

Research paper

COMPARATIVE STUDY ON THE BACTERIA LOAD OF THREE FISH SPECIES SMOKE-DRIED USING RUBBER WOOD (*HEVEA BRASSILLENIS*) IN NIGERIA

BY

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ABSTRACT

Aim: To examine the organoleptic and 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 how fish processed will have little or no significant loss in quality as well as meet set standards for consumers.

Study Design: Completely randomized Design

Place and Duration: Fisheries Department University of Benin between August and September 2011.

Methodology: Fish samples were bought from Ogbe-Ijoh market in Warri, Delta State. Smoking was carried out using rubber wood (*Hevea brassillensis*) in a traditional rectangular mud kiln (Chorkor) and stored on open benches in the laboratory at room temperature. Fish samples were then assessed tri-weekly for various attributes such as colour, fragmentation, odour, taste and texture. Samples from the fish species were also analysed using nutrient agar for bacteria load of the smoke-dried fish during the period of storage.

Results: Significant ($P=0.05$) changes in the colour of the fish samples was observed. Moisture content of the fish samples varied with storage time but was not significant ($P>0.05$). The highest mean bacterial count of (1.48×10^6 and 6.2×10^5) cfu/g were recorded in the gills and muscle of *Chrysichthys walkeri* while the (9.83×10^5) cfu/g was recorded in the skin of *Lutjanus agennes* after six weeks of storage. There was significant ($P=0.05$) difference in the mean bacterial count in the three anatomical parts among the fish samples. The predominant bacterial species isolated from the three different anatomical parts of the three fish samples during the six (6) weeks sampling period was five (5) genera made up of bacteria *Proteus*(30.49%), *Pseudomonas*(26.83%), *Bacillus*(9.76%), *Micrococcus sp* (8.29%) and *Staphylococcus* (7.32%). Conclusion: The study revealed that fish samples were heavily contaminated with micro-organisms and this could pose serious health hazard to consumers. The physico-chemical and nutritional characteristics of the fish samples should be carried out to further understand the changes in the microflora of the fish samples.

Keywords: Bacterial load, *Lutjanus agennes*, *Mugil cephalus*, *Chrysichthys walkeri*, smoke-dried, *Hevea brassillensis*

1.0 INTRODUCTION

Fish is one of the most important animal proteins available in the tropics, and it represents about 14% of all animal proteins on a global basis (Abolagba and Melle, 2008 and Eyo, 2001). In Nigeria, the demand for fish is on the increase due primarily to health benefits of eating fish and secondarily to increase in human population and the rinderpest disaster and drought bane which reduced the availability and affordability of red meat (cattle, sheep and goat) (Adebowale, et al., 2008).

However, fish is an extremely perishable food after catch and therefore requires immediate and proper handling and good preservation to retain its quality (Okonta and Ekelemu, 2005). Fish undergoes spoilage as soon as it is landed. In tropical countries like Nigeria, deterioration sets in earlier. Once spoilage sets in, the odour, flavor, texture and colour and sometimes even its composition changes. Spoilage of fish can result from the biochemical changes taking place in the fish during post-mortem stages and attack of microbes. The type and number of micro-organisms that live in fish vary according to the season, the species and the natural habitat (Encyclopaedia, 2010). Fish may also contain pathogenic (disease-causing) micro-organisms such as *Salmonella*, *Escherichia coli*, *Shigella*, *Leptospira* and *Vibrio* (Obasohan et al., 2009).

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. 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). Martin (1994), Abolagba et al., (2011a) and Abolagba and Uwagbai (2011b) stated that, bacteria such as *Staphylococcus aureus*, *Proteus*, *Bacillus*, *Micrococcus* were the most common micro-organisms associated with smoked fish.

Fuel wood is the main source of energy for fish smoking. Although many wood types may be used as fuel, the particular species of wood used depends on local availability. The fuel wood preferences of most fish smokers are also related to the physical characteristics of the wood and how they affect the smoked product (Nerquaye-Tetteh, 1985; Eyo, 1985; Lartey, et al., 1994).

