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Mycological Evaluation of Smoked-Dried Fishes Sold in Umuahia Markets, Abia State, Nigeria

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Abstract: This study was to identify the fungi commonly involved in contamination and deteriorations of smoked-dried fishes; Stock fish (*Gadus morhua*), Bonga fish (*Ethalmosa fimbriota*), Croaker (*Pseudotolithus typus*), African catfish (*Clarias gariepinus*), mackerel (*Scomber scombrus*) available in Umuahia metropolis, Eastern Nigeria. Sampling was randomly done and pour-plate method was used for inoculation of the sample on the media. The isolates were isolated using Sabouraud Dextrose Agar and growths from the plates were stained using lacto-phenol cotton blue to confirm their identity. Three hundred smoked-dried fish sample sold at four major markets in Umuahia metropolis, Abia state, Nigeria were heavily contaminated with fungi. The associated fungi where *Aspergillus* spp*, Penicillium* spp, *Paecilomyces* spp, *Phialophora* spp, *Cladosporium* spp, yeast spp*, Saprolegnia* spp, *Scedosporium* spp. *Aspergillus* spp had the highest rate of occurrence followed by yeast spp, *Penicillium* spp*, Saprolegnia* spp, *Phialophora* spp, *Paecilomyces* spp and *Scedosporium* spp, respectively. Chi-square, fishers 'exact test and odds ratio where used to determine the strength of association between the type of fish, location of market, where the fish was bought and the presence of fungi in the fish samples. The result and data were further analyzed statistically using one way Analysis of Variance (ANOVA) followed by Duncan multiple range test and there was an insignificant difference for the collection sites and the fish type ($p<0.05$). The high level of contamination can be traceable to handlers and environment to which this fishes are exposed during smoking and storage. Thusthis study recommends proper handling of fish products during processing and selling, also glass boxes should be used for display in the markets.Fish processors and retailers should take extra care when handling and processing the fish.

Key words: Fungi · Fish · Contamination · Processors · Abia

INTRODUCTION

Fish and fish product considered as preferable source of high nutritional values and highly desirable food due to its high quality animal protein content, exceptional richness in calcium and phosphorus and its generous supply of ß- complex vitamins. Fungal contamination of fish is considered the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses [1]. Spoilage proceeds as a series of complex enzymatic, bacterial and chemical changes that begin as soon as the fish dies [2]. Fish processing and preservation is carried out mainly to slow down or prevent the enzymatic, bacterial and chemical deterioration of fresh fish. Akintola *et al*. [3] reported different types of preservation methods; drying, smoking, freezing, chilling and brining. But the most prominent fish preservation method in Nigeria is smoke drying. Drying creates a hard outer layer helping to stop microorganisms from entering the food [2]. The processed fish either salted or smoked may be exposed to contamination by moulds and yeasts derived

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from subsequent handling of fish and/or from the salt or brine used in the processing which undergoes fungal spoilage through utilization of protein and lipids [4]. Food manufacturers and processors usually enumerate these organisms only when a problem occurs, due to off flavors, sliminess, lipolysis and unpalatable taste; that render the product of inferior quality unmarketable or even unfit for human consumption [5]. Dry fish production plants in most countries are located in very remote rural locations, characterized by lack of infrastructure facilities and poor sanitary conditions. The Codex Alimentarius commission recommends that only most fresh fish intended for human consumption should be used for preparing dried fish [6]. Scientific knowledge of the quality and safety of dried fishery products produced in most developing countries is poor. Several studies have attempted to determine the effect of different processing methods and processing conditions [7-9] and storage temperature [10] on the quality of dried fish products. In Nigeria, fish is eaten fresh, preserved or processed [8]. Many different fungal species can spoil food product or produce mycotoxins or both [11]. Mycotoxins are secondary metabolites produced by moulds that are capable of causing disease and death in humans and animals [12]. Drying to moisture content below 15% prevents the growth of many spoilage organisms while mould growth is only suppressed at 10% moisture content [13]. Fish spoilage occurs following the growth and activity of special microorganisms and lipid oxidation which cause off odor and taste by the production of some metabolites changing sensory characteristics and customer acceptability. Fish smoking helps in slowing down fish deterioration thereby giving the commodity a longer shelf-life [14]. The aim of this study was to identify the fungi commonly involved in deterioration of usually available smoke-dried fish in Umuahia metropolis.

