



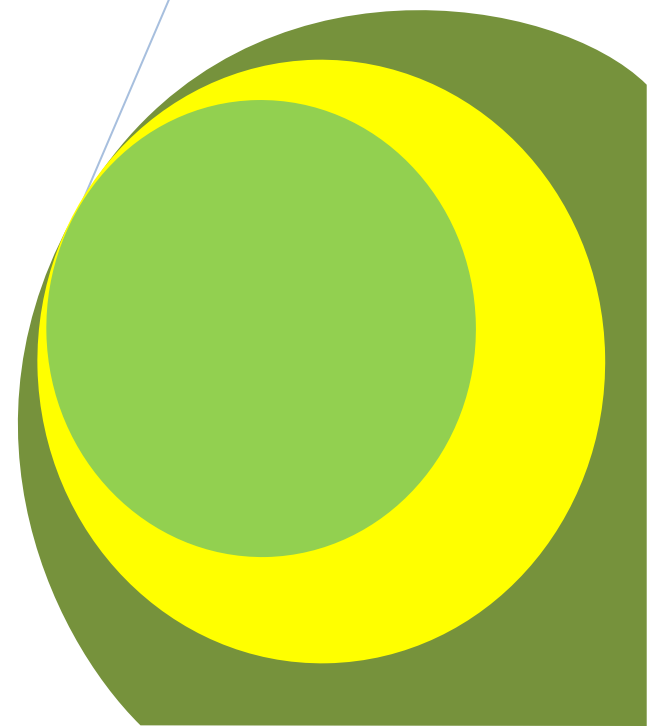
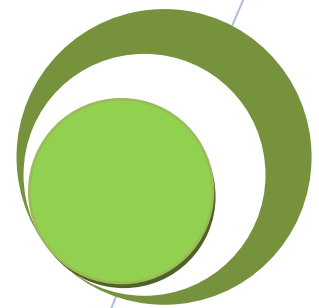
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Mycoflora of Three Fish Species Smoke- Dried Using Rubber Wood (*Hevea Brassillensis*) in Nigeria

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Research Article

Mycoflora of Three Fish Species Smoke-Dried Using Rubber Wood (*Hevea Brassillensis*) in Nigeria

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ABSTRACT

A study of the mycoflora of three fish species: *Lutjanus agennes* (Red Snapper), *Mugil cephalus* (Mullet), *Chrysichthys walkeri* (Catfish) smoke-dried using Rubber wood (*Hevea brassillensis*) was carried out. Fish samples were bought from Ogbe-ljoh market in Warri, Delta State and smoked using a traditional rectangular mud kiln (Chorkor) and stored on open benches in the laboratory at room temperature. Samples from the smoke-dried fish species were assessed tri-weekly using amended potato dextrose agar during the period of storage. Moisture content of the fish samples varied with storage time but was not significant ($P > 0.05$). The highest mean fungi count of (1.08×10^6 , 8.0×10^5 and 1.16×10^6) cfu/g was recorded in the gills, muscle and skin of *Chrysichthys walkeri* during the six weeks of storage. There were significant differences ($P < 0.05$) in the mean fungi counts in the three anatomical parts among the fish species. The predominant fungi species isolated from three different anatomical parts of smoke-dried fish samples during storage was made up of six (6) genera of fungi. *Saccharomyces* (37.69%), followed by *Penicillium italicum* (20.29%), *Penicillium oxalicum* (17.39%), *Mucor sp* (10.15%), *Rhodotorula sp* (7.25%), *Aspergillus sp* (5.79). The study has shown that fish samples could still be consumed after six (6) weeks of storage but were heavily contaminated with micro-organisms and could pose a serious health concern for consumers.