Smoke dried fish are highly relished in Nigerian traditional diets. Dried fish provides an excellent diet in many families. In certain parts of Nigeria, smoke-dried fish of various species form part of the requirements in traditional marriages contracted under native law and customs. This study therefore is aimed at examining the organoleptic and microbial quality of three fish species namely *Lutjanus agennes*, *Mugil cephalus* and *Chrysichthys walkeri* smoke-dried using rubber wood (*Hevea brassillensis*). The study would provide information on how fish processed will have little or no significant loss in quality as well as meet set standards for consumers. The objectives are to; determine the sensory characteristics of fish samples and the quantitative and qualitative bacterial flora that harbors the three anatomical parts (gill, muscle and skin) smoke-dried fish samples.

2.0 MATERIALS AND METHODS

2.1 Sample collection

Three species of fresh fish ~~each~~ were purchased from Ogbe-Ijoh market located in Warri, Delta state, Nigeria. 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.

2.3 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 at a maximum temperature of 100°C, while on the second day smoking lasted for 1hr with a maximum temperature of 45°C. The smoke was produced by burning 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 at room temperature (28 ± 2°C) for periodic bacterial analysis.

2.4 Sensory Analysis

Ten (10) ~~judges~~ were selected ~~and used~~ for organoleptic assessment of the smoke-dried fishes. Questionnaires were used by the panelists and scoring was done on a tri-weekly basis. The questionnaires were prepared using **five (5) points hedonic score** described by (Eyo, 1983) to evaluate changes in colour, fragmentation, odour, taste and texture. Parameters on the questionnaires were as follows: **dislike definitely (bad); dislike mildly (poor); neither like/dislike (fair); like mildly (good); and like definitely (excellent)**

2.5 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.

2.6 Microbiological Analysis

2.6.1 Isolation of bacterial 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 deionised 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 fish samples. Duplicate plates for each dilution were incubated at room temperature (28 ± 2°C) for 24hrs.

2.6.2 Identification of bacterial isolates

All isolates were identified by standard microbiological techniques as described by Cowan and Steel (1970).

2.7 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).

3.0 RESULTS AND DISCUSSION

3.1 Organoleptic (Sensory) Analysis

Organoleptic assessment for colour, fragmentation, odour, texture and taste of fish samples were examined on a tri-weekly basis and the results are presented in (Table 1). There was a general decline in the organoleptic parameters such as colour, fragmentation, odour, texture and taste of fish during storage. This agrees with the results of research into storage of smoke-dried fish (Daramola *et al.*, 2007) and crustaceans (Oyster and shrimps) (Llobreda *et al.*, 1986; Reilly and Dela-cruz, 1986) which revealed quality loss during storage both at ambient temperature and chilling.

The mean sensory scores showed that *Mugil cephalus* had the best colour. *Lutjanus agennes* had the least fragmentation. *Chrysichthys walkeri* had the best texture and taste. Although, the results show that all fish samples had an above average mean sensory score after the storage period. The differences in the quality of fish samples is in agreement with the findings of Sefa-Dedeh (1995) which stated that differences between fish species may be reflected in the quality of the smoked fish.

Statistical analysis revealed significant differences ($P=0.05$) in the colour of fish samples and in the overall attribute score for each sensory characteristics or physical attributes at the end of storage. The attribute that the panelist gave the highest score was taste with a mean score of 3.37 ± 0.08 , followed by texture with a mean score of 3.24 ± 0.03 . However, there were no significant difference ($P=0.05$) in terms of preference for individual fish species after combining all the attributes, *Mugil cephalus* was the most preferred fish by the panelists with a score of 3.12 ± 0.04 . It was followed by *Lutjanus agennes* with a score of 3.11 ± 0.05 and *Chrysichthys walkeri* with a score of 3.06 ± 0.07 . All fish samples studied had an above average score at the end of six (6) weeks of storage, suggesting that they were still acceptable. Taste of the fish samples took precedence over all the parameters in this study. In terms of quality acceptability *Mugil cephalus* was the most acceptable while *Chrysichthys walkeri* had the least acceptable quality.

