

Aflatoxins investigation and mycobiota of selected marketed smoked - dried fish samples in Ado-Ekiti, Nigeria and their environmental health implications

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 Ekiti town, Ekiti State, Fifty samples, ten (10) 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 105°C for 4½h. 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. terreus*, *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 4.031ug.kg⁻¹ and 2.015 to 3.528ug.kg⁻¹ respectively while the fungal count ranged from 4.7x10² to 9.1x10⁴cfu/g. The moisture content also 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* *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 found 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. MATERIAL AND METHODS

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 (10) from each related species were kept in sterile polyethylene bags and transported to the laboratory for analyses.

Mycobiota of sample fish

The three methods used for the isolation of fungal species from the sample fish in this study were direct plating, dilution and washing method.

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 28°C for 5 days as described by Lawal and Fagbohun [7].

Washing method: This was carried out by weighing 10g of randomly sampled fish 90 ml of sterile distilled water in a beaker. This was shaken thoroughly and 1ml 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 28°C for 5 days and were observe for visible fungi growth.

Dilution method: Ten (10) 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 penicillin and streptomycin had been incorporated. They were gently rotated to ensure even dispersion, allowed to solidify and were incubated at 28°C for 5 days.

Identification of fungal species

The pure cultures obtained were identified using morphological characteristics, spore formation, the production of fruiting body and biochemical reactions and were 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.

Moisture content determination

This was determined for all the sampled fish by oven drying at 105°C for 4¹/₂h according to the method of Adebayo-Tayo *et al.*, [2].

Aflatoxins Detection in sampled fish

Extraction of aflatoxin: This was carried out according to the methods recommended by AOAC [8] and described by Alhussaini [9]. 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 (15g) and silica gel (10g). Extracts were air dried and kept in dark vials till chromatographic analysis.

Qualitative Estimation of Aflatoxins: This was carried out according to the methods recommended by AOAC [8] and described by Alhussaini [9]. 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 120°C 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 [10].

Quantitative Determination of Aflatoxins: The dilution-to-extinction [11] and comparison of standards [8] techniques were used for estimation of aflatoxins concentrations [9].

Statistical Analysis

117 Duncan multiple range test (DMRT) was used to compare significant differences between
118 the means as described by Duncan [12] and Oloyo [13].

119 **3. RESULTS AND DISCUSSION**

120

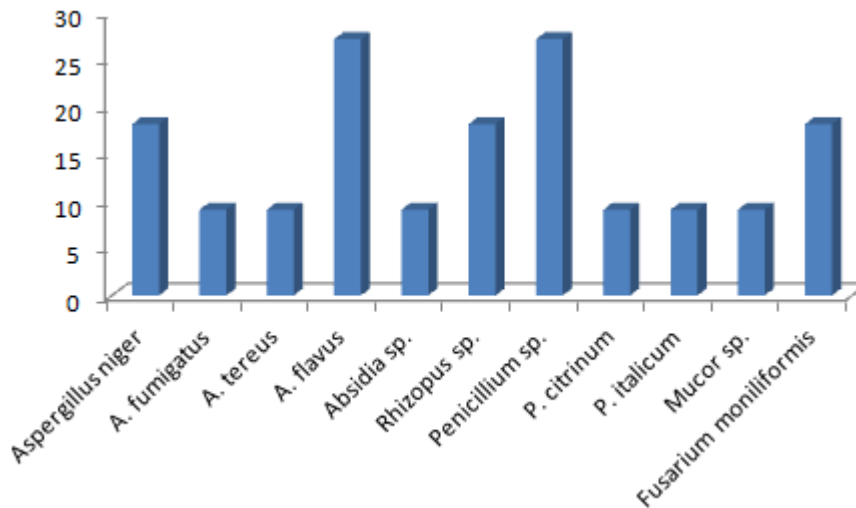
121 **Mycobiota of smoked dried fish samples**