Keywords: Mycoflora, *Lutjanus agennes*, *Mugil cephalus*, *Chrysichthys walkeri*, Smoke-dried fish, *Hevea brassillensis*

INTRODUCTION

Fish is a very important source of animal protein in the diets of man. They constitute about 60% of the total protein intake in adults especially in the rural areas (Adeleye, 1993). In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo et al., 2008). Preserving food and other perishable products like fish and meat generally involves processes that impede growth of micro organisms either by addition of growth inhibiting ingredients or adjusting storage conditions by freezing or drying. Smoking is the preferred method of fish preservation in most rural areas and riverine fishing communities especially in the Delta State of Nigeria. In preserving fish by smoking water activity in the fish is lowered to the point where the activity of spoilage micro organisms is inhibited (Okonta and Ekelemu., 2005; Ighodaro and Abolagba, 2010)

However, the acceptability of smoke depends on the type of wood. According to Olorkor *et al* (2007), hard wood is preferred because it has a higher hemi-cellulose content compared to soft wood. This is usually wood in form of saw dust or wood chips and the shelf-life of smoked products are prolonged if salt-cured, which reduces drying and energy required during smoking (Abolagba, 2006).

Smoke-dried fish of various species form part of requirements in traditional marriages contracted under native law and customs in Delta State, Nigeria. This study therefore is aimed at examining the microbial quality of three fish species namely *Lutjanus agennes*, *Mugil cephalus* and *Chrysichthys walkeri* smoke-dried using rubber wood (*Hevea brassillensis*). To provide information on the fungal species prevalent in smoke dried fish and the health hazard of such micro flora to consumers.

MATERIALS AND METHODS

Sample collection

Three species of fresh fish each were purchased from Ogbe-ljoh market located in Warri, Delta state. The species included; *Lutjanus agennes* (Bleeker, 1863), *Mugil cephalus* (Linnaeus, 1758) and *Chrysichthys walkeri* (Gunther, 1899). They were then put in ice boxes and transported to the Fisheries Department, University of Benin for smoking.

Smoking process

Fresh fish purchased were not gutted, washed thoroughly with clean water and drained before smoking using a traditional rectangular mud kiln (Chorkor) in the Fisheries Department, University of Benin. Smoking of the fishes was carried out for two (2) days. On the first day, smoking lasted for 3 hrs and the maximum temperature in the smoking chamber was 100°C, while on the second day, smoking lasted for 1hr with maximum temperature of 45°C. The smoke was produced by the burning of rubber wood (*Hevea brassillensis*) to stimulate what is practiced by local fish mongers (Abolagba *et al.*, 2002). After smoking, the products were placed in open trays to cool and later transferred to plastic baskets for storage and to prevent rodent and insect infestation. They were then kept on laboratory benches in the open and stored at room temperature at a temperature of 28 ± 2°C.

Moisture content

The moisture content of smoke-dried fish samples were carried out at the initial stage of smoking and tri-weekly for six (6) weeks using A.O.A.C (1990) methods.

Microbiological Analysis

Isolation of fungal isolates from fish samples

A serial dilution method was employed. One gram of each of the anatomical parts used (gills, muscles and skin) was cut out and weighed using a top loading balance. Each weighed samples was transferred into a blender containing 10.0 ml of sterile de-ionised water. The samples were homogenized to prepare the stock suspension. One ml of the stock was serially transferred to six (6) test tubes, each containing ninety (90) ml diluent, one at a time in repeated succession up to the sixth test tube, to obtain 10⁻⁷ dilution (Taylor *et al.*, 1998).

Pour plate method" was employed Ogundana, (1989). Dilutions of 10⁻², 10⁻⁴ and 10⁻⁶ were selected for each of the fish treatments. A pour plate of each of the serial diluted samples were prepared by using approximately 20.0ml of nutrient agar, amended with an antifungal mixture and 0.5ml of dilution suspension from each of the homogenized fish samples. Duplicate plates for each dilution were incubated at room temperature (28 ± 2°C) for 24hrs.

