



Mycoflora of Smoke- dried fish Varieties sold in Eke Awka Market in Anambra State , Nigeria

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Abstract: Dried fish is an aquatic food between 80-85% whose moisture content is between 80-85% and is a relished food item in many dishes in many cities of the world including Awka, Nigeria. This relished food item is prone to invasion by fungi which have been known to produce toxins which have deleterious consequences on human health. It is therefore important to monitor the mycological quality of smoke-dried fishes. This work was aimed at isolating, characterizing and identifying the mycoflora of smoke-dried fish varieties sold in Eke-Awka Market in Anambra State, Nigeria. The mycoflora of the smoke-dried fish varieties was studied using cultural techniques, with Sabouraud dextrose agar as the growth medium. Bonga, Cat, Tilapia, Cod and Croaker fishes were used as the samples. The examination showed that the fishes were contaminated with fungi. The cod fishes had the highest fungal count of 2.6×10^3 cfu/g while the Croaker fishes had the least fungal count of 1.2×10^3 cfu/g. The fungi were characterized and identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solani*, *Mucor mucedo* and *Saccharomyces cerevisiae*. *Aspergillus niger* was isolated from all the fish samples studied, *Saccharomyces cerevisiae* and *Aspergillus flavus* from the Bonga fishes, *Mucor mucedo* from the Cat and Croaker fishes, *Fusarium solani* from the Cod fishes while *Aspergillus* was isolated from Tilapia fishes. *Aspergillus niger* occurred most frequently (79%) while *Saccharomyces cerevisiae* and *Fusarium solani* had the least frequency of occurrence (2%) in the fish samples. The fungi isolated are known to produce toxins that cause cancer of the liver, acute hepatitis, reduction in red blood cells and decreased immune system in man. Prolonged intake of these smoke-dried fish varieties containing these fungi and their toxins may constitute health hazards. Adequate cooking before consumption would reduce the mycoflora of these fishes, hence safeguarding the health of the consuming public.

Keywords: Mycoflora, Smoke-dried fish varieties, Eke Awka Market

INTRODUCTION

Fish is one of the aquatic foods consumed by humans. It is rich in proteins, vitamins and minerals required for supplementing both infant and adult diets¹. The omega 3 fatty acids found in some species of fish are heart friendly and can

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make improvements in brain development and reproduction. Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas². It is widely accepted and forms a more cherished delicacy that cuts across socio-economic, age, religious and educational barriers and is better digested than beef or other types of proteins. Fish is an extremely perishable food. Spoilage proceeds as a series of complex enzymatic, microbial and chemical changes that begin as soon as fish dies. The soft tissues of fishes and the aquatic environment they reside in, make them extremely susceptible to microbial contamination.

At the time of harvest, fish carries a high microbial load on the surface of their skins, in their intestinal tracts and in their gills. The type and number of microorganisms that live in fish vary according to the season, the species and the natural habitat. Additional contamination may occur during the harvesting, handling or processing of the fish.

Dried fishes are those whose moisture contents have been reduced by about 80-85%, to prevent the growth of microorganisms and decay. Water is usually removed by air drying, sun drying, smoking, wind drying and freeze drying. Dried fishes retain all the nutrients only in concentrated form and their shelf life is also prolonged. Drying to moisture content below 15% prevents the growth of many microorganisms while mold growth is suppressed at 10% moisture content.

The invasion of microorganisms into smoke-dried fishes may result from high relative humidity of the air during storage, poor storage and handling conditions, unfavourable temperature and high moisture content of the fish even after drying³. Bukola et al (2008) and Wogu and Iyayi (2011) have revealed that the common fungi mostly found in smoke-dried fishes are *Fusarium* spp, *Rhizopus* spp, *Mucor* spp, *Penicillium* spp, *Aspergillus* spp, *Trichoderma* spp and *Candida* spp^{4,5}.

Smoke-dried fish is a relished food item in many dishes in Nigeria particularly in Awka, Anambra State, Nigeria. It is important therefore to assess its mycological quality to prevent any health hazard that may arise from its consumption, hence in this study, the fungi in the smoke-dried fish varieties sold in Eke Awka Market, Anambra State, Nigeria were isolated, characterized and identified.

