Survey of Mycoflora Associated with Dried Bonga Fish (*Ethmalosa frimbriata*) During Storage

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Abstract: The total number of isolated fungi from the fish stored in various storage medium at different temperature shows that the freezer recorded the least; this may be due to the fact that most of the isolates could not survive mesophilic temperature while ambient temperature and refrigeration have equal number of isolates. The survey of mycoflora on dried Bonga fish stored at refrigeration temperature (4 ± 2 ^{0}C) shows that the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (30.78 %) > *Rhizopus oryzae* 3 (23.08 %) > *Aspergillus niger* 2 (15.38 %) > *Penicillium chrysogenum* 2 (15.38 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(7.69 %) each being the least while at ambient temperature the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 6 (30 %) > *Rhizopus oryzae* 5(25 %) > *Aspergillus niger* 4 (20) > *Penicillium chrysogenum* 3 (15 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(5 %) each being the least. Storage made at freezer temperature (-2 ± 2 ^{0}C) has a reduction in the number of isolated fungi. Only four mycoflora were isolated with. The frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (40%) > *Aspergillus niger* 3 (30 %) > *Rhizopus oryzae* 2 (20 %) > *Penicillium chrysogenum* 1 (10 %) respectively The moisture contents decrease with increase in the storage temperature. Freezer (-2 ± 2 ^{0}C) has the moisture content of 4.6 %, followed by refrigeration (4 ± 2 ^{0}C) with the moisture content of 2.4 % and the least moisture content of 1.6 % was recorded at ambient temperature (25 ± 2 ^{0}C) while the pH of the Bonga fish during the storage conditions was slight acidic. Isolated fungi were statistically analyzed and the test applied was F-test statistic at p= 0.05 level of significant. Preservation could probably be better if the fish product is kept in a hostile environment such as the show glass equipped with heating element or in a regulated oven so as to reduce drastically the moisture content of the fish which is the basic requirement for microbial growth and hence, inhibit the growth of the microorganisms causing fish spoilage.

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1. Introduction

Fish is a highly nutritious food and an excellent source of proteins, vitamins, minerals and essential fatty acids. Interest in fish consumption has increased over the years, due to its recognition as a lean alternative to meat and secondly the health benefits its impacts being a rich source of omega-3-fatty acids that reduces cholesterol levels and the incidence of heart disease and pre-term birth (Adebajo, 1992; Al-jufaili *et al*., 2006). Dried fish are very important parts of the traditionally accepted diet for many in developing countries as well as a major source of protein. They are also often enjoyed for their characteristics flavor and are commonly used as a raw material for seasoned foods such as soups and sauces. Salting, smoking and drying are the oldest known methods used in the preservation of fish (Barnet and Hunter, 1972). Although improvements in the techniques have evolved over time, traditional methods of production continue to be practices (Bababunmi *et al.,* 1978). The processing of raw fish is either dry salted, pickled or boiled in salted water following by smoking at temperature between 40-100^{0}C and or drying but the process may vary considerably depending on a number of factors including the species of fish, type of product desired and the traditional practices in different locations (Bisset, 1998). For tropical countries where most production takes place, direct sun drying is often the method of choice. The process whilst enhancing the flavor and textural properties, results in a product that has reduced water activity and improved microbial stability compared with the raw material (Christianah *et al*., 2010). The reduced water activity of dried foods limits growth of competitive micro-organisms to some moulds and yeast which can cause spoilage. Many filamenteous fungi associated with food products have a role in deterioration contributing to undesirable effects such as discolouration, rotting and production of off odours that could render the food unsalable (Eyo, 2001). However most importantly the natural spoilage mycoflora of foods may include species capable of producing toxic metabolites that could lead to adverse health effects in human and animals (Eyo,