MATERIALS AND METHODS

This study was carried out in Umuahia metropolis in Abia State, South-Eastern Nigeria. Four major markets were food stuffs are sold were selected for purchase of the different kinds of smoked dried fishes.

The smoke dried fish samples were bought randomly from four major markets across Umuahia. The following species of fish where sample; Stock fish (*Gadus morhua*), Bonga fish (*Ethalmosa fimbriota*), Croaker (*Pseudotolithus typus*), African catfish (*Clarias gariepinus*), mackerel (*Scomber scombrus*).

Sample Collection: In each of the market, Seventy five (75) sample of the five (5) species of fishes; Catfish, Croaker fish, Ice fish, Bonga fish and Stock fish were randomly sampled. The fish were carefully packed into labeled sterile polythene bags and transported to the Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike Abia State, Nigeria.

Sterilization of Materials and Media Preparation: All glass wares used in the study were washed withdetergent, rinsed and sterilized in a dry ventilated oven at 160°C for 1 hour. Media used during the cause of the work was sterilized by autoclaving at a temperature of 121°C for 15 minutes. The media used for this study was Saboraud dextrose agar.

Preparation of Samples: The samples from each market were blended with ahigh– speed blender and thoroughly mixed. Then one ml of the powdery samples were weighed, nine milliliter sterile distilled water was added and serially diluted up to 10^{-6} fold. Thereafter, 1ml from each suspension was dropped on a sterile plate.Approximately 20 ml of cooled SDA fortified with streptomycin to inhibit bacteria growth was then poured into sterile petri- dishes. A slight agitation was done toallow for the mixing of the inoculum with the culture medium (SDA), thereafter incubated at 28 ± 2°C and examined daily for 7 days.

Identification of Isolates: Direct observation of culture under the light microscopewas carried out under careful preparation of slides and staining with cotton blue in lacto-phenol. The colonies growing on the plates were identified macroscopically and microscopically using colony colour, type (Compact, loose, aerial hyphae), texture (Velvety, cottonyand coarse), shape and growth pattern according to the detailed drawing of the features and identification manual and guides of Barnet and Hunter [15] and Blackwell [16].

Statistical Analysis: Data obtained from the experiment were subjected to one way analysis of variance (ANOVA) using SPSS 20.0 for windows differences between the means were determined using Duncan's multiple range test at 95% confidence level (P.0.05). Chi-square, fisher' exact test and odds ratio were used to determine the strength of association between the type of fish, location of market where the fish was bought and the presence of fungi in the fish.

RESULTS

A total of 300 of fish were sampled randomly from four (4) different market locations Ndoru, Isi Gate, Ahiaeke and Ubani markets, (75) samples from each of the market. Themycofungiisolated were identified down to the genus level as *Aspergillus* spp, *Yeast* spp, *Penicillium* spp, *Saprolegnia* spp, *Cladosporium* spp, *Paecilomyces* spp, *Phialophora* spp and *Scedosporium* spp (Table 1).

The number of fungi isolated from each of the markets ranges from 4-5 respectively (Table 2). The distribution of fungi isolated from fishes which collected from different markets shows that the prevalent fungus was *Aspergillus* species which was seen in about 76% of the total fish sampled fromUbani, Gate, Ahiaeke and Ndoru markets. species where seen in about 19% of the total fish sampled from, Ubani, Gate, Ahiaeke and Ndoru markets (Table 1) *Paecilomyces* spp affected only 1% of the total fish sampled, same as *Phialophora* spp and *Scedosporium* spp. *Cladosporium* spp where seen in about 5%of the total fish sample while *Saprolegnia* spp and yeast cells where seen in about 9% and 24% of fish samples respectively (Table 1).

Paecilomyces spp, *Phialophora* spp, *Scedosporium* spp where seen in Ubani, Ubani and Ahiaeke markets respectively (Table 2). *Paecilomyces* spp was isolated in a Bonga fish sample while *Phialophora* spp was isolated in croak and *Scedosporium* spp in stock fish.