MATERIAL AND METHODS

Samples Collection: Five smoke-dried fish varieties purchased from Eke Awka Market, Anambra State, Nigeria were used as the samples. They were Cat fish (*Arius heudelotti*), Bonga fish (*Ethmalosa fimbriata*), Cod fish (*Gadus morhua*) Croaker fish (*Pseudotolithus typus*) and Tilapia fish (*Oreochromis niloticus*). The fishes were conveyed in separate sterile polythene bags to the microbiology laboratory for fungal isolation, characterization and identification.

Fungal Isolation: Each sample was homogenized into powder and one gram of the homogenized sample was serially-diluted using sterile distilled water. One milliliter of the serially-diluted sample (10^3) was aseptically introduced into a sterile petridish containing sterile Sabouraud dextrose agar and 2% Chloramphenicol,

added to inhibit bacterial growth. The sample was evenly spread on the surface of the medium and the plate was incubated at room temperature in an inverted position for five days. The colonies that developed after incubation were counted, purified by repeated subculturing and stored in Sabouraud dextrose agar slants for characterization and identification.

Characterization and identification of the isolates: The moulds were characterized macroscopically and microscopically. Microscopic examination was carried out using the lactophenol cotton blue solution. A drop of the lactophenol cotton blue solution was made on a clean grease-free slide and a fragment of the test fungus removed and introduced into it. The fungus was properly spread on the slide with the aid of the sterile needle after which a coverslip was placed on the slide, avoiding bubbles. The slide was thereafter mounted and viewed under the microscope.

Gram staining, motility, germ tube, starch hydrolysis, urease and sugar assimilation tests were also carried out to identify the yeasts. The fungi were identified following the scheme of Oyeleke and Manga⁶.

RESULTS

The fungal counts of the smoke-dried fish varieties studied ranged between 1.2×10^3 cfu/g and 2.6×10^3 cfu/g. The counts were 1.3×10^3 , 1.7×10^3 , 2.6×10^3 , 1.5×10^3 and 1.2×10^3 cfu/g for bonga, cat, cod, tilapia and croaker fishes respectively. The fungi isolated from the samples were *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor mucedo*, *Fusarium solani* and *Aspergillus fumigatus*.

Saccharomyces cerevisiae was isolated from the bonga fishes, *Aspergillus niger* from bonga, cat, cod, tilapia and croaker varieties, *Mucor mucedo* from cat and croaker fishes, *Aspergillus flavus* from cat fishes, *Fusarium solani* from the cod fishes while *Aspergillus fumigatus* was isolated from the dried Tilapia fishes.

Thirteen fungal species were isolated from the bonga fish samples, eight from the cat fish varieties, sixteen from the cod fishes, two from the croaker fishes while four species were isolated from the tilapia fish varieties. A total of forty-three fungal species were isolated from the dried fish varieties.

The percentage distribution of the fungal species in the smoke-dried fish varieties was *Saccharomyces cerevisiae* (2%), *Aspergillus niger* (79%), *Aspergillus flavus* (5%), *Aspergillus fumigatus* (7%), *Mucor mucedo* (5%) and *Fusarium solani* (2%).

Table 1. Fungal counts of the smoke-dried fish varieties

Dried Fish varieties	Fungal counts (cfu/g)
Bonga	1.3×10^3
Cat	1.7×10^3
Cod	2.6×10^3
Tilapia	1.5×10^3
Croaker	1.2×10^3

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Table 2. Fungal species isolated from the smoke-dried Fish Varieties

Dried Fish varieties	Fungal species
Bonga	Saccharomyces cerevisiae Aspergillus niger
Cat	Aspergillus niger Aspergillus flavus Mucor mucedo
Cod	Aspergillus niger Fusarium solani
Tilapia	Aspergillus niger Aspergillus fumigatus
Croaker	Aspergillus niger Mucor mucedo

Table 3. Frequency of isolation of the fungal species from the smoke-dried Fish Varieties.