2001; Immaculate *et al*., 2012) contamination of products with potentially toxigenic fungi should therefore be avoided The mycoflora or the fungi associated with a particular type of food are often specific to that food type (Jay, 2010; Immaculate *et al*., 2012). Generally only a limited number of species, in the order of one to three dominate the spoilage mycoflora of a particular food product (FDF, 2011). This is dependent on many factors such as nutrient availability, water and other prevailing environmental factors. Detailed knowledge of the mycoflora associated with a specific food is crucial for the prevention of spoilage or mycotoxin production in the product. The uncontrolled natural mycoflora growing on the surface of bonga fish (*ethmalosa fimbrinate*) has not been previously characterized (FDF, 2011). Fish can become contaminated by handlers which could be the fishermen, the wholesalers or even the consumers (FDF, 2005). How they are displayed at the selling points, some sellers display these products on open trays, across the gutters where fliers can easily perch on them. The medium of storage such as leather, sieve or even plastics may not be properly cleaned which could also cause contamination. When stored in fridge or freezers the inadequate supply of electricity in Nigeria could also lead to quick spoilage (Ibrahim and Hamid, 2011). The aim of this study was to survey the mycoflora associated with dried Bonga fish (*Ethmalosa frimbriata*) during storage.

2. Materials And Methods

2.1 Study area

This research work was carried out at the Laboratory of the Department of Biology, School of Sciences, Federal Capital Territory College of Education, Zuba-Gwagwalada, Abuja, Nigeria.

2.2 Samples Collection

A total of thirty (30) dried Bonga fish (*Ethmalosa fimbriata*) samples were collected randomly with ten (10) samples each from three (3) different markets in Gwagwalada FCT-Abuja. Samples were collected from Gwagwalada Main market, Kaswandare, Angwandodo market. At each location, the dried Bonga fish samples were collected in the sterile polythene bags and brought to the Microbiology laboratory of the Department of Biological Sciences, University of Abuja, for the survey of mycoflora associated with dried Bonga fish (*Ethmalosa frimbriata*) during storage.

2.3 Storage Medium

The Bonga fish (*Ethmalosa fimbriata*) samples were then stored aseptically in different storage medium which include: leather, sieve and a covered container and then stored at different temperature conditions such as ambient temperature (25 ± 2 ^{0}C), refrigerator (4 ± 2 ^{0}C) and freezer temperature (-2 ± 2 ^{0}C) for 6 consecutive weeks.

2.3.1 Determination of Moisture content

The samples were weighed before storage and recorded as initial weight of sample and also weighed after storage period and recorded as final weight of the sample. The moisture content of the sample was calculated using the following expresion:

$$\%w = \left(\frac{A-B}{B} \quad X\ 100\right)$$

Where A= Initial weight of fish and B= Final weight of fish

2.3.2 Determination of pH content

The pH of the fish samples were determined by the method of Sably (1991). 10g of the fish sample were homogenized with 50mls of distilled water and the pH value of the homogenate was measured by means of a pH meter (HANNA, USA).

2.4 Preparation and sterilization of media

Malt extract agar and Potato dextrose agar were used in this study and they were prepared according to the manufacturer's instructions thus; 48 g of MEA was dissolved in 1000ml of sterile water and then sterilized (autoclaved) at 121ºC and pressure of 15 Psi for 15 minutes. Also, 39 g of Potato dextrose agar was dissolved in 1000ml of sterile water and then sterilized (autoclaved) at 121ºC and pressure of 15 Psi for 15 minutes. Malt extract agar was used for the isolation while Potato dextrose agar was used for the maintenance of pure cultures of fungi.

2.5 Isolation of Mycoflora from Bonga Fish

The fish was macerated in mortar and pastel. One gram (1 g) of macerated Bonga fish was soaked in 10 ml of sterilized peptone water over night to resuscitate dormant micro-organisms in the sample which form the stock solutions. The suspension was diluted up to 5-folds dilutions (10^5 cells/ml). Dilutions 10^5 cells/ml was inoculated on already prepared Malt Extract agar plates. The inoculated Malt Extract agar plate was incubated at ambient temperature (25 ± 2^{0}C) for 48 hours. Colony developments were observed after incubation period. The young fungal colonies were aseptically picked up and transferred to fresh sterile Potato dextrose agar plates to obtain pure cultures.