Identification of bacterial isolates

All isolates were identified based on cultural and morphological characteristics, spore formation, production of fruiting bodies and biochemical reactions as described by (Barnett and Hunter, 1974).

Statistical Analysis

The data were subjected to Two- way Analysis of variance in completely randomized design and the means were tested and compared using Fisher's Least Significance Difference (L.S.D) at 5% level of significance using (Genstat Eighth Edition; 2005 version).

RESULTS AND DISCUSSIONS

In this study, moisture content ranged from (19 - 25.8) % in the fish species studied. There was an increase in the moisture level immediately after smoking up to week 3 in all fish species and then a decrease in week 6 for

L. agennes and *M. cephalus* whereas *Chrysichthys walkeri* increased during storage. However, no significant ($P>0.05$) difference was observed in moisture content with storage time in all fish species. The water activity determines the

storage life of fish. Smoking decreases the water activity of fish tissue (Sveindottir, 1998). The fluctuation in the moisture content of fish samples during storage may have been due to the increased water holding capacity of the samples and evaporation due to storage conditions to some extent because the fish were kept in on open laboratory benches unprotected (Ikeme, 1992; Odour-Odote, 2009).

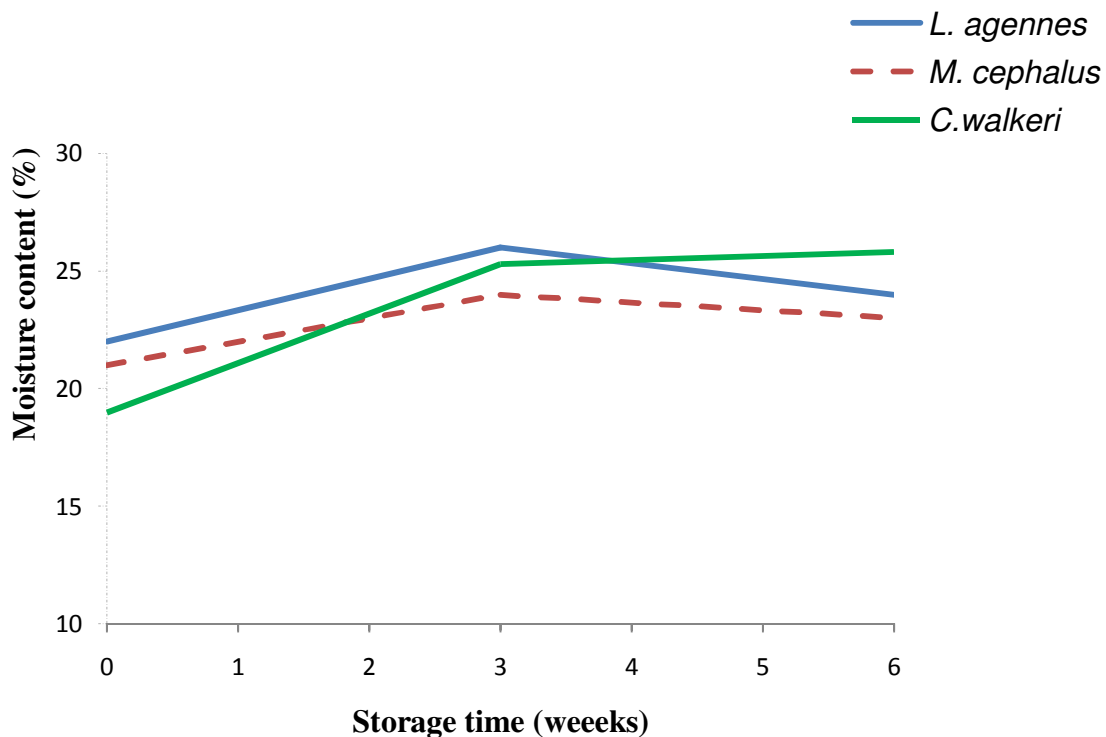


Fig 1: Tri-weekly changes in the moisture level of the fish species during storage

