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AFLATOXIN CONTAMINATION AND NUTRITIONAL EVALUATION OF DRIED JUVENILE FISH (*Clarias gariepinus)* SOLD IN IBADAN METROPOLITAN MARKETS

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ABSTRACT

The contamination of food due to the occurrence of mycotoxigenic fungi is a public health concern. Therefore, fungi and aflatoxin contamination of smoked-dried juvenile fish (SDJF) (*Clarias gariepinus)* were investigated. The samples were purchased from three major markets in Ibadan. The total fungal count ranged from 1.0 x 10^3 to 12.0 x 10^3 CFU/g. Thirty-two fungi were isolated, including *Penicillium* sp., *Rhizopus sp*., *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus ochraceus. A. niger* had the highest frequency of occurrence (38%), while *A. ochraceus* (3%) had the lowest. All the samples were contaminated with aflatoxin. The total aflatoxin content ranged from 0.237 to 1.995 ppb. Of the 11 *Aspergillus* strains obtained from the samples, 5 were aflatoxigenic, while 6 were not. 85 and 94% of the mycobiota were xerophilic and halophilic. The percentage moisture content, crude protein, crude fat, and ash content in the samples ranged from 12.99–17.82%, 68.72–77.62%, 0.49–6.00%, and 6.97–12.24%. Phosphorus and potassium concentrations of the SDJF samples ranged from 388.2–509.6 mg/100g and 1113.8–1517.0 mg/100g, while lead and cadmium were not detected in the samples. Though the level of aflatoxin contamination in this study was within the maximum limit permitted (20 ppb), proper attention is needed for adequate preservation before sales and consumption.

Keywords: Aflatoxin, *Aspergillus* spp., *Clarias gariepinus*, Dried juvenile-fish, Mycotoxigenic fungi

INTRODUCTION

Fish is a highly nutritious food and an excellent source of proteins, vitamins, minerals, and essential fatty acids. It has a relatively 10% calorie content; therefore, its role in nutrition is recognized (Adebami *et al*., 2020; Morris and Mohiuddin, 2021; Rybicka *et al*., 2022). Fish and fish products constitute more than 60% of the total protein intake of adults, especially in rural areas (Egun and Oboh, 2022). 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 as a rich source of Omega-3 fatty acids that reduce cholesterol levels and the incidence of heart diseases and pre-term birth (Ahmad *et al*., 2018).

Dried fish is a very important part of the traditional accepted diet for many in developing countries, as well as a major source of protein (Egun and Oboh, 2022). They are often enjoyed for their characteristic flavour and are commonly used as a raw material for seasoned foods such as soups and sauces. It is widely accepted on the menu card and forms a much-cherished delicacy that cuts across socio-economic, religious, and educational barriers (Dawodu *et al*., 2023).

In Nigeria, smoking is a preferred method of fish preservation in most rural areas and riverine fishing communities due to the limited availability of electricity (Adeyeye, 2016). The breakdown of fish disposal methods for consumption in the artisanal sector is as follows: live fish 7%, fresh fish 27%, smoked-dried 45%, sun-dried 20%, salted and sun-dried 10% (Adebayo-Tayo *et al*., 2008; Osibona *et al*., 2018). The smoked drying method used in Nigeria requires low capital investment and is conducted in fisherman camps using traditional smoking kilns of clay, cement blocks, drums, or iron sheets (Morris and Mohiuddin, 2021). From the processing units of smoked-dried fish to market centers, smoked-dried fish are often contaminated with microorganisms such as bacteria, yeasts, and moulds (Nleya *et al*., 2021; Rybicka *et al*., 2022).

A lot of pathogenic microorganisms isolated from different types of fish can grow and produce their toxic secondary metabolites, which are retained in fish flesh even after salting and storage periods. These toxic substances cause serious systemic dysfunction and public health hazards when consumed (Izzo *et al*., 2022). However, it was also noted that, apart from giving the product a desirable taste and odour, smoked fish provides a longer shelf life through its anti-bacterial and oxidative effects, lowering of pH, imparting desirable colouration, accelerating the drying process, and acting as an antagonist to spoilage agents (Daramola *et al*., 2020).