2.5.1 Identification of fungal isolates

Isolates obtained were characterized and identified on the basis of their colonial and morphological characteristics which include macroscopic and microscopic examinations. Among the characteristics used were colonial characteristics such as size, surface appearance, texture, reverse and pigmentation of the colonies (Shewan, 1999). In addition, microscopy revealed vegetative mycelium including presence or absence of cross-walls, diameter of hyphae, and types of asexual and sexual

reproductive structures. Slide culture method that minimized serious distortion of sporing structures was used. Appropriate references were then made using mycological identification keys and taxonomic description (Harrigan and McCance, 1976).

2.6 Statistical Analysis

Isolated fungi were statistically analyzed and the test applied was F-test statistic at p= 0.05.

3.0 Results

3.1 Physico-chemical parameters

The Table 1 below showed that the moisture contents decrease with increase in the storage temperature. Freezer (-2 ± 2 ^{0}C) has the moisture content of 4.6 %, followed by refrigeration (4 ± 2 ^{0}C) with the moisture content of 2.4 % and the least moisture content of 1.6 % was recorded at ambient temperature (25 ± 2 ^{0}C) while the pH of the Bonga fish during the storage conditions was slight acidic.

Table 1: Physico-chemical parameters of Bonga fish at storage Temperature

	Physico-chemical parameters	
Storage Temperature	**Moisture content (%)**	**pH**
Freezer (-2 ± 2 ^{0}C)	4.6	6.8
Refrigeration (4 ± 2 ^{0}C)	2.4	6.8
Ambient Temperature (25 ± 2 ^{0}C)	1.6	6.9

3.2 Ambient temperature (25 ± 2 ^{0}C)

The survey of mycoflora on dried Bonga fish stored at ambient temperature (25 ± 2 ^{0}C) shows that the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 6 (30 %) > *Rhizopus oryzae* 5(25 %) > *Aspergillus niger* 4 (20) > *Penicillium chrysogenum* 3 (15 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(5 %) each being the least as shown in Table 2.

Table 2: Survey of Mycoflora on dried Bonga fish (*Ethmalosa fimbriata*) stored at Ambient Temperature (25 ± 2 ^{0}C)

	Isolation rate, Number (%)			
Isolates	**Sieve (n=10)**	**Leather (n=10)**	**Container (n=10)**	**Total (n=30)**
Aspergillus flavus	1(10)	2(20)	3(30)	6(30)
Aspergillus niger	1(10)	1(10)	2(10)	4(20)
Aspergillus terrus	0(0)	0(0)	1(10)	1(5)
Penicillium chrysogenum	0(0)	1(10)	2(20)	3(15)
Rhizopus oryzae	1(10)	1(10)	3(30)	5(25)
Microsporum sp	0(0)	0(0)	1(10)	1(5)
Total	3(15)	5(25)	12(60)	20(100)

3.3 Refrigeration temperature (4 ± 2 ^{0}C)

The survey of mycoflora on dried Bonga fish stored at refrigeration temperature (4 ± 2 ^{0}C) shows that the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (30.78 %) > *Rhizopus oryzae* 3 (23.08 %) > *Aspergillus niger* 2 (15.38 %) > *Penicillium chrysogenum* 2 (15.38 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(7.69 %) each being the least as shown in Table 3.

Table 3: Survey of Mycoflora on dried Bonga fish (*Ethmalosa fimbriata*) stored at Refrigeration Temperature (4 ± 2 ^{0}C)

	Isolation rate, Number (%)			
Isolates	**Sieve (n=10)**	**Leather (n=10)**	**Container (n=10)**	**Total (n=30)**
Aspergillus flavus	2(20)	1(10)	1(10)	4(30.78)
Aspergillus niger	1(10)	1(10)	0(0)	2(15.38)
Aspergillus terrus	0(0)	0(0)	1(10)	1(7.69)
Penicillium chrysogenum	1(10)	0(0)	1(10)	2(15.38)
Rhizopus oryzae	1(10)	1(10)	1(0)	3(23.08)
Microsporum sp	0(0)	1(10)	0(0)	1(7.69)
Total	5(38.46)	4(30.77)	4(30.77)	13(100)