The Fungal counts for gill, muscle and skin of fish species during storage are presented in (Table 1). Fungal counts for gill, muscle and skin of fish species ranged from (4.2×10^5 to 1.68×10^6) cfu/g, (1.8×10^5 to 1.88×10^6) cfu/g and (2.6×10^5 to 2.88×10^6) cfu/g respectively. Fungal counts in *Lutjanus agennes* and *Mugil cephalus* decreased immediately after smoking up to week 3 except for *Chrysichthys walkeri* which increased. From week 3 to week 6 there was an exponential increase in the fungal counts of fish samples of *Lutjanus agennes*, *Mugil cephalus* and *Chrysichthys walkeri*.

The fluctuation in fungal load in fish samples suggests that intrinsic factors which include physical, chemical, and structural properties of the fish such as water activity, pH, redox potential (E^h), available nutrients and natural antimicrobial substances and extrinsic factors such as storage time, temperature, humidity, and the composition of storage atmosphere may have played a role (Adams and Moss, 2008).

The microbial load on fish rarely indicate the quality of the fish but gives an indication of the risk of spoilage induced since each of the organisms had different ways of affecting the health conditions of consumers of such contaminated fish (Gram *et al.*, 2000). It is generally accepted that fish with microbial load $>10^6$ cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption (Cheesbrough, 2000).

Table: 1 Fungal count in cfu/g x 10⁴ for gills, muscle and skin of fish species during storage.

Fish parts	Fish species	Storage time (weeks)		
		After smoking	3	6
Gills	<i>Lutjanus agennes</i>	50	42	140
	<i>Mugil cephalus</i>	64	60	124
	<i>Chrysichthys walkeri</i>	72	84	168
Muscle	<i>Lutjanus agennes</i>	30	26	152
	<i>Mugil cephalus</i>	26	18	80
	<i>Chrysichthys walkeri</i>	24	28	188
Skin	<i>Lutjanus agennes</i>	46	38	240
	<i>Mugil cephalus</i>	44	28	128
	<i>Chrysichthys walkeri</i>	44	48	288

Table 2 shows the mean fungi count in the three anatomical parts of the three fish species. There were significant differences ($P < 0.05$) in the three anatomical parts among the fish species. The highest mean fungi count of (1.08×10^6 , 6.2×10^5 and 1.17×10^6) cfu/g was recorded in the gills, muscle and skin of *Chrysichthys walkeri* after six weeks of storage.

The high fungal counts recorded in the gills of fish samples after smoking is an indication that the fish may have recently been feeding and hence the multiplication of the living microorganisms even after death of the fish. Liston (1980), also observed a normal range of 10^2 - 10^7 cfu/ml in his study in the skin surface while Shewan (1962) also stated that the gills and intestines both contain between 10^3 - 10^9 cfu/g. The highest mean fungi counts recorded in the three anatomical parts (gills, muscle and skin) of *Chrysichthys walkeri* suggest that microbial fish spoilage may have commenced at the point of higher microbial counts.

Table 2: Mean Fungi count in cfu/g x 10⁴ for gills, muscle and skin of fish species at the end of six (6) weeks of storage.

Fish parts	Fish species		
	<i>Lutjanus agennes</i>	<i>Mugil cephalus</i>	<i>Chrysichthys walkeri</i>
Gills	78.50 ^b	84.33 ^b	108 ^a
Muscle	41.33 ^b	69.33 ^a	80 ^a
Skin	60.67 ^b	108 ^a	116.67 ^a

Different letters in the row indicate significant difference ($p < 0.05$).