Moulds are multicellular fungi that form thin thread-like structures called hyphae, which are widely distributed in nature (Adeyeye, 2016). Fungi cause major spoilage of foods and feedstuffs. The proliferation of various fungi in agricultural products leads to a reduction in yield and quality with significant economic losses (Awuchi *et al*., 2021).

They produce secondary metabolites, which are referred to as mycotoxins and are present in most food substances (Izzo *et al*., 2022). Mycotoxins are low-weight metabolites that cause harm known as mycotoxicosis in livestock, domestic animals and humans, and therefore are of public health significance (Awuchi *et al*., 2021). The production of mycotoxins is stimulated by certain environmental factors. Therefore, the extent of contamination will differ with geographic location, agricultural methods, and the susceptibility of commodities to the penetration of fungi during storage and processing periods (Jonathan and Esho, 2010). Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking (Awuchi *et al*., 2021).

Numerous pathogenic strains grow and produce toxic secondary metabolites like aflatoxins, produced by certain *Aspergillus* species, which are found to be carcinogenic, teratogenic, and mutagenic to several species of experimental animals (Navale *et al*., 2021). As a result, smoked-dried juvenile fish may harbor mycotoxins that cause

serious system dysfunction and public health hazards (Singh and Nsokolo, 2020).

There is a dearth of information on smoked-dried juvenile fish (SDJF) (*C. gariepinus)n*popularly called '*Lori Amala'* in Yoruba, Nigeria. There is, therefore, a need to study the mycobiota, aflatoxin B1 level, and quality due to the lack of awareness of the dangers posed by the fish as well as the public health implications of this mycoflora for a detailed assessment of the mycological quality and mycotoxin level in the samples. This study was aimed at investigating fungi contamination and total aflatoxin levels of SDJF and determining the proximate and mineral composition of the fish, sampled from selected major markets in Ibadan metropolis, Oyo State, Nigeria.

MATERIALS AND METHODS

Collection and Identification of Smoked-dried Juvenile Fish

Smoked-dried juvenile fish (SDJF) were purchased from retail stands in three different major metropolitan market sites (Bodija, Oje, and Itamerin) in Ibadan, Nigeria (7.3775° N, 3.9470° E). A total of 27 of these fish were randomly purchased from three different sellers in each of the markets in December, March, and June (2021–2022). The fish were subsequently packaged, labeled, and transported to the laboratory for analysis in a sterile polyethylene bag.

Isolation and identification of fungi

The isolation of fungi from the SDJF was done using serial dilution and the pour plating method. Ten grams of the fish samples were homogenized, and 1.0 mL of the homogenate was serially diluted. One millilitre of the diluent was aseptically plated on sterile molten potato dextrose agar (PDA) incorporated with streptomycin (30 mg/L) to prevent bacterial growth. The inoculated plates were incubated at 28 ± 1 ºC for 3 days. Discrete colonies were isolated in pure culture by sub-culturing, and the fungal isolates were identified based on their morphological and cultural characteristics as recommended by Domsch and Gams (1980) and Singh and Rekib (1991) and on the basis of their mycelial and spore characteristics (Rathnan *et al*., 2012).

Determination of proximate and mineral composition

The moisture content (method no. 925.09), crude protein (method no. 978.04), crude fat (method no. 930.09), ash (method no. 930.05), crude fiber, and carbohydrate of the smoked-dried fish samples were determined using the Association of Official Analytical Chemists' procedures (AOAC, 2005). To estimate the crude fibre, 5.0 g of the powdered material was extracted with hexane in a thimble for six hours to remove the fat. The digestible nutrition in the fat was then removed by adding 200 mL of 1.25% sulphuric acid to three grams (3.0 g) of free fat. Using a Buchner funnel, the resultant mixture was filtered. The filter paper residue was put in a muffle furnace at 600 ºC for 30 minutes. The sample was weighed after cooling in a desiccator (Olayinka and Etejere 2018). The carbohydrate content was calculated using the subtraction method. This was accomplished by adding the moisture content, crude protein, crude fat, ash, and crude fiber and subtracting from 100.

