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Research Article

**MYCOLOGICAL ASSESSMENT OF DRIED FISH AND STOCK
FISH SOLD IN OZORO MARKET**

Orogu J.O.* and Akpobire, D.

Department of Science Laboratory Technology, Delta State Polytechnic Ozoro, Delta State,
Nigeria**Abstract:**

Dried fish and stock fish are largely consumed as a source of nutrient by man. It has been established that fish food can act as vehicle for transmission of some mycological pathogens especially in immune compromised individuals. Sixteen (16) different samples of fish were bought from Ozoro central market in four different locations. The samples were labeled sample A (dried fish) and sample B (stock fish). A total of four (4) fungi species were isolated from the test samples: Aspergillus niger, Candida albican, Penicillium species and Rhizopus oryzae. Aspergillus niger has the highest plate count of 7.7×10^3 , and 5.6×10^3 and 0.3×10^3 for both samples. While Penicillium species has the least plate count of 0.3×10^3 Aspergillus niger have the highest percentage (%) occurrence for both samples of 52.275%, while penicillium species have the least percentage (%) occurrence of 08.33%. The total mean cfu/g count for sample A is 10.08cfu/g while for sample B is 09.08cfu/g. the occurrence of Aspergillus spp, Penicillium spp, and Candida spp could lead to mycotoxin Elaboration and when consumed, they induce gastrointestinal and metabolic disturbance.

Key words; *Mycological, dried fish, stock fish, Assessment, Market.***Corresponding author:****Orogu Joshua Othuke,**

Department of Science Laboratory Technology,

Delta State Polytechnic Ozoro, Delta State,

Nigeria

Email- joeorogu4real2000@yahoo.com and joshuaorogu4@gmail.com

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INTRODUCTION:**BACKGROUND OF THE STUDY**

Fish is an aquatic vertebrate with fins, gills and skin with glandular secretion that decreased friction. A typical fish is torpedo-shape and usually limbless with a head containing a brain or sensory organ and muscular tail. Most fish have tail and are poikilothermic [1].

Fish is highly perishable food, if long shelf life is demanded and that it retains good quality and nutritional value, good handling and preservation is necessary [2]. Fish processing refers to processes associated with transforming fish from raw material into product that is delivered to the consumer. Fish handling is a subdivision of fish processing which is the preliminary processing of raw fish, and the product of fish products [2].

Fresh fish rapidly deteriorate unless some way can be found to preserve it. Drying is a method of food preservation that works by removing water from the fish which inhibits the growth of microorganisms [3]. Open air drying using sun and wind has been practice since ancient times to preserve food. Water is usually removed by evaporation (air drying, sun drying, smoking, or wind drying) [4]. But in the case of freezing-drying, food is first frozen and then the water is removed by supplementation. Bacteria, yeast and molds need the water in the food to grow and drying effectively prevent them from surviving in the food.

Preservation of fish is carried out after caught from water bodies. Dried fish and stock fish can be eaten without further cooking. From the processing to the market centers, dried fish and stock fish are often contaminated with microorganisms such as bacteria, yeasts and molds [5]. Numerous pathogenic agents isolated from different types of fish were able to grow and produce their toxic secondary metabolites, which are retained in fish flesh even after salting and storage periods. These toxic substances causes serious systematic dysfunctional and public hazard is a serious problem that could affect fish farmers in Africa. These contaminations may be primarily fungi (the initial agent) or as a secondary invasion of mechanical agents [6]. Several foods borne moulds and possible yeast may also be hazardous to human health because of their ability to produce toxic metabolite known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking [7] even though the contaminant organism may not survive food preparation, the performed toxin may still be present. Certain moulds and yeast may also elicit allergic reactions or may cause infection in humans. Although most food borne are not infectious, some species can cause infections in immune compromised

populations such as children, the elderly and depilated individuals, diabetic patients, HIV-infected individuals and persons receiving long terms anti biotic therapy [8]. These pose serious health implications especially when these microorganisms especially when these microorganism and / or toxin find their way into the digestive and circulatory system.