All the fish samples examined in this study contained fungal isolates of diverse spp. Fig 1 shows the comparative frequency of occurrence of fungi isolates from three different anatomical parts of smoke-dried fish samples during storage. The fungi species isolated from the three different anatomical parts smoke-dried fish samples during storage was made up of six (6) genera of fungi. *Saccharomyces* (37.69%) was the most predominant fungi species followed by *Penicillium italicum* (20.29%), *Penicillium oxalicum* (17.39%), *Mucor sp* (10.15%), *Rhodotorula sp* (7.25%), *Aspergillus sp* (5.79%). In smoke dried *Lutjanus agennes*, the muscle specimen accounted for 42.11% of the total fungi isolated, whereas 36.84% and 21.05% were recorded for gills and skin respectively. *Saccharomyces sp.* 9(47.36%) was the most frequently isolated fungi from the three different anatomical parts followed by *Penicillium italicum* 3(15.78%), *Penicillium oxalicum* 3(15.78%), *Mucor sp.* 3(15.78%) and *Aspergillus niger* 1(5.26%). In smoke-dried *Mugil cephalus* the gill specimen accounted for 45.83% of the total fungi isolated, whereas 29.17% and 25.00% were recorded for skin and muscle. *Saccharomyces sp.* 9(37.5%) was the most frequently isolated fungi from the three different anatomical parts followed by *Penicillium italicum* 4(16.66%), *Penicillium oxalicum* 4(16.66%), *Mucor sp.* 3(12.5%), *Aspergillus niger*, *Cercospora sp.*, *Rhodotorula sp.* and *Trichoderma sp* with 1(5.26%) each. Whereas,

in smoke-dried *Chrysichthys walkeri*, the gill specimen accounted for 38.46% of the total fungi isolated, whereas 30.77% were recorded for muscle and skin respectively. *Saccharomyces sp.* 8(30.77%) was the most frequently

isolated fungi from the three different anatomical parts followed by *Penicillium italicum* 7(26.92%), *Penicillium oxalicum* 5(19.23%), *Rhodotorula sp* 4(15.39), *Aspergillus niger* 2(7.69%) and *Mucor sp.* 1(3.85%).