Table 1: Organoleptic characteristics of fish samples smoke-dried during storage.

Parameters	Fish species	Storage time(weeks)			Means
		After smoking	3	6	
Colour	<i>Lutjanus agennes</i>	4.0	3.2	2.3	3.17 ^b
	<i>Mugil cephalus</i>	4.5	3.3	2.4	3.40 ^a
	<i>Chrysichthys walkeri</i>	3.2	2.9	1.5	2.53 ^a
Fragmentation	<i>Lutjanus agennes</i>	3.3	3.0	2.2	2.83 ^a
	<i>Mugil cephalus</i>	3.4	3.0	2.4	2.93 ^a
	<i>Chrysichthys walkeri</i>	4.0	2.7	2.4	3.03 ^a
Odour	<i>Lutjanus agennes</i>	3.3	2.8	2.7	2.93 ^a
	<i>Mugil cephalus</i>	3.7	3.3	1.8	2.93 ^a
	<i>Chrysichthys walkeri</i>	3.9	2.6	2.1	2.87 ^a
Texture	<i>Lutjanus agennes</i>	3.6	3.3	2.6	3.17 ^a
	<i>Mugil cephalus</i>	3.7	3.4	2.6	3.23 ^a
	<i>Chrysichthys walkeri</i>	3.7	3.4	2.9	3.33 ^a
Taste	<i>Lutjanus agennes</i>	4.0	3.3	3.1	3.47 ^a
	<i>Mugil cephalus</i>	3.3	3.1	2.9	3.10 ^a
	<i>Chrysichthys walkeri</i>	4.0	3.3	3.3	3.53 ^a

* Different letters in the same column indicate significant difference ($P=0.05$).

Table 2: Mean scores of attributes for *L. agennes*, *M. cephalus* and *C. walkeri*.

Parameters	Fish species			Overall attribute score
	<i>L. agennes</i>	<i>M. cephalus</i>	<i>C. walkeri</i>	
Colour	3.17 ± 0.34	3.40 ± 0.25	2.53 ± 0.31	3.03 ± 0.15 ^{ab}
Fragments	2.83 ± 0.40	2.93 ± 0.24	3.03 ± 0.21	2.93 ± 0.03 ^b
Odour	2.93 ± 0.44	2.93 ± 0.24	2.87 ± 0.15	2.91 ± 0.01 ^b
Texture	3.17 ± 0.24	3.23 ± 0.09	3.33 ± 0.05	3.24 ± 0.03 ^{ab}
Taste	3.47 ± 0.19	3.10 ± 0.09	3.53 ± 0.07	3.37 ± 0.08 ^a
Overall acceptability	3.11 ± 0.05 ^a	3.12 ± 0.04 ^a	3.06 ± 0.07 ^a	

* Different letters in the same column or row indicate significant difference ($P=0.05$).

3.2 Moisture content

The water activity determines the storage life of fish. Smoking decreases the water activity of fish tissue (Sveindottir, 1998). In this study, Moisture content ranged from (19 - 25.8) % in the fish samples studied. There was an increase in the moisture content immediately after smoking up to week 3 in all fish samples and then a decrease in week 6 for *L. agennes* and *M. cephalus* whereas *C. walkeri* increased during storage. However, no significant ($P=0.05$) difference was observed in moisture content with storage time in all fish species.

