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Mycofloral of Smoke-Dried Fishes Sold in Uyo, Eastern Nigeria

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Abstract: This study was aimed to estimate the mycoflora and aflatoxin contamination of smoked dried fishes of Stock (*Gadus morhua*), Skip jack tuna (*Katsuworus pelamis*), Croaker (*Pseudotolithus typhus*), Sting ray (*Dasyatis margarita*), Cat (*Arius hendeloti*), Bonga (*Ethalmosa fimbriota*), Ribban fish (*Triuchurius trichurius*), Stark (*Carchanas faunis*), Thread fin (*Pentanemis qumquarius*), Sole (*Cynoglossus browni*), Spade (*Drepane africana*) in Uyo, eastern Nigeria. Fifty-five smoke-dried fishes samples sold at three different markets in Uyo town, located at main markets, Itam and Akpan Adem in Uyo, Akwa lbom state, Nigeria were heavily contaminated with moulds. Twelve different fungi were found associated with the smoked dried fishes samples sold in the three different markets. The associated fungi were *Aspergillus flavus, A. tereus. Aspergillus fumigatus, Absidia* sp., *Rhizopus* sp., *A. niger, Mucor* sp., *Cladosporium* sp, *Penicillium Italiculum, Penicillium viridatus Candida tropicalis and Fusarium moniliformis, Aspergilus flavus* and *A. tereus* had the highest rate of occurrence among the isolated fungi. Aflatoxin B_1 and G_1 were found associated with the samples. The Aflatoxin B_1 and G_1 concentrations in the sample were between 1.5^{g} – 8.1^{a} µg/kg and 1.8^{j}- 4.5^{a}µg/kg respectively. The fungal counts were between 3.0×10^2 - 4.8×10^4 cfu /g. The moisture content and the pH were between 22.7 – 27.6% and 3.0 – 6.0 respectively. In conclusion smoked dried fishes stored for sale in Uyo markets were heavily contaminated with aflatoxigenic fungi.

Key words: Smoked dried fish · Aflatoxin · *Aspergillus flavus* · Mycoflora

INTRODUCTION

Fish supplies a good balance of protein, vitamins and minerals. It has a relatively 10% calories content hence its role in nutrition is recognized [1].

Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas [2]. They are widely accepted on the menu card and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers [2]. Fish flesh is one of the best sources of protein. Its flesh is tender due to bundles of muscle fibers, which are held together by fibrous material when heated [3]. It is better digested than beef or other types of protein.

In Nigeria, fish is eaten fresh, preserved or processed. The percentage composition of the different methods of fish disposed for consumption in the artisanal sector according to Tobor [4] are as follows live fish 7%, fresh fish 27%, smoke dried 45%, sun dried 20% salted and sun dried 10%. Smoke drying methods used in Nigeria requires 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 [5]. This result in a very short shelf life and low market value as well as inability to withstand handling and transportation by retailers [1].

For long, fungi were regarded as causing only anesthetics spoilage of food. But during 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 metabolic produced by certain *Aspergillus* species have been found to be carcinogenic tetratogenic and mutagenic to several species of experiment animals [6-8]. Aflatoxin occurs in a variety of crops and animal product. 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 [9]. Aflatoxins have a high melting point i.e. 250°C. It has been

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proved that food items do carry residue of the toxin. Thus, it's certain that human beings are exposed to aflatoxins through contaminated food items among which fish is an important component [10].

This research was embarked upon to investigate the mycoflora associated with smoke dried fishes in Uyo, eastern Nigeria and the presence of aflatoxins in the smoke-dried fishes.

MATERIALS AND METHODS

Sample Collection: Smoke dried fishes (Stock, Skip jack tuna, Croaker, Sting ray, Cat, Bonga, Ribban, Stark, Thread fin, Sole and Spade) were randomly sampled and purchased from three different marketing sites located at main markets, Itam and Akpan Adem in Uyo town Akwa lbom State, Nigeria. Fifty-five samples of related species were grouped together to make eleven composite samples, they were subsequently kept in sterile polyethylene bags, which were used for analyses.

Isolation of Fungi: Attempts to isolate fungi from the smoke-dried fish's samples were made aseptically on Saboraud dextrose agar. Ten grams of the fishes samples obtained from each of the markets were weighed aseptically and macerated in 90 ml sterile watery agar (0.2%) using a Warring blender. From this, subsequent tenfold dilution was made up to 10^{-5} [11]. One milliliter of each dilution was dispensed in triplicate in sterile Petri dishes. Molten Saboraud dextrose agar to which penicillin and streptomycin had been incorporated were added to the Petri dishes, which were gently rotated to ensure even dispersion. The plates were allowed to solidify and were incubated at 28±1°C for 3-5 days. All observed colonies were subculture to obtain pure cultures which were subsequently isolated and identified using morphological characteristics, spore formation, the production of fruiting bodies and biochemical reactions [12-14] and by compares with already identified cultures, which were obtained from the plant pathology laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University Moor Plantation, Ibadan, Nigeria. The moisture content was determined by oven drying at 105°C for $4^{1}/2$h.

