MYCOFLORA AND AFLATOXIN B1 ANALYSIS AND PROXIMATE MINERAL COMPOSITION OF SMOKED DRIED JUVENILE FISH (*Clarias gariepinus* (*Lori amala*)) FROM SOME MAJOR MARKETS IN IBADAN METROPOLIS, OYO STATE,NIGERIA

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Introduction

Fish is a highly nutritious food and an excellent sources of proteins, vitamins, minerals and essential fatty acids. It has a relatively 10% calories content therefore its role in nutrition is recognized. (Akande and Tobor, 1992). Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas (Adeleye, 1992). Interest in fish consumption has increased over the years, due to its recognition as a lean alternative to meat and secondly the health benefits it imparts being a rich source of Omega-3 fatty acids that reduces cholesterol levels and the incidence of heart diseases and pre-term birth (He, 2009).

Dried fish are very important parts of the traditionally accepted diet for many in developing countries as well as a major source of protein, their characteristics flavour and for seasoning foods. In Nigeria, smoking is a preferred method of fish preservation in most rural areas and riverine fishing communities (Akinola *et al*., 2006) described several methods of fish preservation by smoking in most fishing communities owing to non-availability of electricity. It requires low capital, investment and it is conducted in fisherman camps and fish processing centuries in traditional smoking kilns of clay, cement blocks, drums or iron sheets (Eyo, 1992). It was also noted that apart from giving the product desirable taste and odour, smoking provides longer shelf-life through its anti-bacterial and oxidative effects, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage agents (Sengor *et al*., 2004).

In Nigeria, fish is eaten fresh, preserved or processed.Smoked dried fishes are contaminated during processing and also handling. Several microorganisms ranging from bacteria, yeasts and moulds. Numerous pathogenic agents isolated grow and produce their toxic secondary metabolites like aflatoxins produced by certain *Aspergillus species* which are found to be carcinogenic, tetratogenic and mutagenic to several species of experimental animals. As a result, smoked dried juvenile fish may harbour mycotoxins which can cause serious system dysfunction and public health hazards.

Materials and Method

A total of 20 smoked dried juvenile fish samples were randomly purchased from hawkers and open markets in different major markets sites in Ibadan, Oyo State. Fungi associated with the samples were isolated using a sterile Potato Dextrose Agar (PDA). The isolated fungi were identified using morphological and microscopic characteristics. Determination of aflatoxigenic fungi was carried out using Coconut Cream Agar (CCA) (Davis *et al*., 1987). Proximate (crude protein, crude fat, crude fibre and moisture content), mineral and heavy metal composition of the samples were evaluated (AOAC., 2000). Quantitative Determination of Total Aflatoxin in Smoked Dried Fish Samples done using ELISA kit. Veratox for quantitative analysis of total aflatoxin test NEOGEN Crop, Lansing, MI. The assay kit was based on Competitive Direct Enzyme Linked Immunosorbent Assay.

Results

A total of 32 fungi were obtained from the smoked dried juvenile fish samples. The total fungi count ranged from 1.0 – 12.0 $\times 10^3$ CFU/g. The fungi were identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus orchraceus*, *Rhizopus* sp., *Alternaria* sp. and *Penicillum* sp. (Table 1)

Samples obtained from all the markets had *Aspergillus niger* with the highest frequency of occurrence of 37.50%. This was followed in order by *Aspergillus flavus* (31.25%), *Penicillium* sp. (12.50%), *Rhizopus* sp. (9.375%), *Alternaria* sp. (6.25%), and *Aspergillus orchraceus* (3%) *had* the least frequency of occurrence (Figure 1).

Table 1: Fungi Associated with smoked- dried juvenile fish samples

Growth	Colony Colour	Character of Hyphae	Conidial Head	Probable Organism
Rapid	Yellowish green with golden edges	Septate	Globose	*Aspergillus flavus*
Rapid	Blackish	Septate	Globose	*Aspergillus niger*
Rapid	Whitish with black spores	Non Septate	Spherical	*Rhizopus* sp.
Moderate	Blackish gray	Septate	Ovoid	*Alternaria* sp.
Rapid	Golden Yellow	Septate	Globose	*Aspergillus orchraceus*
Rapid	Dark-green, cream yellow reverse	Smooth	Ellipsoidal	*Penicillium* sp.

The pH of the dried fish samples ranged from 5.4– 7.5.