% Carbohydrate = [100 - % (moisture + crude protein + crude fat + ash + crude fiber)]

The mineral composition as well as heavy metals in the fish samples were determined (Ayanda *et al.*, 2019). The micro Kjeldhal technique was used to determine the nitrogen (N) content (AOAC, 2005; method no. 978.04). The Flame Photometer (Sherwood, UK, Model: 410) was used to determine sodium (Na) and potassium (K), while the Atomic Absorption Spectrophotometer (Shimazzu, Japan, Model: AA-6800) was used to evaluate calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), lead (Pb), and cadmium (Cd).

Production and quantification of the total aflatoxin production

The *Aspergillus* spp. isolated from the samples were sub-cultured onto PDA plates and incubated at 28°C for 21 days to evaluate aflatoxin production (Kana *et al*., 2013). Aflatoxin was extracted from approximately 5 g of PDA containing the fungal colonies in 25 mL of 70% methanol and sterile distilled water, respectively, using the standard ELISA extraction protocol essentially described by the kit manufacturer (NEOGEN Crop, Lansing, MI, USA). Aflatoxin was quantified using an ELISA kit.

Determination of aflatoxigenic, xerophilic, and halophilic potential

The aflatoxigenic potential of the isolated fungi was determined using Coconut Cream Agar (CCA) (Davis *et al*., 1987). Production of an orange-yellow pigmentation on the mycelium indicates a positive result for toxigenic isolates. The isolates were screened for their ability to grow under low water activity using Malt Extract-Yeast Extract 5% Salt 12% Glucose Agar (MY5-12) and high salt content using Dichloran 18% Glycerol Agar (DG18), respectively. The sterile agar plates were inoculated with the isolates and incubated at 25 ºC for 7 days. The growth of an isolate on the agar plates indicates its xerophilic and halophilic potential.

Quantitative determination of total aflatoxin

The quantitative analysis of total aflatoxin was carried out using a commercially available immunoassay kit (Veratox, Neogen Crop, Lansing, MI). The kit was based on the Competitive Direct Enzyme-Linked Immunosorbent Assay. The extraction of the filtrate from the fish samples was done using a standard extraction technique. Total aflatoxin content in the samples was quantified using an ELISA kit (Veratox, Neogen Crop, Lansing, MI). The total amount of aflatoxin was read and calculated using Neogen Veratox® software (version 3.3.0.5) (Adeyeye, 2016).

Statistical analysis

The data generated from the investigations were subjected to statistical analysis of variance using the SPSS software program, version 20, Chicago, USA. A Duncan multiple range test was used to compare significant differences between the means.

RESULTS AND DISCUSSION

A total of 27 smoked-dried juvenile fish (SDJF) were sampled and cultured in the lab. The total fungal counts were recorded as shown in Table 1. The fungal count ranged from 1.0×10^3 to 12.0×10^3 CFU/g, with the highest recorded in the sample obtained from Oje Market (OJA12). 22 of the samples (81.48%) were found to have fungal counts of $\geq 3.0 \times 10^3$ CFU/g, while only 5 samples (18.52%) had fungal counts of $< 3.0 \times 10^3$ CFU/g.

Thirty-two (32) fungi were isolated from the SDJF samples, as shown in Table 2. *A. niger* was the most prevalent (40.6%) in all the samples. This was followed in order by *A. flavus* (28.1%), *Penicillium* sp. (12.5%), *Rhizopus* sp. (9.4%), *Alternaria* sp. (6.3%), and *A. ochraceous* (3.1%).

