Aflatoxins in Smoked-dried Fish sold in Abeokuta, Ogun State, South-west Nigeria

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Abstract

This study estimated the aflatoxin contamination of smoked-dried fish samples of *Alestes nurse* (Silverside fish)*, Synodontis budgeti* (Catfish)*, Ilisha Africana* (West African Shad)*, Gymnallabes typus* (Catfish)*, Ethmalosa fimbriata* (Bonga)*, Chrysichthys nigrodigitatus* (Siver Catfish)*, Schilbe uranoscopus* (Butter fish)*, Cynoglossus browni* (Sole), *Clarias gariepinus* (Mud Catfish)*, Calamoichthys calabaricus* (Rope fish) in Abeokuta, Ogun State, Southwestern Nigeria. Fifty smoked-dried fish samples sold at two different markets in Abeokuta town, Lafenwa and Itoku in Abeokuta, Ogun State, Nigeria were found to be lightly contaminated with aflatoxin ($P<0.05$), after testing for their aflatoxin levels using Veratox quantitative aflatoxin test. The aflatoxin concentrations in the samples were between 0.030ppb-1.150ppb with a mean of 0.5980±0.1050 ppb. Rope fish had the lowest aflatoxin concentration while Mud catfish had the highest aflatoxin concentration. Aflatoxins are known to be carcinogenic (causing hepatoma – cancer of the liver), acute hepatitis, reduced red blood cell and decreased immune system in man. Prolonged intake of smoked fish with these metabolites may constitute potential public health hazard. Smoked-dried fishes stored for sale in Abeokuta markets were not heavily contaminated with aflatoxins.

Keywords: Smoked-dried fish, Aflatoxin, Aflatoxicosis, Cancer, *Aspergillus species*

Introduction

In Nigeria, fish smoking is the most practiced preservation method. Practically all species of fish available in the country can be smoked and it has been estimated that 70-80 percent of the domestic marine and freshwater catch is consumed in smoked form. Smoke drying methods used in Nigeria require low capital investment and it is conducted in fishermen camps and fish processing centuries in traditional smoking kilns of clay, cement blocks, drums or iron sheets

Smoked fish constitute a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural population (Eyo, 1992). Aflatoxin is a toxic compound produced by *Aspergillus flavus* and *A. parasiticus.* The molds can grow in improperly stored feeds and feeds with inferior quality of ingredients. Aflatoxins represent a serious source of contamination in foods and feeds in many parts of the world. These toxins have been incriminated as the cause

of high mortality in livestock and in some cases of death in human beings. Aflatoxin B1 is known to be the most significant form that causes serious risk to animals and human health (Murjani, 2003). Before now, fungi were regarded as causing only anesthetics spoilage of food. But in 1966, when the famous "Turkey X" diseases killed 10,000 turkey poultry in Great Britain, western world became aware that common spoilage molds could produce significant of toxigenic fungi and potentially toxic compounds have been discovered. Aflatoxins, a group of toxic metabolites produced by certain *Aspergillus species* have been found to be carcinogenic, tetratogenic and mutagenic to several species of experiment animals. Aflatoxin occurs in a variety of crops and animal products. The conditions that contribute to fungal growth and production of aflatoxins are a hot and humid climate, moisture content of 16% and above, favorable substrate characteristics and factors that decrease the host's immunity such as insect damage. Aflatoxins have a high melting point of 250°C. It has been proved that food items do carry residue of the toxin. Thus, it is certain that human beings are exposed to aflatoxins through contaminated food items among which fish is an important component (Murjani, 2003).

According to ICRI (2000), humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid because fungal growth in foods is not easy to prevent. Although heavily contaminated food supplies are not permitted in market places in developed countries, concern still remains for the possible adverse effects resulting from long-term exposure to low levels of aflatoxins in the food supply. Evidence of acute aflatoxicosis in humans has been reported from many parts of the world. The syndrome is characterized by vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and death with cerebral oedema and fatty involvement of the liver, kidney, and heart.