Determination of pH: Two grams each of the macerated fish's samples were weighed in triplicates. Water was added and mixed thoroughly to make a fish slurry. The pH readings were taken using digital pH meter equipped with a glass electrode (digital thermo pH meter mod B-E105). The electrode was rinsed and immersed into the fish slurry. The pH reading were then recorded. Determination were done in triplicates and the mean value were obtained.

Aflatoxins Detection in Smoke Dried Fish's Samples: Aflatoxins were extracted from the samples according to the method of Seitz and Mohr [15]. Ten grams of the fishes samples obtained from each of the markets were weighed aseptically and macerated using a Warring blender and were extracted with chloroform and concentrated. Thin layer chromatography of aflatoxin and samples extracted were performed on silica gel DG 254. Of the extracted samples 5, 10 and 15 µl were spotted on three different points on a ruled base line of the TLC plates. Also 5, 10 and 15 µl of the aflatoxin standard were spotted on another three points near the previous sample extract spotted points. The plates were developed first with diethyl ether and then with chloroform: acetone (9:1v/v). Aflatoxin was identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent color under long Ultra Violet (UV) illumination was at 360 nm. The fluorescent spots of aflatoxins were scraped off the TLC and eluted by chloroform: methanol (9:1 v/v). The solvent was evaporated under nitrogen to dryness and the residue was dissolved in methanol. The concentration of aflatoxins (B_1 and G_1) in solution was determined by measuring its absorbance at 360 nm then calculated according to the method of Masri *et al*., [16].

Confirmatory Tests for Aflatoxin: Three different derivatives were prepared by treating portions of the isolated toxin or the aflatoxin standard with formic acid thionyl chloride, acetic acid-thionyl chloride and tri-fluoroacetic acid. The test was then continuing according to the method of Stoloff and Friedman [17].

Statistical Analysis: Duncan multiple range test was used to compare significant differences between the means [18].

RESULTS AND DISCUSSION

Results obtained from this study showed that *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* were found to be associated with smoked dried fishes sold in different market in Uyo. As stated above, *Aspergillus flavus* and *Aspergillus tereus, A. fumigatus*

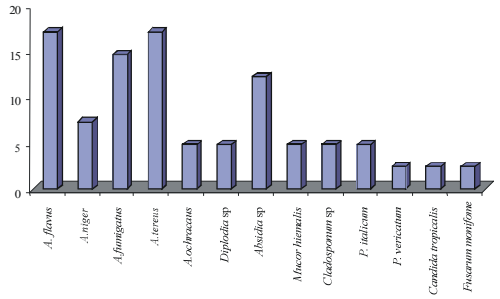

Fig. 1: The rate of occurrence (%) of fungi associated with marketed smoked dried fish samples Fungi associated with smoked dried fish samples

were the dominant mycoflora in decreasing sequential order. Adebayo-Tayo *et al.,* [19] reported similar result in marketed bush mango seeds (*Irvingia* spp.) stored for sale in Uyo. *Penicillium viridatus, Candida tropicalis and Fusarium moniliformis* occurred less frequently (fig 1). The presence of *A. flavus* in the samples might probably make its consumption hazardous to health. According to Akande and Tobor [1], 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 right from the aquatic ecosystem. Handling fishes are also prone to microbial attack especially in artisanal fishery due to unhygienic methods of reducing temperature. 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 fungal attack [5]. During storage of smoked dried fish products, good storage practices are not adhering by wholesaler hence stores are not well ventilated and pest 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 microbial contamination. Very often, retailers display the smoke-dried fish samples in open trays beside the gutter on refuse heaps, this also encourages fungi attack and subsequent production of toxins. This is in agrement with the report of Akande and Tobor [1]. The result also revealed that the average mould count ranged from 3.0×10^2-8.4×10^4 cfu/g (Table1). The microbial levels obtained in this report which is 10^4 could be considered hazardous to consumers because of the possibility of the presence of enterotoxigenic strains. The pH ranged between 3.0 – 6.0 and also the moisture content ranged from 22.7% - 27.6%. Specimen E had the lowest moisture content while specimen H had the highest moisture content as shown in Table 1.