Table 1: Total fungi count (x10^3 CFU/g) of SDJF obtained from some major markets in Ibadan

Sample Source	Sampling /code / Total Fungal Count (×10^3 sfu/g)					
	First Sampling (December)	Total Fungal Count (×10^3 sfu/g)	Second Sampling (March)	Total Fungal Count (×10^3 sfu/g)	Third Sampling (June)	Total Fungal Count (×10^3 sfu/g)
Bodija A	BOA12	3.0	BOA03	3.0	BOA06	1.0
Bodija B	BOB12	8.0	BOB03	4.0	BOB06	3.0
Bodija C	BOC12	6.0	BOC03	4.0	BOC06	3.0
Itamerin A	ITA12	5.0	ITA03	3.0	ITA06	2.0
Itamerin B	ITB12	6.0	ITB03	3.0	ITB06	2.0
Itamerin C	ITC12	4.0	ITC03	3.0	ITC06	3.0
Oje A	OJA12	12.0	OJA03	4.0	OJA06	2.0
Oje B	OJB12	4.0	OJB03	3.0	OJB06	1.0
Oje C	OJC12	5.0	OJC03	3.0	OJC06	3.0

Code: *OJA, OJB, OJC – samples from Oje market; ITA, ITB, ITC – samples from Itamerin market; BOA, BOB, BOC – samples from Bodija market.*

Table 2: Distribution of the fungal strains associated with the SDJF

Fungal Strains	Markets/Sampling codes									Total isolates	% occurrence
	BOA	BOB	BOC	ITA	ITB	ITC	OJA	OJB	OJC		
Aspergillus niger	3	1	1	1	2	1	2	1	1	13	40.6
Aspergillus flavus	3	1	-	1	-	-	2	2	-	9	28.1
Alternaria sp.	-	-	-	1	-	-	-	1	-	2	6.3
Penicillium sp.	1	1	1	1	-	-	-	-	-	4	12.5
Aspergillus ochraceus	-	-	-	1	-	-	-	-	-	1	3.1
Rhizopus sp.	-	1	-	-	1	-	-	1	-	3	9.4

Codes*: - = absent; OJA, OJB, OJC – samples from Oje market; ITA, ITB, ITC – samples from Itamerin market; BOA, BOB, BOC – samples from Bodija market.*

The proximate composition of the SDJF is shown in Table 3. Sample OJC06 had the highest moisture content (17.82%), BOB03 had the highest crude protein (77.62%), BOA12 had the highest crude fat (6.00%), and ITC06 had the highest ash content (12.24%).

Table 3: Proximate composition of the smoked-dried juvenile fish (SDJF)

Isolates codes	Proximate content (%)					
	Moisture Content	Crude protein	Crude fat	Crude fiber	Ash	Carbohydrate
BOA12	14.51±0.02^e	70.52±0.05^g	**6.00±0.02^a**	0.00	8.50±0.02^f	0.00
BOB03	12.99±0.02^i	**77.62±0.03^a**	1.49±0.02^g	0.00	7.90±0.03^h	0.00
BOC06	14.70±0.03^d	73.76±0.04^c	0.49±0.01^i	0.00	11.05±0.01^b	0.00
ITA12	15.35±0.01^c	76.51±0.02^b	1.15±0.01^h	0.00	6.97±0.01^i	0.00
ITB03	16.12±0.12^b	72.41±0.08^e	3.02±0.02^f	0.00	8.45±0.05^g	0.00
ITC06	14.06±0.01^g	69.51±0.04^h	4.19±0.01^b	0.00	**12.24±0.02^a**	0.00
OJA12	14.43±0.01^f	73.10±0.02^d	3.99±0.01^c	0.00	8.48±0.01^e	0.00
OJB03	13..45±0.02^h	68.72±0.02^i	3.31±0.01^d	0.00	10.1±0.02^c	0.00
OJC06	**17.82±0.12^a**	71.77±0.05^f	3.11±0.01^e	0.00	10.09±0.01^d	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

Code: *BOA, BOB, BOC – samples from Bodija market; ITA, ITB, ITC – samples from Itamerin market; OJA, OJB, OJC – samples from Oje market; - = Not detected*