4.4 Freezer temperature (-2 ± 2 ^{0}C)

The survey of mycoflora on dried Bonga fish stored at freezer temperature (-2 ± 2 ^{0}C) shows that the storage made at freezer temperature (-2 ± 2 ^{0}C) has a reduction in the number of isolated fungi. Only four mycoflora were isolated with. The frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (40%) > *Aspergillus niger* 3 (30 %) > *Rhizopus oryzae* 2 (20 %) > *Penicillium chrysogenum* 1 (10 %) respectively as shown in Table 4.

Table 4: Survey of Mycoflora on dried Bonga fish (*Ethmalosa fimbriata*) stored at Freezer Temperature (-2 ± 2 ^{0}C)

	Isolation rate, Number (%)			
Isolates	**Sieve (n=10)**	**Leather (n=10)**	**Container (n=10)**	**Total (n=30)**
Aspergillus flavus	2(20)	1(10)	1(10)	4(40)
Aspergillus niger	1(10)	1(10)	1(0)	3(30)
Penicillium chrysogenum	0(0)	0(0)	1(10)	1(10)
Rhizopus oryzae	1(10)	1(10)	0(0)	2(20)
Total	(40)	3(30)	3(30)	10(100)

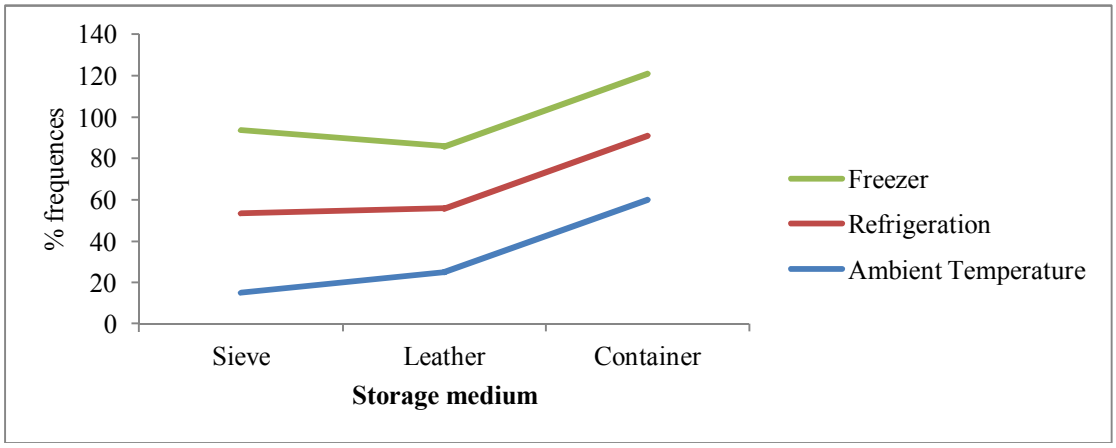

Figure 1: Mycoflora on dried Bonga fish (*Ethmalosa fimbriata*) at different storage conditions

4. Discussions

It is important to state that majority of the fungal isolates obtained in this study are of veterinary and medical importance. *A. flavus* and *Microsporum* sp are probably the most notorious of the common isolates because of their high potentials in producing aflatoxin and causes dermatophytosis respectively. Aflatoxin has been reported to cause acute hepatitis (aflatoxicosis) while dermatophyte is responsible for dermatophytosis which is in agreement with Christianah *et al.* (2010). The occurrence of *Aspergillus spp, Rhizopus sp* and *Penicillium* could be due to the fact that during storage, the fish product reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to contamination during processing, handling and display on the market stalls. This study showed that the moisture contents decrease with increase in the storage temperature. Freezer (-2 ± 2 ^{0}C) has the moisture content of 4.6 %, followed by refrigeration (4 ± 2 ^{0}C) with the moisture content of 2.4 % and the least moisture content of 1.6 % was recorded at ambient temperature (25 ± 2 ^{0}C). The pH of the Bonga fish during the storage conditions was slight acidic and they include pH 6.9 for the fish stored at ambient temperature while the pH 6.8 each was recorded for the Bonga fish stored at freezer (-2 ± 2 ^{0}C) and refrigeration (4 ± 2 ^{0}C) temperature. Six fungal species made of four genera: *Aspergillus, Penicillium, Rhizopus* and *Microsporium* were isolated. The genus *Aspergillus* consisted of *Aspergillus niger*, *Aspergillus terrus* and *Aspergillus flavus*. Within 6 weeks of storage, all the fungal isolates developed in most of the fish samples examined. The survey of mycoflora