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

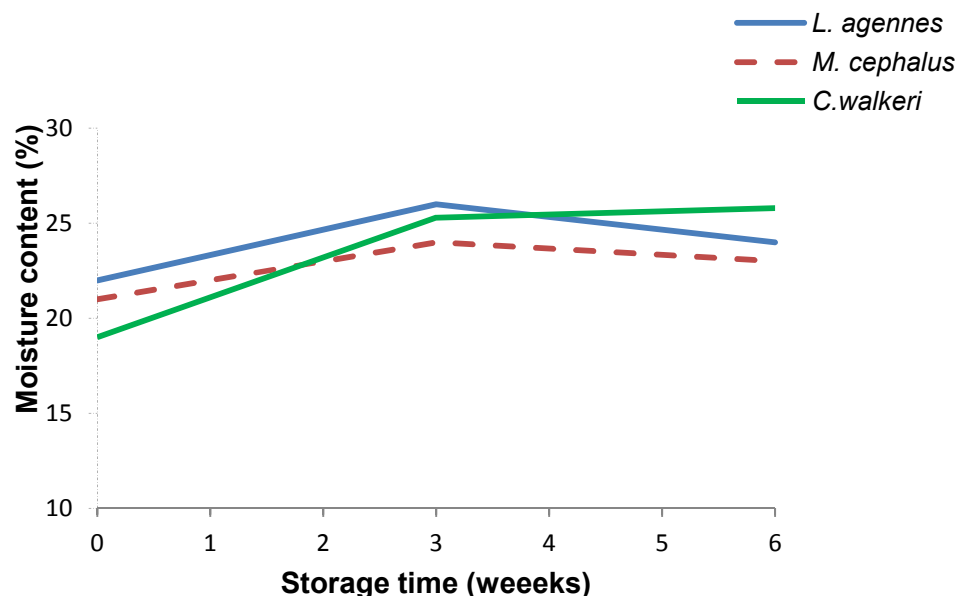


Fig 1: Tri-weekly changes in the moisture level of the fish species during storage

3.3 Quantitative Analysis of bacteria

The bacterial counts for gill, muscle and skin of fish species during storage, are presented in (Table 3). There was variation in the bacteria count among the anatomical parts. Bacterial counts for gills, muscle and skin of fish species ranged from $(8.0 \times 10^5 \text{ to } 3.9 \times 10^6)$ cfu/g, $(3.8 \times 10^5 \text{ to } 1.4 \times 10^6)$ cfu/g and $(2.6 \times 10^5 \text{ to } 2.4 \times 10^6)$ cfu/g respectively. Bacterial counts of all fish samples increased immediately after smoking up to the third week. From the third week to the sixth week there was a decrease in the bacterial count of fish samples of *Lutjanus agennes* and *Mugil cephalus*, whereas bacterial count in *Chrysichthys walkeri* was on the increase up to the sixth week. The fluctuation of bacterial count in *Lutjanus agennes* and *Mugil cephalus*, 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 bacterial 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 unfit for consumption (Cheesbrough, 2000).

The high bacteria 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

-10⁷ cfu/ml in his study in the skin surface while Shewan (1962) also stated that the gills and intestines both contain between 10³ -10⁹ cfu/g.

Table 3: Bacteria 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>	90	100	80
	<i>Mugil cephalus</i>	108	184	138
	<i>Chrysichthys walkeri</i>	160	192	392
Muscle	<i>Lutjanus agennes</i>	38	74	56
	<i>Mugil cephalus</i>	58	84	100
	<i>Chrysichthys walkeri</i>	60	80	144
Skin	<i>Lutjanus agennes</i>	78	160	26
	<i>Mugil cephalus</i>	70	104	90
	<i>Chrysichthys walkeri</i>	64	156	240

Table 4 shows the mean bacterial count in the three anatomical parts of the three fish species studied. There were significant differences ($P=.05$) in the three anatomical parts among the fish species. The highest mean bacterial count of 1.5×10^6 and 6.2×10^5 cfu/g were recorded in the gills and muscle of *Chrysichthys walkeri* while the 9.8×10^5 cfu/g was recorded in the skin of *Lutjanus agennes* after six weeks of storage respectively. The highest mean bacterial counts recorded in the three anatomical parts (gills, muscle and skin) of *Chrysichthys walkeri* suggest that bacterial fish spoilage may have commenced at the point of higher bacterial counts.

Table 4: Mean Bacteria 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	60.67 ^c	85.33 ^b	148.67 ^a
Muscle	40.67 ^b	48 ^b	62.50 ^a
Skin	98.33 ^a	48.17 ^b	89.33 ^a

- Different letters in the row indicate significant difference ($P=.05$).