The expression of aflatoxin- related diseases in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation. Ingestion of aflatoxin, viral diseases, and hereditary factors has also been suggested as possible aetiological agents of childhood cirrhosis. There are evidences to indicate that children exposed to aflatoxin-infested breast milk and dietary items such as unrefined groundnut oil, may develop cirrhosis. Malnourished children are also prone to childhood cirrhosis on consumption of contaminated food. Several investigators have suggested aflatoxin as an aetiological agent of Reye's syndrome in children in Thailand and New Zealand, though; there is no conclusive evidence yet. Epidemiological studies have shown the involvement of aflatoxins in Kwashiorkor, an evidence of malnourishment in children. The diagnostic features of Kwashiorkor are oedema and damage to liver, among others. Hence, it is very important to reduce the dietary intake of aflatoxins by following the procedures for monitoring levels of aflatoxins in foodstuffs. The principal target organ for aflatoxins is the liver. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes, leading to necrosis or liver cell death.

Materials and Methods

Sample Collection

Smoke dried fishes were randomly sampled and purchased from two different marketing sites, Itoku and Lafenwa in Abeokuta town, Ogun State, Nigeria. Five samples of related species were grouped together to make ten composite samples totaling fifty samples in all. They were subsequently packed in sterile polyethylene bags and tagged accordingly, and taken to Zartech Veterinary laboratory for analyses.

Assay Principles

Veratox for Aflatoxin is a direct competitive ELISA in a microwell format which allows the user to obtain exact concentrations in part per billion (ppb). Free aflatoxin in the samples and controls are allowed to compete with enzyme-labeled aflatoxin (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue colour. Bluer colour means less aflatoxin. The test is read in a micro-well reader to yield optical densities of the controls from the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of aflatoxin.

Sample Preparation and Extraction

The collected samples (smoked-dried fish) were ground into powdery form with the use of high-speed blender, thoroughly mixed together and made into composite, followed by weighing on an electronic scale. 5 grams of the representative sample was put into an extraction cup. 25ml of 70% methanol was added, the extraction cup was covered and manually shaken for 3 minutes; the mixture was then allowed to settle down. The extract was filtered by pouring 5ml through a Whatman #1 filter syringe, and filtrate was collected as a sample. The sample was now ready for testing.

Test Procedure

The kit (containing coated well, mixing well, conjugate, subscript and stop solution) was set at room temperature (20^0C). A well holder was obtained and 1 red-marked mixing well for each sample tested plus 4 red-marked wells for controls, and placed in the well holder. An equal number of antibody-coated wells were removed. One end of strip was marked with a “1”, and strip was placed in the well holder with the marked end on the left. Marking the inside or bottom of the wells was avoided. Each reagent was mixed swirling the reagent bottle prior to use. 100µl of conjugate from the blue-labeled bottle was placed in each red-marked mixing well. Using a new pipette tip for each, 100µl of controls and samples were transferred to the red-marked mixing wells as described below:

0	5	15	50	S1	S2	S3	S4	S5	S6	S7	S8	Strip 1
S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Strip2

Using a 12-channel pipettor, mix the liquid in the well and swirled up and down for 3 times. 100µl was transferred to the anti-body coated wells, then the red-marked mixing wells. The timer was set for 2 minutes, mixing the wells by forward pipetting and set at room temperature for incubations by sliding the mixing wells back and forth on a flat surface without splashing the wells. The content of the antibody wells was shaken vigorously, filled with distilled water and

dumped out. Then, wells were turned upside-down and tap-dried with towel until the remaining water has been removed. With new tips on the 12-channel pipettor, 100µl of substrate was added into the wells. The timer was set for 3 minutes; the wells were mixed by sliding back and forth on a flat surface. The mixture was then discarded and the reagent boat was rinsed with distilled water. Red Stop solution was poured from the red-labeled bottle into the labeled reagent boat. Excess substrate from the 12-channel pipette tips was ejected, and 100µl of Red Stop solution was pipetted into each well, mixed back and forth on a flat surface and discarded. The bottom of the micro-wells was wiped with a dry towel such that there was no fluid remaining; the plate was taking into the micro-well reader using a 650nm filter. Air bubbles were eliminated, as they could affect analytical results.