Saprolegnia is the most important fungi contaminant of fish, although a number of other species have been implicated. Martin (2008)[9] stated that bacteria (*Staphylococcus aureus*), yeast (*Saccharomyces cerevisiae*) and moulds (*Penicillium* and *Aspergillus*) were the most common microorganisms associated with dried fish and stock fish. The threshold colony forming unit (CFU) responsible for clinical diseases depends on immunity of individuals and largely vary with the type of fungal ingested. In most cases, 5×10^4 CFU/g of total source (e.g, food, water and air), are responsible for clinical diseases, stated by Hedges, 2012 [10]. Any kind of fish can be smoked or dried.

The aim of this research is to provide an over view of fungi growth in dried fish and stock fish and to examine the fungi in dried and stock fish.

MATERIALS AND METHOD:**STUDY AREA**

The study was conducted in Ozoro Isoko North Local Government Area of Delta State, Nigeria.

Collection of sample

Sixteen (16) different samples (dried fish and stock fish) were used for the experiment. The samples (dried fish and stock fish) was purchased from the market in Ozoro Isoko North, Delta State, and it was labeled A (dried fish) and B (stock fish). Both samples were transferred to the laboratory for analysis

MATERIALS

The material used in this study includes laboratory coat, gloves weighing balance, cotton wool foil paper measuring cylinder (50ml,250ml, 500ml), and beakers (50ml, 200ml,500ml), pipette, petri dishes test tubes conical flask, Bunsen burner, potato dextrose Agar (PDA), wire loop, water etc.

Sterilization of Glasswares

The glasswares that were used for this project were washed with detergent, rinsed thoroughly and sterilized using autoclave at 121°C for 15 minutes.

METHOD**Analysis****Preparation of samples**

The samples (A and B) bought from the market were blended and serial dilution was carried out according to the method of Cheesbrough (2002). 0.1ml of 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} dilution were transferred to plate of

potato dextrose Agar (PDA). Media prepared was according to the manufacturer instruction and the use for enumeration of isolated fungi. The plates were incubated at 23⁰ C for 48 hours. The fungi were identified based on colonial / morphological characteristics.

Preparation of Media

The media (potato dextrose Agar) used were prepared according to the manufacturer instructions into a conical flask plugged with cotton wool wrapped into aluminum foil and sterilized in an autoclave at 121⁰ for 15 minutes

Isolation of Fungal Isolate

The serial dilution techniques (Cheesbrough, 2002) were employed, 1g of blended fish (dried fish and stock fish) was dissolved in 9ml of distilled water. 0.1ml of the diluted sample was withdrawn from test

tube into well labeled petri dishes .The molten agar i.e. Potato Dextrose Agar (PDA) was dispensed into sterile petri dishes to over lay the sample which was then soared properly and allow to solidify and incubates at 23⁰ C for 48 hours

Characterization and Identification

Morphological and microscopic characteristics of the culture were used for the identification of the isolate following standard references. The colony morphology used includes; color of spores, present or absent of pigmentation, elevation, and nature of mycelia. Microscopic Characteristics used for the identification include the type and the shape of asexual and sexual spores, present or absent of cross walls in hyphae, presence or absence of sterigmata and the sporangiospores

RESULT:

Aspergillus niger, *Candida albican*, *Penicillium species* and *Rhizopus oryzae* were isolated from the samples

Table1: Morphological and microscopic characteristics of the fungal isolated

Morphological characteristics	Microscopic characteristics	Micro organism
The colony is circular and about 4.0-4.5cm in diameter. Color is yellowish green becoming green with age. Reverse is creamish yellow.	Stripe is long, vesicle is dome shaped, Metulae is small Candida is globose, rough and yellowish green.	<i>Aspergillus niger</i>
The colony are cream without profuse growth.	Hyphae and conidiospores are non-septate	<i>Candida albicans</i>
Blue-green fluffy growth on plate	Blue-green condition spores borne in multilink chain	<i>Penicillium species</i>
Growth rate is very rapid and colonies are typically cotton-candy like in texture. The surface colony color is initially white becoming gray to yellowish brown in time reverse is white to pale	Non- septate or scarcely septate broad hyphae with diameter ranging from 6-15µm, rhizoids, sporangiophores, sporangia, and sponrangiophores are present. Sporangiphore appear in clusters	<i>Rhizopus Oryzae</i>

Table 2: shows the occurrence of fungi isolates and its total heterotrophic count