Figure 1: The fungal isolates and their frequency of occurrence (%)

Table 2: Detection and concentration of Aflatoxin B_1 (ppb) in the smoked dried juvenile fish samples

Sample	First Sampling		Second Sampling		Third Sampling	
	Code	AFB1 Conc.	Code	AFB1 Conc.	Code	AFB1 Conc.
Oje A	OJA25	0.841^f	OJA08	1.789^a	OJA10	0.237^e
Oje B	OJB25	0.606^j	OJB08	1.590^b	OJB10	0.654^c
Oje C	OJC08	0.832^g		-		-
Itamerin A	ITA25	1.995^a	ITA08	1.377^c	ITA10	0.602^d
Itamerin B	ITB25	0.849^e	ITB08	1.394^d	ITB10	1.415^a
Itamerin C	ITC10	0.878^d		-		-
Bodija A	BOA25	1.064^b	BOA08	1.025^f	BOA10	1.194^b
Bodija B	BOB08	0.648^h	BOB10	1.580^c		-
Bodija C	BOC08	0.937^c		-		-

Mean values followed by the same lowercase letter(s) along each vertical column are not significantly different by Duncans' Multiple range Test at P<0.05

KEYS: OJA, OJB, OJC- Oje market; ITA, ITB, ITC- Itamerin market; BOA, BOB, BOC- Bodija market. ppb- part per billion.

Table 3: Proximate analysis of the smoked dried juvenile fish samples

Isolates codes	%Moisture Content	%Crude protein	%Crude fat	%Crude fibre	%Ash	%Carbohydrate
OJC08	17.82±0.12^a	68.72±0.02^i	3.31±0.01^d	-	10.1±0.02^c	-
ITA08	16.12±0.12^b	72.41±0.08^e	3.02±0.02^f	-	8.45±0.05^g	-
BOA08	12.99±0.02^i	77.62±0.03^a	1.49±0.02^g	-	7.90±0.03^h	-
ITA10	14.06±0.01^g	69.51±0.04^h	4.19±0.01^b	-	12.24±0.02^a	-
OJB10	13..45±0.02^h	71.77±0.05^f	3.11±0.01^e	-	10.09±0.01^d	-
BOB10	14.70±0.03^d	73.76±0.04^c	0.49±0.01^i	-	11.05±0.01^b	-
ITB25	15.35±0.01^c	76.51±0.02^b	1.15±0.01^h	-	6.97±0.01^i	-
OJA25	14.43±0.01^f	73.10±0.02^d	3.99±0.01^c	-	8.48±0.01^e	-
BOA25	14.51±0.02^e	70.52±0.05^g	6.00±0.02^a	-	8.50±0.02^f	-

Mean values followed by the same lowercase letter(s) along each vertical column are not significantly different by Duncans' Multiple range Test at P<0.05

KEYS: OJA, OJB, OJC- Oje market; ITA, ITB, ITC- Itamerin market; BOA, BOB, BOC- Bodija market

Table 4: Mineral and Heavy metal composition (mg/100g) of smoked dried juvenile fish samples

Isolates codes	Mineral Composition (mg/100g)							Heavy metal(mg/100g)	
	P	K	Ca	Mg	Fe	Cu	Zn	Pb	Cd
OJC08	509.6^a	1113.8^i	1177.5^f	131.5^h	30.80^d	0.755^c	5.855^c	0.00	0.00
ITA08	432.8^e	1410.0^e	1372.5^c	168.0^d	13.37^i	0.467^f	5.515^f	0.00	0.00
BOA08	388.2^i	1517.0^a	1191.3^e	161.9^e	27.90^e	0.805^a	5.856^d	0.00	0.00
ITA10	418.1^g	1275.0^h	1491.3^b	241.8^a	61.25^a	0.770^b	7.690^a	0.00	0.00
OJB10	468.9^c	1452.5^d	1008.8^h	151.1^g	36.25^b	0.625^e	5.375^h	0.00	0.00
BOB10	473.9^b	1455.0^c	1518.8^a	184.3^b	31.42^c	0.697^d	7.210^b	0.00	0.00
ITB25	436.6^d	1503.6^b	1099.0^g	157.4^f	16.85^g	0.443^g	5.228^i	0.00	0.00
OJA25	425.5^f	1392.5^f	1237.5^d	168.8^c	16.25^h	0.193^i	6.540^c	0.00	0.00
BOA25	411.3^h	1338.7^g	928.7^i	129.0^i	17.40^f	0.350^h	5.470^g	0.00	0.00

Mean values followed by the same lowercase letter(s) along each vertical column are not significantly different by Duncans' Multiple range Test at P<0.05.