Table 4 shows the mineral composition of the SDJF. The macroelements such as phosphorus, potassium, calcium, and magnesium compositions of the fish samples ranged from 388.2^i - 509.6^a mg/100g, 1113.8^i - 1517.0^a mg/100g, 928.7^i - 1518.8^a mg/100g, and 129.0^i - 241.8^a mg/100g, respectively. Samples OJB03, BOB03, BOC06, and ITC06, respectively, had the highest phosphorus, potassium, calcium, and magnesium contents. The composition of trace elements such as iron, copper, and zinc ranged from 13.37^i - 61.25^a mg/100g, 0.193^i - 0.805^a mg/100g, and 5.228^i - 7.690^a mg/100g. Samples ITC06, BOB03, and ITC06, respectively, had the highest. Meanwhile, lead and cadmium were not detected in the fish samples.

Table 4: Mineral and Heavy metal composition (mg/100g) of SDJF

Isolates codes	Mineral Composition (mg/100g)							Heavy metal (mg/100g)	
	P	K	Ca	Mg	Fe	Cu	Zn	Pb	Cd
BOA12	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
BOB03	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
BOC06	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
ITA12	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
ITB03	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
ITC06	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
OJA12	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
OJB03	509.6^{a}	1113.8^{i}	1177.5^{f}	131.5^{h}	30.80^{d}	0.755^{c}	5.855^{e}	0.00	0.00
OJC06	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

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

The detected aflatoxigenic strains from the fish samples using Coconut Cream Agar (CCA) are shown in Table 5. Some of the *Aspergillus* strains showed aflatoxin production with the formation of orange-yellow pigmentation in the medium. 15.63% of the isolated fungi were aflatoxigenic, while 84.37% were not. Out of the 23 *Aspergillus* species isolated, only five (5) of the strains of *A. niger* and two (2) of *A. flavus* were found to produce aflatoxin.

Table 5: Aflatoxigenic potential of fungal isolates from SDJF

Isolate Codes	Fungi strains	CCA
BOA03	*A. niger*	+
BOA06	*A. flavus*	+
ITA12	*A. niger*	+
ITB03	*A. niger*	+
ITB12	*A. niger*	+
OJB03	*A. niger*	+
OJC06	*A. flavus*	+

Codes: + = Present, - = Absent; OJA, OJB, OJC – samples from Oje market; ITA, ITB, ITC – samples from Itamerin market; BOA, BOB, BOC – samples from Bodija market; CCA- Coconut cream agar

The determination of the growth of halophilic and xerophilic fungi obtained from the SDJF is shown in Table 6. Malt Extract-Yeast Extract-Salt Glucose agar (5% salt and 12% glucose) supported the growth of *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. Ninety-four percent (94%) of the isolates from the SDJF were halophilic. Dichloran Glycerol Medium (DGM) (18% glycerol) used in the analysis of the isolates for the xerophilic fungi showed that 86% of the fungi were xerophilic. All the *Aspergillus* and *Penicillium* species were able to grow on the medium.

Table 6: Halophilic and Xerophilic potentials of the SDJF

Markets	Isolate Codes	Fungi Strains	MY5-12 AGAR	DG-18 AGAR
Bodija	BOA03$_{a}$	*A. flavus*	+	+
	BOA03$_{b}$	*A. niger*	+	+
	BOA06$_{a}$	*A. niger*	+	+
	BOA06$_{b}$	*Penicillium* sp.	+	+
	BOA06$_{c}$	*A. flavus*	+	+
	BOA12$_{a}$	*A. flavus*	+	+
	BOA12$_{b}$	*A. niger*	+	+
	BOB03$_{a}$	*Penicillium* sp.	+	+
	BOB06$_{a}$	*A. niger*	+	+
	BOB06$_{b}$	*A. flavus*	+	+
	BOB06c	*Rhizopus* sp.	+	-
	BOC03$_{a}$	*A. niger*	+	+
	BOC03$_{b}$	*Penicillium* sp.	+	+
Itamerin	ITA03$_{a}$	*Alternaria* sp.	-	-
	ITA06$_{a}$	*A. ochraceus*	+	+
	ITA06$_{b}$	*Penicillium* sp.	+	+
	ITA12$_{a}$	*A. niger*	+	+
	ITA12$_{b}$	*A. flavus*	+	+