Sample	Fungi isolates	Cfu/g	Cfu/ml in standard form
A	<i>Aspergillus niger</i>	77	7.7x 10 ³
	<i>Candida albicans</i>	25	2.5x10 ³
	<i>Rhizophus oryzae</i>	0.6	0.6x10 ³
B	<i>Aspergillus niger</i>	56	56x10 ³
	<i>Candida albicans</i>	32	3.2x10 ³
	<i>Penicillium species</i>	18	1.8x10 ³
	<i>Rhizopus oryzae</i>	03	0.3x10 ³

Table 3: Shows bacterial isolates, number of occurrence and percentage of Occurrence.

sample	Bacterial isolates	Number of occurrence	Percentage(%)of occurrence
A	<i>Aspergillus niger</i>	6	54.55
	<i>Candida albicans</i>	2	18.18
	<i>Rhizopus oryzae</i>	3	27.27
B	<i>Aspergillus niger</i>	6	50.00
	<i>Candida albicans</i>	4	33.33
	<i>Penicillium species</i>	1	08.33
	<i>Rhizopus oryzae</i>	1	08.33

DISCUSSION:

We were able to isolate fungi from our test samples. This is not surprising because according to Akande and Tobor 2012 [11], freshly caught fish, covered with damp sacks mixed with wet grass or water weeds to reduce the temperature makes fish, prone to contamination with microorganisms such as bacteria and fungi. This indicates that spoilage of fish start from the aquatic ecosystem.

Fungi isolated in this study are in consonance with findings by other authors however, Refai *et al.* 2004 [12] reported that *Penicillium spp*, *Aspergillus spp* and *Rhizopus spp* (table1) are normal mycoflora present in most fish. Notwithstanding, many fungal genera have virulence factor which cause toxin elaboration under favourable predisposing environment. Ecology is also an important factor which influences the diversity of fungus genera on fish and their eggs [13]. According to Pailwal *et al.* (2000) [14], diversity of water molds depends upon the interaction of physiochemical factors.

The fungi isolated from our dried fish and stockfish were somewhat specific in that while *Aspergillus spp.* and *Mucor spp.* were observed in all the fish species, *Candida albicans* and *Penicillium spp.* (table 3) occurred in dried fish while stockfish harbours most of different species fungal isolates. Conversely, stockfish harbor least fungal agents. This fungal may be due to the differences in the biochemical composition of these fish species and to which different moulds and yeast react differently [15].

It is important to state that majority of the fungal agents isolated were of medical significance. The occurrence of *Aspergillus spp.* (table 3) could lead to mycotoxin elaboration and when consumed, they induce gastrointestinal and metabolic disturbance [9]. The source of fungal contamination can also be a result of consumption of fungal contaminated feed present in the pond. Moreover, the decomposition of these feed also add to increase in fish contamination: particularly, poor pond management, injured fish, or fish having other forms of diseases. Fungal pose wide

contamination threat in fish farming mainly due to mismanagement of ponds [16].

Handling of fish could also engender microbial contamination especially in artisanal fishery due to unhygienic methods of reducing temperature. During the drying period, smoke kilns used in artisanal fishery and the over loading of the fish in the trays leads to improper processing which in turn encourages fungal attack .Eyo,2012 and Akande and Tobor,2012 [6,11]. During storage of dried fish product, good storage product practices are not observed by most wholesalers such as improper ventilated and easy access of pest into the storage environment.

The environment where fish are displayed in the market are usually unhygienic and this could constitute another avenue for microbial dried and stock fish samples in open trays beside refuse heaps ,this encourages fungal attack through air droplet [11].

CONCLUSION:

Finding from this study indicate the presence of fungal contamination in samples analyzed. When consumed, they might be source of infection in humans. This suggest the need for veterinary and public health intervention through fish regulatory programs, ,more so fish processors should be educated on safe methods of preservation in order to prevent or minimize fungal contamination.

On a general note, health education/enlightening will be great significance to fishermen, fish handlers, vendors, and buyers that good processing and availability of storage facilities are crucial to minimize general microbial contamination

RECOMMENDATIONS

In view of the heavy fungal contaminants isolated from dried fish and stock fish in this study, fishermen and marketers should adapt better method of preservation and better smoking kilns should be provided to them at subsidized rates. More so, stored

fish product should be well kept. It is important that mycological examination of fish be carried out, especially by environmental public health workers and regulatory bodies like National Agency for Food and Drug Administration and Control (NAFDAC) at regular intervals and the health implications of fungal agents and their mycotoxins be emphasized to fish handlers and consumers.

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