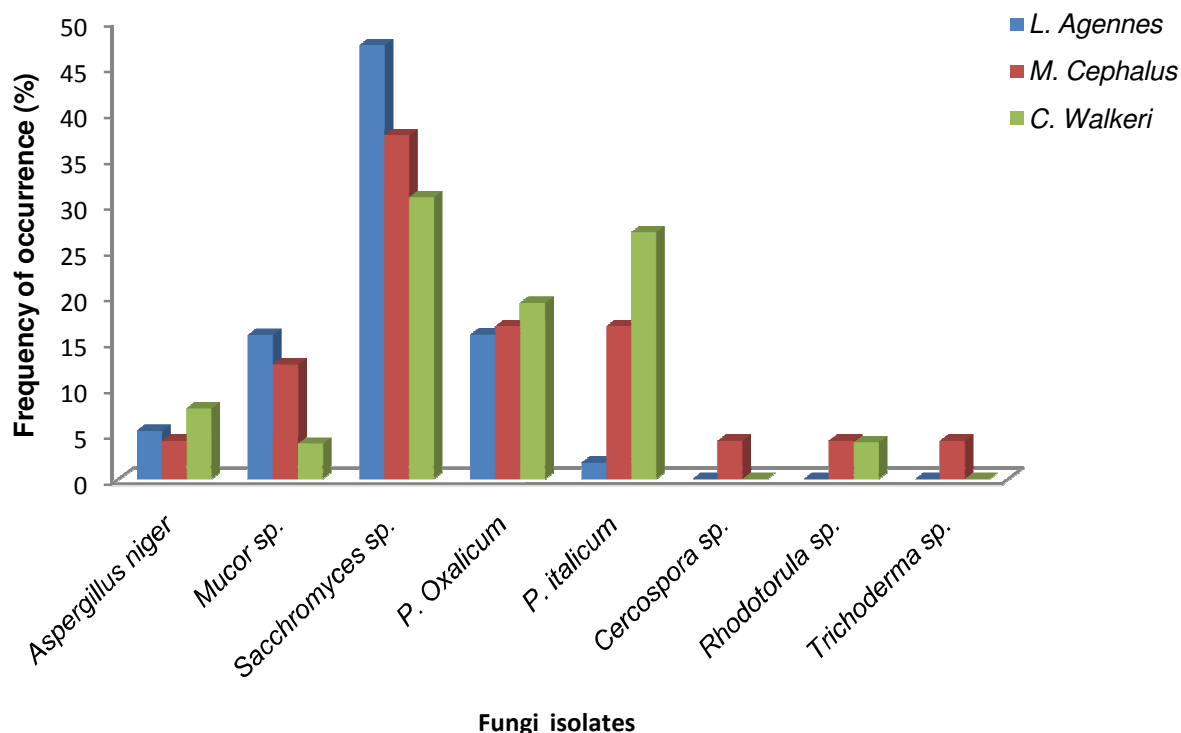


Fig 1: Comparative frequency of occurrence of fungi species isolated from smoke-dried fish samples during storage.

The occurrence of *Saccharomyces spp.* may be due to its ubiquitous form in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. *Saccharomyces spp.* is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables (Phaff et al., 1966). The only adverse effect to the environment noted in the literature was the presence of the "killer toxins" which is active against other strains of *Saccharomyces spp.* The occurrence of *Aspergillus sp.* and *Penicillium sp.* could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and display on the market stalls (Christianah et al., 2010). The occurrence of yeast (*Saccharomyces sp.*), moulds (*Penicillium sp.* and *Aspergillus niger*) in the smoke-dried fish samples were in accordance with Martin (1994), when he stated that these organisms were the commonest microorganisms associated with smoked fish and these microorganisms were also reported by Abolagba et al., (2011) in the microbial assessment of smoked fish (*Clarias sp.*) sold in Benin metropolis. Several species of yeast and *Aspergillus* have been isolated from salted and dried meat and fish products (Graikoski, 1973) and these species are known to produce toxic substances. Adebayo-Tayo et al., (2008) detected aflatoxins B1 and G1 concentrations ranging from 1.50- 8.10 µg/kg and 1.81 -4.5 µg/kg respectively. This finding is instructive as consumption of contaminated smoked fish could pose serious health problems. Aflatoxins have been implicated in cases of acute hepatitis in man and they are also known to be carcinogenic causing hepatoma (Eaton and Gorpman, 1994). The presence of *Trichoderma sp.* can also be as a result of the fish which has been kept on contaminated soil because according to Wikipedia, *Trichoderma sp.* is mainly isolated from forest and soils. This is in line with the findings of Wogu and Iyayi (2011) who observed this

organism in some smoked fish samples in Benin City. The occurrence of *Cercospora sp.* in smoke-dried fish samples is an indication that fish samples may have been contaminated by atmosphere during storage. All these

microorganisms isolated in this study are of food processing and public health concerns and hence hazardous and injurious to human health if consumed.

The disappearance and appearance of some microbial species in fish samples during the period of storage may have been as a result of antagonism, that is, production of antibiotic/antifungal or hyphal interference or competition for nutrient in the medium by successive isolates. Appearance of new species would have come as a result of the product of metabolism of the fish nutrient by microbial activity during the first sampling which would have introduced a metabolite as a substrate of the new species, hence a tangible reason for the disappearance and appearance of species.

CONCLUSION

The study has shown that fish samples could still be consumed after six (6) weeks of storage but were heavily contaminated with micro-organisms. The fact that microbial isolates from the fish samples during shelf storage showed high microbial quality and quantity is an implication which could pose a serious health concern for consumers and public health workers. As such better preservation methods and less direct handling should be adopted by fish processors.

Also the higher microbial load observed in the skin of fish samples suggests that most of the poor handling and storage could have increased proliferation of the organisms. The microbial dynamics of fish samples within the sampling period is an indication that moisture, available nutrient and conditions may have played a significant role.

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