KEYS: OJA, OJB, OJC- Oje market; ITA, ITB, ITC- Itamerin market; BOA, BOB, BOC- Bodija market. P-Phosphorus, K- Potassium, Ca- Calcium, Mg- Magnesium, Fe- iron,Pb- Lead, Cu- Copper, Zn- Zinc, Cd- Cadmium.

Table 5: Hallophilic and Xerophilic potential of the isolates

Fungi Strains	Xerophilic Fungi (DG18 AGAR)	Halophilic fungi (MY5-12 AGAR)
Aspergillus flavus	+	+
Aspergillus niger	+	+
Rhizopus sp.	-	+
Alternaria sp.	-	-
Aspergillus orchraceus	+	+
Penicillium sp.	+	+

Table 6: pH of the dried fish samples

Sample code	pH	Sample code	pH
OJA25	6.8	BOB08	7.6
OJB25	5.4	BOC08	6.5
ITA25	6.8	OJA10	6.5
ITB25	6.0	OJB10	6.5
BOA25	6.9	ITA10	6.8
OJA08	6.1	ITB10	6.8
OJB08	6.8	ITC10	6.7
ITA08	7.5	BOA10	6.7
ITB08	6.8	BOB10	6.5
BOA08	6.7		

Discussion

The isolated fungal can be classified as storage fungi and the presence of toxigenic fungi increases the risk for mycotoxin production. 94% and 85% of the isolates were halophilic and xerophilic respectively. Detection of aflatoxin in the all the smoked dried juvenile fish samples is in agreement with the work of Akinyemi *et al*. (2011) and Adebayo *et al*. (2008). The aflatoxin level in the food samples is below the acceptable limit of 20 ppb (FDA)

Conclusion

The smoked dried juvenile fish sold in some major markets in Ibadan are contaminated with mycotoxigenic fungi and aflatoxin. Therefore, it is likely that a percentage of the population of consumers in Ibadan metropolis and its environs have been ingesting an amount of partially unhealthy food, since many consumers at the point of purchase with no further processing consume with little processing. Though in small quantity, the continuous consumption of these mycotoxin may pose health hazards. The moulds obtained were from the smoked dried juvenile fish samples, thereby the need for proper harvesting, processing and preservation methods to eliminate them.

Enlightenment to the general public of proper storage of food products especially dried products after purchase and proper cooking before consumption is essential.

References

Adeleye, O.A. 1992. *Conservation needs of fisheries resources and reorientation for sustainable captive and culture practices.* Proceedings of the 10th annual conference fisheries society of Nigeria, 230-234.

Akande, G.R. and Tobor, J.G. 1992. *Conservation needs of fisheries resources and re-orientation sustainable captive and cultural practices*. Proceedings of the 10th annual conference of Fisheries Society of Nigeria 230 – 234.

Akinyemi, A.A. and Buoro, O.O. 2011. Occurrence of Bacteria Found in Gills, Skin, Buccal Cavity of *Lutjans agennes*, *Pseudotolithus elongatus* and *Sphyraena barracuda* from Lagos Lagoon, Nigeria. *Journal of Fisheries and Aquatic Science*. 6: 555-562

Adebayo - Tayo, B.C., Onilude, A.A.and Patrick, U.G. 2008. Mycoflora of Smoke Dried Fishes Sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Science*. 4(3): 346-350.

He, K. 2009. Fish long-chain omega-3 polyunsaturated fatty acids and prevention of cardiovascular diseases—Eat fish or take fish oil supplement? *Progress in cardiovascular Diseases*. 52:95 -114.

Akinola, O. A., Akinyemi, A. O. and Bolaji, B. O. 2006. Evaluation of traditional and solar drying systems towards enhancing fish storage and preservation in Nigeria (Abeokuta Local Government as a case study). *Journal of Fish. International.* 2.1: 99 – 103.

Eyo, A.A. 1992. *Traditional and improved fish handling, preservation and processing techniques.* NAERLS/NIFER national workshop on fish processing, storage, marketing and utilization, 15.

Sengor GF., Kalafatoglu H. and Gun H 2004. The Determination of Microbial Flora, Water Activity and Chemical Analysis in Smoked Mussels (*Mytilus galloprovincialis*, L.) *Turkish Journal of Veterinary Animal Science* 28: 793 797.

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