Statistical Analysis

The result was read and calculated using Neogen's Veratox software, while T-test was used to test for the significant level of the means.

Results

Sample Analysis

The result of the total aflatoxin concentrations expressed in part per billion (ppb) obtained from the sampling of ten smoked-dried fish species (Table 1) purchased from local markets in Abeokuta are shown in Table 2 and Figure 1. Aflatoxin concentrations of the smoked-dried fish samples ranged from 0.030 ppb to 1.150 ppb with a mean of 0.5980±0.1050 ppb.

Table 1: Identified smoked-dried fishes sampled from the two markets (Itoku and Lafenwa) totaling fifty

Scientific names	Common names
Alestes nurse	Silverside fish
Synodontis budgeti	Catfish
Ethmalosa fimbriata	Bonga fish
Schilbe uranoscopus	Butterfish
Clarias gariepinus	Mudcatfish
Gymnallabes typus	Catfish
Ilisha Africana	West African Shad
Chrysichthys nigrodigitatus	Silver catfish
Cynoglossus browni	Sole
Calamoichthys calabaricus	Rope fish

PLATES SHOWING THE SAMPLED SMOKED-DRIED FISH SPECIES

Plate 1: ***Ethmalosa fimbriata***

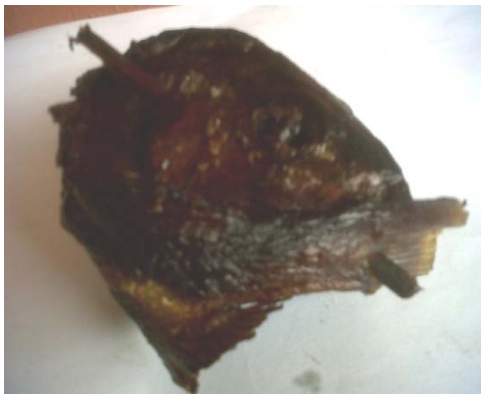

Plate 2: ***Synodontis budgeti***

Plate 3: ***Ilisha africana***

Plate 4: ***Calamoichthys calabaricus***

Plate 5: ***Clarias gariepinus***

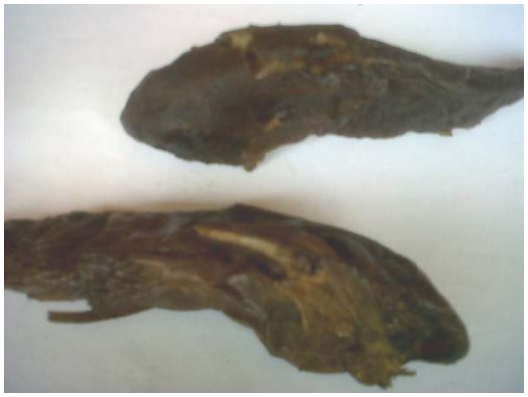

Plate 6: ***Schilbe uranoscopus***

Plate 7: ***Chrysichthys nigrodigitatus***

Plate 8: ***Gymnallabes typus***

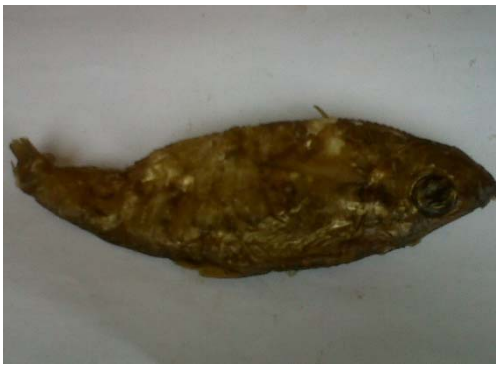

Plate 9: ***Cynoglossus browni***

Plate 10: ***Alestes nurse***

Table 2: Result of Laboratory Analysis for the Aflatoxin Levels of the Smoked-dried fishes sampled from the two markets