3.4 Qualitative Analysis of bacteria

All the fish samples examined in this study contained bacterial isolates of diverse spp. Fig 2 shows the comparative frequency of occurrence of bacteria isolates from smoke-dried fish samples during storage. It shows that *Proteus* (30.12%) was the most predominant bacteria isolate followed by *Micrococcus* sp (18.07%), *Pseudomonas* (26.51%), *Bacillus* (9.64%), *Staphylococcus* (7.23%), *Escherichia coli* (4.82%) was isolated from *Lutjanus agennes* and *Chrysichthys walkeri* while *Staphylococcus epidermidis* (2.41%) and *Streptococcus* sp (1.22%) were isolated from only *Lutjanus agennes*. In smoke-dried *Lutjanus agennes*, the most frequently isolated bacteria was *Pseudomonas* sp 8(28.57%) followed by *Proteus* sp. 7(25%), *Micrococcus* sp. 5(17.86%), *Bacillus* sp 3(10.71%), *Staphylococcus epidermidis* 2(7.14%),

Escherichia coli 2(7.14%), *Staphylococcus aureus* and *Streptococcus sp* with 1(3.57%) respectively. In smoke-dried *Mugil cephalus*, the most frequently isolated bacteria was *Proteus sp.* 9(34.62%) followed by *Pseudomonas sp* 8(30.77%), *Micrococcus sp.* 4(15.39%), *Bacillus sp* 3 (11.54%) and *Staphylococcus aureus* 2(7.69%) respectively. Whereas in smoke-dried *Chrysichthys walkeri*, the most frequently isolated bacteria was *Proteus sp.* 9(32.14%), followed by *Micrococcus sp.* and *Pseudomonas sp* with 6(21.43%), *Staphylococcus aureus* 3(10.71%), *Bacillus sp* 2 (7.14%), and *Escherichia coli* 2(7.14%).

The predominant bacterial isolates from the three different anatomical parts of the three fish samples during the period of storage was made up of five (5) genera of bacteria *Proteus*(30.29%), *Pseudomonas*(26.83%), *Bacillus*(9.76%), *Micrococcus sp* (8.29%) and *Staphylococcus* (7.32%).

Most of the organisms isolated from the fish samples are those commonly found in soil, water and air. The highest frequency of occurrence of isolates from the fish samples was obtained from *Chrysichthys walkeri* which constituted 35.76% while *Mugil cephalus* and *Lutjanus agennes* 33.11% and 31.13% respectively. The pathogens isolated in this present study are similar to the microorganisms reported by Abolagba and Uwagbai (2011) who reported the presence of *Proteus*, *Micrococcus*, *Staphylococcus aureus*, *Bacillus*, among other organisms. Bankole *et al.*, (2004, 2005) also reported the presence of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus sp*, *Escherichia coli*, *Enterobacter sp.*, *Pseudomonas sp.*, *Shigella sp.*

The occurrence of *Proteus sp* may also be due to contamination of soil and water. *Proteus sp.* is an opportunistic human pathogen and has simple nutritional requirements. They are known for their propensity to swarm over the plating media, making the isolation of other organisms in mixed cultures difficult. They are important causative agents in community-acquired and nosocomial urinary tract infections (Abott, 2007). The occurrences of *Bacillus sp.* can be said to be as a result of prevalence of their spores in the environment most especially in the soil (Jay, 1978; Adebayo-Tayo *et al.*, 2009). *Bacillus* species are spore formers whose spores could survive high temperatures of fish processing. *Bacillus sp* causes a toxin-mediated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Bergdoll, 1981; Adebayo-Tayo *et al.*, 2006, 2009). *Bacillus sp.* have been isolated from non-alcoholic beverages (Osuntogun and Aboaba, 2004; Amusa *et al.*, 2005). These organisms are present in most raw materials used in food manufacturing at concentration of 10^3 /g or less. The infectious dose has been estimated to be 10^5 /g (Adeleke and Odelola, 1997; Adebayo-Tayo *et al.*, 2009). *Pseudomonas sp* on the other hand is prevalent among patients with wounds, burns, cystic fibrosis hence are likely to have been introduced into the aquatic environment by swimmers and infected individuals who use these waters for recreational purposes (Adebayo-Tayo *et al.*, 2006).