122 The results obtained from this study showed that *Aspergillus flavus*, *Penicillium* sp.,
123 *Aspergillus niger*, *Rhizopus* sp., *Fusarium moniliformis*, *Aspergillus fumigatus*, *Aspergillus*
124 *tereus*, *Absidia* sp., *Penicillium citrinum*, *Penicillium italicum*, and *Mucor* sp. are associated
125 with smoked dried fish samples from different marketing site in Ojo Oba, Ado Ekiti, Nigeria
126 as shown in fig 1. A total of eleven (11) fungal species belonging to six (6) different genera
127 were isolated. The occurrence of the fungal species varied with the fish sample types.
128 *Aspergillus niger* was found to be associated with bonga fish and West African shad while
129 *Rhizopus* sp. was isolated from bonga fish and cat fish. *Aspergillus flavus* was isolated from
130 Cat fish, West African shad and Sole fish while *Penicillium* sp. was isolated from West
131 African shad, butter fish and sole fish. *Fusarium* sp. was isolated from Bonga fish and Sole
132 fish samples while *Absidia* sp. and *Aspergillus tereus* were isolated from bonga fish and cat
133 fish samples respectively. In addition, *Penicillium italicum* was isolated from cat fish only
134 while *P. citrinum* and *Mucor* sp. were isolated from West African shad.

135

136 This is similar to the work of Laciakova [14] who reported the isolation of *Aspergillus*,
137 *Penicillium* and *Fusarium* from heat processed meat in summer months in some countries.
138 Adebayo-Tayo et al.,[2] also reported the isolation of *Aspergillus flavus*, *Aspergillus tereus*,
139 *A.fumigatus*, *Absidia* sp., *Rhizopus* sp., *Aspergillus niger*, *Mucor* sp. *Cladosporum* sp.,
140 *Penicillium italicum*, *Penicilium viridatus*, *Candida tropicalis* and *Fusarium moniliformis* from
141 selected smoked fish from different markets sites in Uyo, Akwalbom state. Hassan [6] also
142 reported the isolation of several species of fungi belonging to the genera *Penicillium*,
143 *Aspergillus*, *Fusarium*, *Rhizopus* and *Mucor* from smoked dried fish sold at market place in
144 Giza Governorate, Egypt.

145



146

147 Fig 1: Percentage occurrence of fungi associated with marketed smoked dried fish samples
148 fungi associated with smoked dried fish samples

149

150 The occurrence of these fungal contaminations in smoked dried fish samples using the three
151 methods of isolation as shown in Table 1 could be attributed to improper sanitation from the
152 catching stage to the smoking stage. Moreover, there is ever increasing demand for smoked
153 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 also clean or hygienic, some display them in open trays without coverage [6]. This in turn allows the dusts and fungal spores to settle on the product which may lead to fungal invasion, production of toxins and spoilage.

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

Methods of isolation	Fungal species
Washing method	<i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Fusarium</i> sp., <i>Penicillium</i> sp., <i>Penicillium citrinum</i> , <i>Penicillium italicum</i> , <i>Mucor</i> sp.
Direct plating	<i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i> , <i>Aspergillus terreus</i> , <i>Absidia</i> sp., <i>Penicillium</i> sp., <i>Penicillium citrinum</i> , <i>Penicillium italicum</i> , <i>Mucor</i> sp.
Dilution method	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , <i>Fusarium moniliformis</i>

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 4.7×10^2 in Butter fish to 9.1×10^4 in West African Shad. This microbial level is considerable high and could be 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 needed by fungi for growth. 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].

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

Fish samples	Fungi count cfu/g	Moisture content (%)
Bonga fish (<i>Ethmalosa fimbriata</i>)	7.1×10^3	26.1
Cat fish (<i>Gymnallabes typhus</i>)	5.7×10^4	28.4
West African Shad (<i>Ilisha africana</i>)	9.1×10^4	28.8
Butter fish (<i>Schilbe uranoscopus</i>)	4.7×10^2	21.1
Sole fish (<i>Cynoglossus browni</i>)	6.3×10^3	22.1

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 2.731µg/kg in Sole fish to 4.031µg/kg in West African Shad while that of aflatoxin G1 ranged from 2.015µg/kg in Sole fish to 3.528µg/kg 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.