Slope: -2.090 **Correlation Co-efficient** **Units: ppb**

Sample	Description	Optical Density	Preliminary Result	Dilution Factor	Final Result
1	0 ppb	1.220	0.0000		
2	5 ppb	0.752	5.290		
3	15 ppb	0.497	13.480		
4	50 ppb	0.203	52.620		
5	A	1.184	0.190	1.0	0.190 ± 0.105
6	B	1.121	0.620	1.0	0.620 ± 0.105
7	C	1.117	0.650	1.0	0.650 ± 0.105
8	D	1.127	0.570	1.0	0.570 ± 0.105
9	E	1.056	1.150	1.0	1.150 ± 0.105
10	F	1.096	0.810	1.0	0.810 ± 0.105
11	G	1.154	0.380	1.0	0.380 ± 0.105
12	H	1.083	0.910	1.0	0.910 ± 0.105
13	I	1.114	0.670	1.0	0.670 ± 0.105
14	J	1.214	0.030	1.0	0.030 ± 0.105

Keys:

A: *Alestes nurse*
B: *Synodontis budgeti*
C: *Ethmalosa fimbriata*
D: *Schilbe uranoscopus*
E: *Clarias gariepinus*
F: *Gymnallabes typus*
G: *Ilisha Africana*
H: *Chrysichthys nigrodigitatus*
I: *Cynoglossus browni*
J: *Calamoichthys calabaricus*