Fungal species	Bonga	Cat	Cod	Croaker	Tilapia	Total
Saccharomyces cerevisiae	1	-	-	-	-	1
Aspergillus niger	12	5	15	1	1	34
Aspergillus flavus	-	2	-	-	-	2
Aspergillus fumigatus	-	-	-	-	3	3
Mucor mucedo	-	1	-	1	-	2
Fusarium solani	-	-	1	-	-	1
Total	13	8	16	2	4	43

- = Not detected

Table 4. Percentage distribution of the fungal species in the smoke-dried fish varieties

Fungal species	Number isolated	Percentage distribution
Sacchamyces cerevisiae	1	2
Aspergillus niger	34	79
Aspergillus flavus	2	5
Aspergillus fumigatus	3	7
Mucor mucedo	2	5
Fusarium solani	1	2
TOTAL	43	100

DISCUSSION

The mycoflora of the smoke-dried fish varieties sold in Eke-Awka Market, Anambra State, Nigeria was studied to determine their suitability for human consumption. The fish varieties were bonga, cat, cod, tilapia and croaker fishes. The fungal counts ranged between 1.2×10^3 cfu/g and 2.6×10^3 cfu/g. Bukola et al (2008) studied the mycoflora of some smoke-dried fish varieties in Uyo, Southern Nigeria and reported the fungal counts of 4.8×10^3 , 1.7×10^3 and 2.3×10^3 cfu/g for cod, croaker, cat and bonga fishes respectively⁴. Wogu and Lyayi (2011) also studied the mycoflora of some smoke-dried fish varieties in Benin City, Nigeria and reported the mean fungal counts of 12.5×10^3 , 17.8×10^3 , 15.3×10^3 , 14.3×10^3 and 13.5×10^3 cfu/g for the bonga, tilapia, cod, croaker and cat fishes respectively⁵.

The fungi isolated from the dried fish varieties were *Saccharomyces cerevisiae*, *Aspergillus niger*, *Mucor mucedo*, *Aspergillus flavus*, *Fusarium solani* and *Aspergillus fumigatus*. Junaid et al (2010) isolated and identified the fungi associated with stock fish contamination in Jos metropolis. The associated fungi were *Mucor* spp, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*⁷. Wogu and Lyayi (2011) also isolated *Aspergillus*

niger, *Aspergillus flavus* and *Fusarium* spp from their smoked fish varieties⁵. Bukola et al (2008) reported the presence of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor* sp and *Fusarium* sp in the smoked dried fishes they examined⁴.

Aspergillus niger was the dominant fungus isolated from the dried fishes. Bukola et al (2008) however reported that *Aspergillus flavus* had the highest rate of occurrence among the fungi isolated from the smoke-dried fishes from Uyo, Akwa Ibom State which included Stock, Croaker, Cat and Bonga fishes⁴. Junaid et al (2010) also reported that *Mucor* had the highest rate of occurrence among the fungi isolated from the stock fish samples sold in Jos, Nigeria⁷.

Wogu and Iyayi (2011) reported that the most frequently isolated fungi in the smoked fish varieties examined was *Aspergillus niger*. Its dominance may be as a result of its ubiquitous nature.

Fungi produce spores which are moderately resistant to drying and therefore easily contaminate and spoil dry materials. Dry fishes have low moisture content and this makes them prone to fungal colonization. The molds isolated from the dried fishes in this study are known to be common in the air and soil and have been linked with the production of various toxins under various conditions⁸. *Aspergillus* sp has been reported to produce aflatoxins and ochratoxins.

Mari and Riccioli (2004) reported *Aspergillus niger* and *Aspergillus fumigatus* as being allergenic⁹. Bukola et al (2008) also reported that the consumption of dried fishes contaminated with *Aspergillus niger* and *Aspergillus fumigatus* poses serious damage to health since the fishes may contain toxin metabolites produced by the fungi⁴. These metabolites may cause severe damage to the liver and kidney.

The presence of these fungi in the dried fishes may be due to improper processing, unhygienic handling and poor storage conditions. Dried fishes are usually displayed in exposed trays, pans and tables. This encourages the entry and proliferation of the fungi and the subsequent production of toxins.

Aflatoxins are highly carcinogenic and cause the cancer of the liver and acute hepatitis in man especially in the developing countries such as Nigeria due to poor environmental management, therefore adequate drying and effective storage methods must be adopted to reduce the incidence of these fungi in the dried fish varieties.

CONCLUSION

This study showed that all the smoke-dried fish varieties examined were contaminated with fungi indicating that their consumers may have been consuming one toxic metabolite or the other. Prolonged intake of these metabolites may constitute a health hazard. It is therefore imperative that appropriate methods of preservation and storage of the dried fishes must be adopted. In addition, stored smoke-dried fishes must be cooked properly before consumption.

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