Table 2 shows the probable fungal isolates from smoked /dry fishes from selected markets in Umuahia. In ubani, six fungal species namely; *Aspergillus* spp, *Penicillium* spp, *Paecilomyces* spp, *Phialophora* spp, *Cladosporium*spp and yeast cells while in Isi-gate, (Five) 5 fungal species was identified. In Ahiaeke, four (4) fungal species was identified. Finally, in Ndoru market, only three (3) fungal specieswas identified, see in (Table 2) below.

All the fungi isolated from the various markets had different growth characteristics with the exception of samples from Ahiaeke and gate market having *Aspergillus* spp which exhibited similar shades of greenish colonies *Paecilomyces*spp and *Phialophora*spp where isolated only in Ubani market.

Cladosporium spp where isolated from both Ubani and gate markets and both of these species has the same morphologic appearance of dark green colour with irregular rugae. *Scedosporium*spp where isolated from only Ahiaeke and was very distinct. *Saprolegnia*spp was isolated in Isi- gate and Ndoru market. Yeast cells were isolated in samples from all the markets with the exception of Ndoru (Table 2).

Table 3; shows a slight difference in the percentage of fungal infestation on the different types of fish sampled. Here Bonga fish and Croaker fish has the highest percentage of fungal infestation of (90%) followed by Stock fish with the (75%) and then Cat fish and Ice fish with (80%) and (70%) respectively.

Table 4; shows a significant difference in the percentage of fungal load in the collection site where Isi-gate market has the highest percentage of fungal load with (88%) followed by Ndoru market which has (84%) and Ahiaeke and Ubani market (80%) and (72%) respectively.

Table 1: Distribution of fungi isolated from fishes

S/N	Type of fungi isolated	Percentage of occurrence
1.	*Aspergillus* spp	76%
2.	Yeast spp	24%
3.	*Penicillium* spp	19%
4.	*Saprolegnia* spp	9%
5.	*Cladosporium* spp	5%
6.	*Scedosporium* spp	1%
7.	*Phialophora* spp	1%
8.	*Paecilomyces* spp	1%

Table 2: Fungal isolates from differently sourced smoked fishes from the selected markets

S/N	Fungi isolates	Ubani	Isi-gate	Ahiaeke	Ndoru
1.	*Aspergillus* spp	+	+	+	+
2.	*Penicillium* spp	+	+	+	+
3.	*Paecilomyces* spp	+	-	-	-
4.	*Phialophora* spp	+	-	-	-
5.	*Cladosporium* spp	+	+	-	-
6.	*Scedosporium* spp	-	-	+	-
7.	*Saprolegnia* spp	-	+	-	+
8.	Yeast spp	+	+	+	-

Key = + (Positive) – (Negative)

Table 3: Prevalence of fungal infestation based on type of fish

S/N	Type of fish	No. Sampled	No positive %	OR	95% Cl on OR
1	Cat Fish	60	48 (80%)	0.9231	0.2695 - 3.1617
2	Stock Fish	60	45 (75%)	0.6364	0.1985 - 2.0400
3	Bonga Fish	60	54 (90%)	2.4107	0.5124 - 11.5112
4	Croaker Fish	60	54 (90%)	2.4107	0.5124 - 11.5112
5	Mackerel Fish	60	42 (70%)	0.4527	0.1469 - 1.3957
	Total	300			

*Not significant at $p < 0.05$

Table 4: Prevalence of fungal infestation based on market location

S/N	Market locations	No. Sampled	No positive %	OR	95% Cl on OR
1	Ndoru	75	63 (84%)	1.3125	0.3915 - 4.4003
2	Ahiaeke	75	60 (80%)	0.918	0.2938 - 2.8682
3	Isi Gate	75	66 (88%)	1.9887	0.5277 - 7.4952
4	Ubani	75	54 (72%)	0.4898	0.1681 - 1.4269
	Total	300			

*Not significant at $p < 0.05$

DISCUSSION

The study established that all the smoked fishes sampled from Ubani, Isi-gate, Ahiaeke and Ndoro markets harbored one fungus or the other within the following list *Aspergillus* spp *Penicillium* spp, *Scedosporium* spp, *Cladosporium* spp, *Saprolegnia* spp, *Paecilomyces* spp, *Phialophora*spp and yeast spp.

