatus, Aspergillus niger, Aspergillus tereus, Absidia* sp., *Penicillium* sp., *Penicillium citrinum, Penicillium italicum, Mucor* sp. |
| Dilution method | *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium moniliformis* |

**Table 2. Fungi count and moisture content (%) in different fish samples**

| Fish samples | Fungi count $cfu.g^{-1}$ | Moisture content (%) |
|---|---|---|
| Bonga fish (*Ethmalosa fimbriata*) | $7.1 \times 10^3$ | 26.1 |
| Cat fish (*Gymnallabes typhus*) | $5.7 \times 10^4$ | 28.4 |
| West African Shad (*Ilisha africana*) | $9.1 \times 10^4$ | 28.8 |
| Butter fish (*Schilbe uranoscopus*) | $4.7 \times 10^2$ | 21.1 |
| Sole fish (*Cynoglossus browni*) | $6.3 \times 10^3$ | 22.1 |

**Table 3. Aflatoxins concentrations in smoked fish samples**

| Samples | Aflatoxin B1 (ug.kg$^{-1}$) | Aflatoxin G1 (ug.kg$^{-1}$) |
|---|---|---|
| Bonga fish (*Ethmalosa fimbriata*) | - | - |
| Cat fish (*Gymnallabes typhus*) | 3.905$^{b}$ | 2.617$^{b}$ |
| West African Shad (*Ilisha africana*) | 4.031$^{a}$ | 3.528$^{a}$ |
| Butter fish (*Schilbe uranoscopus*) | - | - |
| Sole fish (*Cynoglossus browni*) | 2.731$^{c}$ | 2.015$^{c}$ |

*Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

Aflatoxins are highly carcinogenic and have been reported to be associated with cancer of the liver (hepatoma), acute hepatitis and paralysis in developing world [4]. Aflatoxins have been reported in smoked dried fish in Uyo [2], Abeokuta [16], Lagos [4] all in Nigeria. Fish is a rich substrate for the growth of fungi. The fungi introduced to the fish either through the environment or poor personal hygiene or improper processing use the fish as substrate to grow and produce their metabolites such as aflatoxins which are toxic and detrimental to the consumers well being [17]. This smoked dried fish samples are consumed directly by consumers and as a results, they consume the mycobiota and the metabolites [18]. These metabolites (aflatoxin) has the tendency of crossing the placenta to the developing foetus while some can also be passed across to the suckling child via breast milk which may results into retarded growth, paralysis or death in this children [19].

## 4. CONCLUSION

This study showed that smoked dried fish samples from different sites in Ojo Oba, Ado Ekiti were contaminated with aflatoxins producing fungi which when consumed may pose a great threat to the health of the consumers and dependants as in the case of a breast feeding mother. The fungi isolated are major airborne contaminant which might have invaded the smoked dried fish samples as a result of exposure to air, poor storage conditions and poor personal hygiene. However, fish samples should be well smoked and dried to reduce the moisture content, the samples for sale should be kept in a covered container or show glass to reduce settling of droplets and spores, gloves should be worn by wholesalers and retailers to reduce direct inoculation and storage in a well ventilated environment to reduce contaminations.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Zaki MS, Sharaf NE, Mostafa HS, Fawzi OM. Diminution of aflatoxicosis in *Tilapia niloticus* fish by dietary supplementation with fix in toxin and Nigella sativa oil. American-Eurasian Journal of Agriculture and Environmental Science. 2008;3(2): 211-215.
2. Adebayo-Tayo BC, Onilude AA, Ukpe GP. Mycoflora of smoke-dried fishes sold in Uyo, Eastern Nigeria. World Journal of Agricultural Sciences. 2008;4(3):346-350.
3. Ali EH, Hashem M, Al-salahy MB. Pathogenicity and oxidative stress in Nile Tilapia caused by *Aphonomyces laevis* and *Phoma herbarium* isolated from farmed fish. Dis. Aquat. Org. 2011;94:17–28.
4. Olajuyigbe OO, Akande GR, Ezekiel CN, Ezekiel MO. Aflatoxigenic moulds and aflatoxin contamination of retailed fishery products in Lagos markets Mycotoxicology. 2014;1:57-63.
5. Onyuka JHO, Kakai R, Ofulla AVO. Bacterial contamination dynamics and fungal contamination levels of fish *Rastrineobola argentea* and *Oreochromis niloticus* from Lake Victoria Basin of Kenya. Photon. 2014;106:179-188.
6. Hassan AA, Hassan MA, El Shafei HM, El Ahl RM, Abd El-Dayem RH. Detection of aflatoxigenic moulds isolated from fish and their products and its public health significance. Nature and Science. 2011;9(9):106-114.
7. Lawal OU, Fagbohun ED. Studies on bio-deterioration, aflatoxin contamination and nutritive values of processed cashew (*Anacardium occidentale* L) nuts during

Storage. Nature and Science. 2014;11(9): 127–133.
8. AOAC. Official methods of analysis of association of official agricultural chemists. 13th Ed., Washington D.C., USA. Chapt. 1984;26:481-483.
9. Dugan FM. The idenfication of fungi: An illustrated introduction within Keys, Glossary, and Guide literature. American Phytopathological Society: St. Paul. Mn. 2006: 176.
10. Alhussaini MS. Mycobiota and mycotoxins of nuts and some dried fruits from Saudi Arabia. Journal of American Science. 2012;8(12):525-534.
11. El-Bazza ZE, Zedan HH, Toama MA, El-Tayeb OM. Isolation of aflatoxins producing fungi from Egyptian food and feed commodities. Proc. Int. Symb. Mycotoxins, Cairo. 1982;12:443-454.
12. Coomes TJ, Crowther PC, Francis BJ, Stevens L. The detection and estimation of aflatoxin in groundnut and groundnut materials. Analyst. 1965;90:492-494.
13. Duncan PB. New multiple range and multiple F-tests in Biometrics. 1956;11:1-42.
14. Oloyo RA. Fundamentals of research methodology for social and applied sciences. ROA Educational Press, Ilaro, Nigeria. 2001;53-200.
15. Laciakova A, Pipova M, Mate D. Luciak V. Microbial analysis of meat products focused on the occurrence of microscopic filamentous fungi. The University Veterinary Medicine, Kovenskeho. 2004;6: 24–28.
16. Adejola QA. Presence of aflatoxins in smoked-dried fish sold in Abeokuta, Ogun State, South-Western Nigeria. B.Sc project submitted to the Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Nigeria. 2011;35.
17. Adebayo-Tayo BC, Onilude AA, Ogunjobi AA, Gbolagade JS, Oladapo MO. Detection of fungi and aflatoxin in shelved bush mange seeds (*Irvingia* spp) stored for sale in Uyo, Eastern Nigeria. EJEAFche. 2006;5(5):1569–1574.
18. Essien JP, Ekpo MA. Brooks AA. Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (*Chlamydoselachus anguincus*). Journal of Applied Science and Environmental Management. 2005;9(3):53-57.
19. Sage LS, Krivobok E, Delbos F, Seigle M, Creppy EE. Fungal flora and ochratoxin a production in grapes and musts from France. Journal of Agricultural and Food Chemistry. 2002;50:1306.



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