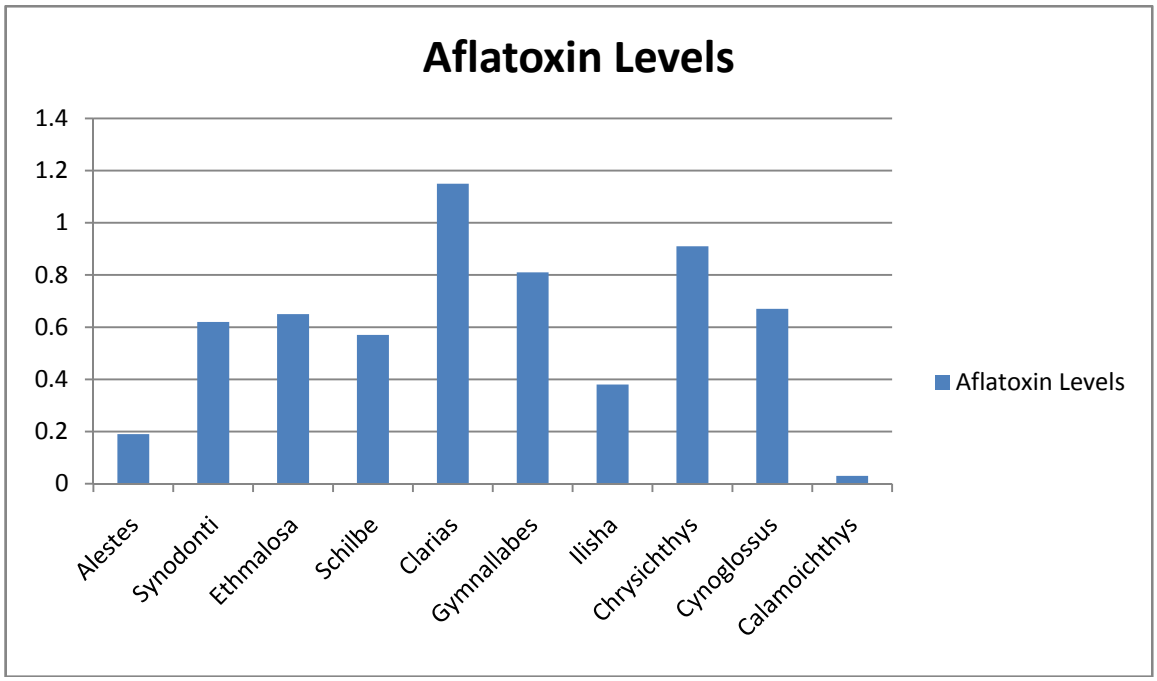

Figure 1: Aflatoxin concentrations in the different smoked-dried fish samples

Discussion

Results obtained from this study showed that aflatoxins were found to be associated with smoked-dried fishes sold in different markets in Abeokuta, but in non-significant levels ($P<0.05$) (0.030ppb-1.150ppb) that may not be toxic to human health, according to the regulatory levels for aflatoxins issued by the Food and Administration (FDA) of the United States (The FDA regulatory levels for aflatoxin intake for humans and all animal species is maximum of 20 ppb). Adebayo-Tayo *et al.* (2006), reported different results in marketed smoked-dried fish stored for sale in Uyo, Akwa-Ibom State, whereby the presence of aflatoxins were in higher concentrations (The aflatoxin concentrations were between 1.5ppb – 8.1ppb) in the samples, which might make their consumption hazardous to health. Such a higher difference in aflatoxin concentrations might be as a result of higher relative humidity usually recorded in Uyo, unlike Abeokuta town. However, this could favour the accumulation of aflatoxin due to high moisture content when displayed for sale in the market. The processes of handling fishes are also prone to aflatoxin contamination especially in artisanal fishery due to unhygienic methods of preservation. According to Akande and Tobor (1992), in artisanal fishery, freshly caught fish are covered with damp sacks and at times, they are mixed with wet grass or water weeds to reduce the temperature. Fish treated this way is prone to contamination with microorganisms such as bacteria and fungi. This indicates that spoilage of fish starts shortly after harvesting. During the smoke-drying period, smoking kilns used in artisanal fishery and the overloading of the fishes on the trays leads to improper processing which in turn encourages aflatoxin contamination (Eyo, 1992). During storage of smoked-dried fish products, good storage practices are not adhered to, hence stores are not well ventilated and pests can easily gain access into the stores. The environment in which fishes are displayed in the market is not always hygienic and this is another avenue for aflatoxin contamination. Very often, retailers display the smoked-dried

fishes in open trays beside the gutter on refuse heaps; this also encourages fungal attack and subsequent production of toxins. This is in agreement with the report of Akande and Tobor (1992). The result also revealed that aflatoxins were detected in all of the samples. The aflatoxin concentration ranged from 0.030ppb-1.150ppb. Rope fish had the lowest aflatoxin concentration while Mud catfish had the highest aflatoxin concentration.

The extracted smoked-dried fish samples produced bluish and greenish spots during laboratory analysis. Sharma (2002) reported that the two major metabolites of *Aspergillus sp*. called aflatoxins were designated B and G because they fluoresce blue (B) and green (G) when exposed to long-wave ultraviolet light.

Aflatoxins are highly carcinogenic causing hepatoma (cancer of the liver) and have also been associated with acute hepatitis in man, mostly the developing world (Eaton and Groopman, 1994). Aflatoxin have been reported in grapes in France (Sage *et al.,* 2002), edible nuts and nut products, milk and milk products (Prasad, 1992), bush mango seeds (Adebayo-Tayo *et al.*, 2006). The implication of this report is that, though in Abeokuta most of the populace feed on smoked-dried fishes, it can be confirmed that most of the consumers would have been consuming aflatoxins. Though, in relatively small amount, prolong intake of these aflatoxins may constitute a health hazard. Goldbatt and Stolloff (1983) reported that aflatoxins occurred in the human diet and this could pass from feed to milk. Since improper smoking and drying of fishes may lead to insect infestation, fungal attack, fragmentation and degradation of the product (Eyo, 1992), it is therefore important that both the artisanal fishermen and the marketers should adopt a better method of preservation. Better smoking kilns should be provided for artisanal fishermen at subsidized rates and fish product should be well stored.

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