Table 1: Fungi count, pH and moisture content (%) in different fish samples

Samples	Fungi count	pH	Moisture content (%)
A	4.8 x 10^3	3.0	25.2
B	5.8 x 10^3	5.0	25.7
C	1.72 x 10^4	3.0	26.6
D	6.4 x 10^3	5.0	26.3
E	3.0 x 10^2	6.0	22.7
F	2.3 x 10^3	3.0	24.9
G	4.2 x 10^4	6.0	27.6
H	9.0 x 10^2	6.0	24.3
I	8.4 x 10^4	5.0	27.2
J	2.8 x 10^4	4.0	27.3
K	7.6 x 10^3	5.0	26.1

Keys:
A: Stock fish (*Gadus morhua*)
B: Skipjack tuna (*Katsuworus pelamis)*
C: Croaker (*Pseudotolithus typhus)*
D: Sting - ray (*Dasyatis margarita)*
E: Catfish (*Arius hendeloti)*
F: Bonga fish (*Ethalmosa fimbriota)*
G: Ribban fish (*Triuchurius trichurius)*
H: Stark (*Carchanas faunis)*
I: Thread - fin (*Pentanemis qumquarius)*
J: Sole (*Cynoglossus browni)*
K: Spade - fish (*Drepane africana)*

Table 2: Aflatoxin B_1 and G_1 concentrations in the samples

Samples	AF B_1µgkg^{-1}	AF G_1µgkg^{-1}
A	4.750^{c}	3.550^{c}
B	3.55^{d}	3.050^{d}
C	2.100^{i}	2.8200e
D	3.005^{e}	2.515^{g}
E	1.505^{g}	1.8100^{j}
F	2.5150^{g}	2.205^{h}
G	3.0005^{e}	3.505^{c}
H	2.205^{h}	2.000^{I}
I	2.805^{f}	2.715^{f}
J	7.525^{b}	3.710^{b}
K	8.105^{a}	4.51^{a}

Each value represents a mean of three replicates. Means followed by the same letter are not significantly different by Duncan's multiple range tests.

The spots from the extracted smoke dried fish's samples and the standard aflatoxin fluorescence produced bluish and greenish sport. Sharma [20] reported that the two major metabolites of *Aspergillus* sp. called aflatoxins were designated B_1 and G_1 because they fluoresce blue (B_1) and green (G_1) when exposed to long-wave ultraviolet light.

Aflatoxin was detected in all of the samples. The concentration of aflatoxin B_1 and G_1 ranged between 1.505^{g}-8.105^{a} µg/kg and 1.810^{j}-4.51^{a} µg/kg respectively. As shown in Table 2 above samples E had the lowest AFB_1 and AFG_1 concentration while sample K had the highest

AFB_1 and AFG_1 concentration. This indicate that the smoke-dried fishes samples have been contaminated by fungi especially *Aspergillus flavus* which produced the toxins. *A. flavus* is known to produce aflatoxins [21]. Aflatoxins are highly carcinogenic, causing hepatoma (cancer of the liver) and have also been associated with acute hepatitis in man, mostly the developing world [22-24]. Aflatoxin have been reported in grapes and musts in France [25], edible nuts and nut products, milk and milk products [24], bush mango seeds [19].

The implication of this report is that, though in Nigeria most of the populace feed on fishes, it can be confirmed that most of the consumers would have been consuming this and other metabolites. Golbatt and Stolloff [26] reported that aflatoxins occurred in the human diet and this could pass from feed to milk. Prolong intake of this metabolites may constitute a health hazard. It is therefore important that both the artisanal fisher-men and the marketers should adapt a better method of preservation and better smoking kilns should be provided for artisanal fishermen at subsidized rates and stored fish product should be well stored. Equally complicated aflatoxin analysis procedure should be replaced with commercial kits such as veratose and Afla B^{+m} that are easy to run and health regulatory bodies such as NAFDAC should carry this out so that the toxin can be easily detected and samples containing them discarded.

Improper smoking and drying of fishes may lead to insect infestation, fungal attack, fragmentation and degradation of the product [5]. Since most of the moulds isolated are probably contaminants rather than originating in the fishes sample, better methods of preservation (drying and storage) will reduce their incidence or eliminate them.

CONCLUSION

Smoked dried fishes samples stored for sale in Uyo markets were heavily contaminated with aflatoxigenic fungi and they are not acceptable for consumption due to the presence of aflatoxin B_1 and G_1 contents and prolong intake may constitute a health hazard. Since most of the moulds isolated are probably contaminants rather than originating in the fishes sample, better methods of preservation (drying and storage) will reduce their incidence or eliminate them.

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