on dried Bonga fish stored at ambient temperature (25 ± 2 ^{0}C) shows that the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 6 (30 %) > *Rhizopus oryzae* 5(25 %) > *Aspergillus niger* 4 (20) > *Penicillium chrysogenum* 3 (15 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(5 %) being the least as shown in Table 2. The occurrence of *Aspergillus spp, Rhizopus sp*, *Penicillium* and *Microsporum canis* could be due to the fact that moisture reabsorbed from the environments during storage of the dried fish supported the growth of the microorganisms in addition to contamination during processing, handling and display on the market stalls. The survey of mycoflora on dried Bonga fish stored at refrigeration temperature (4 ± 2 ^{0}C) shows that the frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (30.78 %) > *Rhizopus oryzae* 3 (23.08 %) > *Aspergillus niger* 2 (15.38 %) > *Penicillium chrysogenum* 2 (15.38 %) respectively, while *Aspergillus terrus* and *Microsporum* sp have equal frequencies of occurrence of 1(7.69 %) each being the least as shown in Table 3. Within 6 weeks of storage, isolates such as *Rhizopus oryzae, Aspergillus niger, Penicillium chrysogenum* and *Aspergillus flavus* developed with *Aspergillus flavus* covering all the skin of the fish samples, this result is agreement with Eyo, 2011. This result is consistent with the fact that *Aspergillus flavus* proliferated in the tissue of the fish flesh than any of the isolates. *Microsporum sp* and *Aspergillus terrus* did not grow appreciably on the fish flesh stored in the freezer and fridge (Table 4). This indicates that they lack the enzyme required to breakdown the fish tissue during storage. The isolates might probably be a contaminant acquired during bargaining in the market or acquired during the course of storage. The survey of mycoflora on dried Bonga fish stored at freezer temperature (-2 ± 2 ^{0}C) shows that the storage made at freezer temperature (-2 ± 2 ^{0}C) has a reduction in the number of isolated fungi. Only four mycoflora were isolated with. The frequencies of occurrence of the fungal isolates were in the order of *Aspergillus flavus* 4 (40%) > *Aspergillus niger* 3 (30 %) > *Rhizopus oryzae* 2 (20 %) > *Penicillium chrysogenum* 1 (10 %) respectively as shown in Table 4. This is in an agreement with the report of Konstantinos, (2005). The total number of isolated fungi from the fish stored in various storage medium at different temperature shows that the freezer recorded the least, this may be due to the fact that most of the isolates could not survive mesophilic temperature while ambient temperature and refrigeration have equal number of isolates. Fish stored in the sieve has the least percentage followed by the leather which recorded a slightly higher percentage of fungal isolates while the highest percentage of occurrence of fungal isolates was recorded for the fish stored in the container as seen in Figure 1.

5. Conclusion

It is important to state that majority of the fungal isolates obtained in this study are of veterinary and medical importance. *A. flavus* and *Microsporum* sp are probably the most notorious of the common isolates because of their high potentials in producing aflatoxin and causes dermatophytosis respectively. It is therefore suggested that better preservation method (agro waste drying) should be employed and good storage condition fashioned out for the fish product. Preservation could probably be better if the fish product is kept in a hostile environment such as the show glass equipped with heating element or in a regulated oven so as to reduce drastically the moisture content of the fish which is the basic requirement for microbial growth and hence, inhibit the growth of the microorganisms causing fish spoilage.

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