	ITB03$_a$	*A. niger*	+	+
	ITB06$_a$	*Rhizopus* sp.	+	-
	ITB12$_a$	*A. niger*	+	+
	ITC06$_a$	*A. niger*	+	+
Oje	OJA03$_a$	*A. niger*	+	+
	OJA06$_a$	*A. flavus*	+	+
	OJA12$_a$	*A. flavus*	+	+
	OJA12$_b$	*A. niger*	+	+
	OJB03$_a$	*A. niger*	+	+
	OJB03$_b$	*A. flavus*	+	+
	OJB06$_a$	*Alternaria* sp.	-	-
	OJB12$_a$	*A. flavus*	+	+
	OJB12$_b$	*Rhizopus* sp.	+	-
	OJC03$_a$	*A. niger*	+	+

KEYS: *+ = Present, - = Absent; BOA, BOB, BOC – samples from Bodija market; ITA, ITB, ITC – samples from Itamerin market; OJA, OJB, OJC – samples from Oje market; MY5-12 Agar – Malt Extract Yeast Extract 5% Salt 12% Glucose Agar; DG-18 – Dichloran 18% Glycerol Medium.*

Moreover, using the ELISA kit, aflatoxin was detected in all the samples. The aflatoxin concentrations ranged from 0.237 to 1.995 ppb.

Table 7: Concentrations of Aflatoxin B1 (ppb) in the smoked-dried juvenile fish

	Sampling interval								
	First Sampling			Second Sampling			Third Sampling		
Markets	**Sampling Codes**	**Afb1 (ppb)**	**Conc.**	**Sampling Codes**	**Afb1 (ppb)**	**Conc.**	**Sampling codes**	**Afb1 (ppb)**	**Conc.**
Bodija	BOA12	1.194^b		BOA03	1.025^e		BOA06	0.878^d	
	BOB12	1.580^c		BOB03	0.648^h		BOB06	0.878^d	
	BOC12	1.064^b	-	BOC03	0.937^f		BOC06	0.602^f	
Itamerin	ITA12	1.995^a		ITA03	1.377^d		ITA06	0.602^f	
	ITB12	1.394^c		ITB03	0.849^c		ITB06	0.602^f	
	ITC12	0.937^f		ITC03	0.937^f		ITC06	0.878^d	
Oje	OJA12	1.789^a		OJA03	0.841^c		OJA06	0.237^g	
	OJB12	1.590^b		OJB03	0.606^d		OJB06	0.654^e	
	OJC12	0.832^g		OJC03	0.849^c		OJC06	0.849^c	

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

Codes: *- = Not detected; BOA, BOB, BOC – samples from Bodija market; ITA, ITB, ITC – samples from Itamerin market; OJA, OJB, OJC – samples from Oje market*

Discussion

Smoked-dried juvenile fish (SDJF) samples obtained from some major markets in Ibadan have fungal strains that are of major public health concern. Isolated fungi can be classified as storage fungi, such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., and *Rhizopus* sp. The fungal strains or their spores could have contaminated the SDJF samples during any of the phases of processing (Bankole *et al*., 2003), especially during the storage period, as a result of inadequate storage facilities, marketing, or transportation (Hernández-Cortez *et al*., 2017).

The detection of aflatoxin without the presence of aflatoxigenic strains could mean that toxin may be present without the producing mold (Nleya *et al*., 2021); however, the presence of toxigenic fungi increases the risk for mycotoxin production. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking (Tournas *et al*., 2001). Though the contaminant organism may not survive food preparation, the produced toxin may still be present.

Moisture content is of great importance in fish storage due to the dehydration of water molecules present. The low moisture content of smoked-dried fish extends its shelf life to about 4-6 months. Higher percentage moisture content (4.67–21.69% and 12.99–17.82%) was reported in smoked-dried *C. gariepinus*, sampled from an earthen pond and concrete tank systems (Olubunmi *et al*., 2015).