An organism like *Escherichia coli* is an indication of faecal contamination of fish samples which is of great health significance. *Staphylococcus aureus* have been found to be relatively resistant to drying which is a property that favours their transmission from one host to another Nester *et al.*, (2003) and Prescott *et al.*, (2008), they are however able to grow in concentrations of sodium chloride up to 15%. The presence of *Staphylococcus aureus* in fish samples according to

Okonko *et al.*, (2008) might have been through handling as it is a normal flora of the skin. This is also in accordance with the assertion of Dunn *et al.*, (1995) and Omemu and Bankole, (2005) that improper handling and improper hygiene might lead to the contamination of ready-to-eat food such as vegetable salad and this might eventually affect the health of the consumers. The disappearance and appearance of some microbial species in fish samples during the period of storage may have been as a result of succession which can be related to the fluctuating nutritional values of the samples as a result of spoilage setting in.

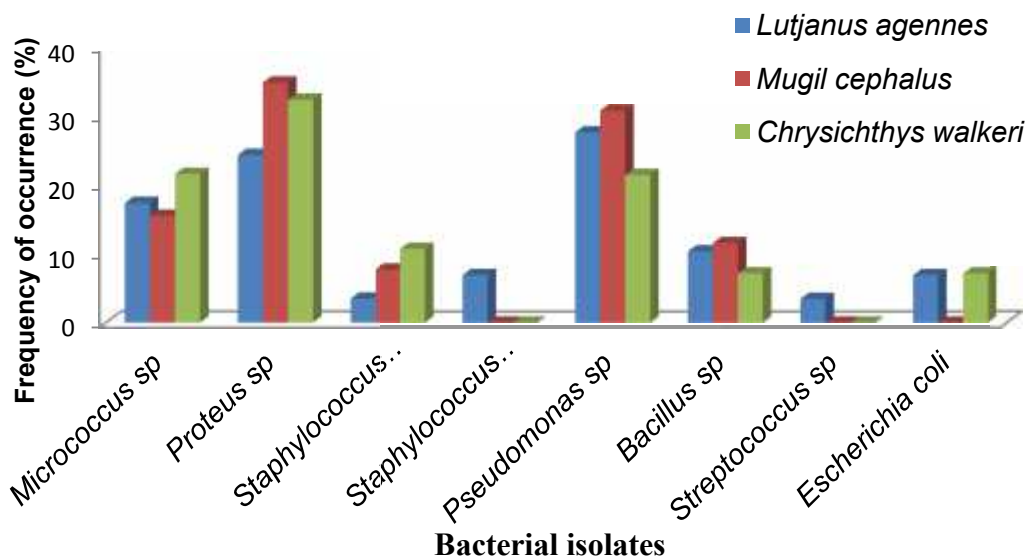


Fig 2: Comparative frequency of occurrence of bacterial isolates from three smoke-dried fish samples during storage.

4.0 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 lower the population diversity of micro organisms, the longer the shelf life of fish sample. The fact that most of the organisms isolated from the fish samples during shelf storage were from soil, water and air could pose a serious health concern for consumers. Also the higher microbial load observed in the gills of fish samples suggests that spoilage could have already started in the gills. 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.

Based on the findings of this study, the physico-chemical and nutritional characteristics of the fish samples should be carried out to further understand the changes in the microflora of the fish samples. Education of the fish processors/consumers by food surveillance agencies, including National Agency for Food and Drug Administration and Control (NAFDAC), World Health Organization (WHO), United Nations International Children Educational Fund (UNICEF) and United Nations on the health implication of consuming not properly stored fish products is

imperative. Processors/handlers/sellers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of smoke-dried fish.

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