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 [15], 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 [16]. This smoked dried fish samples are consumed directly by consumers and as a results, they consume the mycobiota and the metabolites [17]. 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 [18].

Table 3: Aflatoxins concentrations in smoked fish samples

Samples	Aflatoxin B1 (ug.kg ⁻¹)	Aflatoxin G1 (ug.kg ⁻¹)
Bonga fish (<i>Ethmalosa fimbriata</i>)	-	-
Cat fish (<i>Gymnallabes typhus</i>)	3.905 ^b	2.617 ^b
West African Shad (<i>Ilisha africana</i>)	4.031 ^a	3.528 ^a
Butter fish (<i>Schilbe uranoscopus</i>)	-	-
Sole fish (<i>Cynoglossus browni</i>)	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

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.

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

- 236 7. Lawal OU, Fagbohun ED. Studies on Bio-Deterioration, Aflatoxin Contamination and
237 Nutritive Values of Processed Cashew (*Anacardium occidentale* L) Nuts during
238 Storage. *Nature and Science*, 2014; 11(9): 127 – 133.
- 239 8. AOAC. Official Methods of Analysis of Association of Official Agricultural Chemists.
240 13th Ed., Washington D.C., USA 1984; Chapt. 26: 481-483.
- 241 9. Alhussaini MS. Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from
242 Saudi Arabia. *Journal of American Science* 2012; 8(12): 525-534.
- 243 10. El-Bazza ZE, Zedan HH, Toama MA, El-Tayeb OM. Isolation of aflatoxins producing
244 fungi from Egyptian food and feed commodities. *Proc. Int. Symb. Mycotoxins*, Cairo,
245 1982; 12: 443-454.
- 246 11. Coomes TJ, Crowther PC, Francis BJ, Stevens L. The detection and estimation of
247 aflatoxin in groundnut and groundnut materials. *Analyst* 1965; 90: 492-494.
- 248 12. Duncan PB. New multiple range and multiple F-tests in Biometrics, 1956;11: 1-42.
- 249 13. Oloyo RA. Fundamentals of research methodology for social and applied sciences.
250 ROA Educational Press, Ilaro, Nigeria 2001; 53-200.
- 251 14. Laciakova A, Pipova M, Mate D, Luciak V. Microbial analysis of meat products
252 focused on the occurrence of microscopic filamentous fungi. *The University*
253 *Veterinary Medicine*, Kovenskeho 2004; 6: 24 – 28.
- 254 15. Adejola QA. Presence of aflatoxins in smoked-dried fish sold in Abeokuta, Ogun
255 State, South-Western Nigeria. B.Sc project submitted to the Department of
256 Aquaculture and Fisheries Management, University of Agriculture, Abeokuta,
257 Nigeria. 2011; 35.
- 258 16. Adebayo-Tayo BC, Onilude AA, Ogunjobi AA, Gbolagade JS, Oladapo MO.
259 Detection of fungi and aflatoxin in shelved bush mange seeds (*Irvingia* spp) stored
260 for sale in Uyo, Eastern Nigeria. *EJEAFche*. 2006;5(5): 1569 – 1574.
- 261 17. Essien JP, Ekpo MA, Brooks AA. Mycotoxigenic and proteolytic potential of moulds
262 associated with smoked shark fish (*Chlamydoselachus anguincus*). *Journal of*
263 *Applied Science and Environmental Management*, 2005;9 (3): 53- 57.
- 264 18. Sage LS, Krivobok E, Delbos F, Seigle M, Creppy EE. "Fungal flora and ochratoxin
265 A production in grapes and musts from France". *Journal of Agricultural and Food*
266 *Chemistry*, 2002; 50: 1306.