Similarly, Dike *et al.* [17] reported that the microbial load in smoked fish sold in Owerri Nigeria was A*spergillusflavas*, *Penicillium* spp, *Fusarium* spp, *Biospora* spp, *Botryodioplodia*, *Cladosporium* spp, *Paecilomyces* spp, *Scedosporium* spp etc. Most of these organisms found in these smoked fish are those commonly found in soil and water. The fungi isolated in this study are also similar to the micro-organisms reported by Olawale *et al.* [18] and Adesokan *et al.* [19]. In this study *Aspergillus* spp is by far the highest occurring fungi species seen, affecting a total of about 76% of the total fish samples collected and this can be as a result of the organism being able to thrive in high osmotic pressure and high level of sugar and salt [20]. Micro-organisms were also reported byAbolagba and Igbinevbo [21] in smoked fish (*Clarias* spp) sold in Benin metropolis.

The results could not establish whether contamination occurred before or after smoking. Venugopal [22] established that contamination of fish, particularly by pathogens may occur prior to harvest, during capture, processing, distribution and/or storage. Other studies dealing with different processing methods have similarly concluded that the plant and processing environment may be the source of product contamination rather than the raw material. However, this does not exclude the possibility that the fresh fish is an important initial source for contaminating processing environment and the environment [23].

The occurrence of *Aspergillu*s, *Penicillium* and *Cladosporium* species could be due to absorption of moisture during storage, the stored fish might have reabsorbed moisture from the environment which then supported the growth of the micro-organisms. In addition to the contamination during processing, handling and display on the market stall [24].

The results obtained in this study indicated that smoked fish samples from Ubani market had the lowest fungal load of about 72% (Table 4.4) and thus would be safer for consumption and this might be as result of the good sanitary measures adopted in the smoked fish processing chain and storage system and also due to the fact that ubani market is well planned unlike the other three markets and this is in agreement with the work done by Abolagba *et al*. [25] in Benin Metropolis. Also from the results obtained in this study there is indication that fish samples from Isi-gate hasthe highest percentage of fungal load of about 88% (Table 4) and this also might be as a result of poor sanitary measures adopted in the processing chain and storage or poor environmental and personal hygiene of the retailer. This is in agreement with the findings of Abolagba and Iyeru [26] who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a very high microbial load.

The statistical analysis using chi-square, fisher's exact test and ANOVA to determine the strength of the association between the type of fish, location of the market where the fish was bought and presence of fungi

in the fish; using 95% confidence interval and values of $P<0.05$ considered significant, shows that their association is not significant. Where the chi-square value of the relationship between the market locations and the presence of fungi is (0.7515) and that of fisher's exact test (0.783) and ANOVA (0.573) and the chi- square, fisher's exact test and ANOVA values of the relationship between the fish types and the presence of fungi are (4.159), (4.037) and (0.859) respectively.

On the other hand, the analysis using odds ratio shows that there is a stronger association between the presence of fungi and Cat fish, bonga fish, stock fish and croaker fish. (Which are significant) and a lower association between the ice fish and the presence of fungi (Which is not significant) see in (Table 3). This might be as a result of the fungi organisms not being able to growth or thrive in an extreme cold environment /condition like the one seen in ice fish before smoking them unlike in bonga, croaker, cat and stock fishes which where smoked right after harvest from a mild warm environ [25]. Also in the relationship between the presence of fungi and the market locations the odds ratio values indicates that samples from Ndoru, Ahiaeke and Isi-gate have a significant relationship with the presence of fungi. While that of Ubani market does not (Table 4).

Thus it is more preferable to use the odds ratio in the determination of the relationship/association between the two groups and the presence of fungi.

CONCLUSION

In conclusion, fungal contamination or re-contamination of smoked fish products has been seen to vary from one market to another and even within the same locality from one fish type to another. Thus, fungal contamination of smoked fish has been found to be due to several factors such as poor smoking of fish products (i.e. inappropriate temperature control or application), poor personal hygiene of processors/seller, poor hygiene/sanitary practices relating to smoked fish products, smoked/workhouse, packaging and storage, poor market planning as well as the use of inadequate and inefficient traditional processing facilities. Poor environmental sanitation and high human traffic are also implicated.

Recommendation: The high level of contamination can be traceable to handlers and environment to which this fishes are exposed during smoking and storage. Thusthis study recommends proper handling of fish products during processing and selling, also glass boxes should be used for displaying the smoke dried fish in the markets instead of using open trays. Fish processors and retailers should take extra care when handling and processing the fish.

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