Proteins form the largest quantity of dry matter in fish, and the content increased with a reduction in moisture content (Oladipo and Bankole, 2013). Dried fish had higher crude protein due to the dehydration of water molecules present between the proteins, causing aggregation of the protein molecules, thus resulting in higher protein content (Tan and Xie, 2021). The crude protein range of 68.72 to 77.62% for the SDJF observed in this study was lower than what was reported (71.46–89.13%) by Olayemi *et al*. (2011) on *Clarias gariepinus* and Egbal *et al*. (2017) on six commercial fish species (*Lates niloticus, Bagrus bayad, Oreochromis niloticus, Synodontis schall, Labeo niloticus, and Hydrocynus froskalii*) obtained from Jebl Awlia reservoir, Sudan. Effiong *et al*. (2011) reported lower crude proteins (21.62–60.57%) from *Clarias gariepinus, Oreochromis niloticus, Lates niloticus, Bagrus bayad,* and *Citharinus cithrus* obtained from Lake Kainji, Nigeria.

The crude fat content of the samples agreed with the report of Egbal *et al*. (2017) on dry fish samples. Fabiola *et al*. (2012)

reported that dried fish has a lower fat content than fresh fish; this could be due to the temperature difference, stage of life, environmental salinity, food type, and species. The fluctuation of fat content has been reported to be significantly associated with fish age, feed, and sexual cycle (Egbal *et al*., 2017).

Crude fibre and carbohydrates were not present in all the smoked-dried juvenile fish samples. This may be due to the low carbohydrate content of *Clarias gariepinus*, and since they are juvenile, they are very active and may have utilized all the carbohydrate content in their feed for metabolism (Akinwumi, 2014; Aderolu *et al*., 2018).

Ash content is a good source of minerals such as calcium, potassium, zinc, iron, and magnesium (Amer *et al*., 2022). Ash is a measure of the mineral content of food items (Oladipo & Bankole, 2013; Amer *et al*., 2022). Huda *et al*. (2011) reported that the nutrient content of fish is influenced by several factors, including smoking method and time.

Phosphorus, calcium, magnesium, and iron were detected in the samples. However, all the elements play significant roles in nutrition. For instance, phosphorus is an essential nutrient used to maintain fluid and electrolyte balance, while calcium is good for the growth and maintenance of bones, teeth, and muscle (Serna and Bergwitz, 2020). Normal extracellular calcium concentration is necessary for blood coagulation and the integrity of intracellular cement substances (Wim *et al*., 2007). Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions. Magnesium is also required for energy production, oxidative phosphorylation, and glycolysis (Fiorentini *et al*., 2021). Iron is essential for metabolic reactions, regulation of cell growth and differentiation, and an important constituent of haemoglobin (Camaschella *et al*., 2020). Copper has a value that is incorporated into a variety of proteins and metalloenzymes, which perform essential metabolic functions. Lead and cadmium are heavy metals and were not detected in the fish samples analyzed. The absence of lead and cadmium in the fish samples is in agreement with the report of Hashim *et al.* (2014).

The detection of aflatoxin in the range of 0.237–1.995 ppb in all the SDJF samples was in agreement with the work of Akinyemi *et al.* (2011) and Adebayo-Tayo *et al.* (2008), who reported 0.030–1.150 ppb and 1.5–8.1 ppb in smoked-dried fish samples obtained from The aflatoxin content was below the acceptable level recommended by the FDA (2000). The FDA regulatory levels for aflatoxin intake for humans and all animal species are a maximum of 20 ppb.

CONCLUSION

Smoked-dried juvenile fish sold in some major markets in Ibadan were contaminated with mycotoxigenic fungi and aflatoxins B1. The AFB1 in the samples was below the acceptable limit, but prolonged intake may constitute a health hazard. The fish samples were rich in nutrients, but proper attention is needed for adequate preservation before sale and consumption to prevent continuous consumption of aflatoxin.

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