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Comparative Study on Quality Attributes of Three Fish Species Smoke-Dried Using Rubber Wood (*Hevea brassillensis*) in Nigeria

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Authors' contributions

This work is a concerted effort of all authors. Authors OGA and MME designed the study, performed the laboratory analysis and wrote the first draft of the manuscript. Author OGA performed the statistical analysis. Author OGA supervised the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To examine the organoleptic and microbial quality of three fish species namely African red snapper (*Lutjanus agennes),* Mullet (*Mugil cephalus)* and Catfish (*Chrysichthys walkeri)* smoke-dried using rubber wood (*Hevea brassillensis*).
Study Design: Factorial experiment laid in 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 (28 ± 2ºC). Fish samples were assessed tri-weekly for moisture content and sensory attributes. Three anatomical parts of fish samples were also analyzed for bacteria load during the six weeks period of storage.
Results: There was a general decline in all sensory attributes during storage. Moisture content of the fish samples varied with storage time but was not significant ($P \geq 0.05$). The highest mean bacterial counts of 3.9×10^6, 1.4×10^6 and 2.4×10^6cfu/g was recorded in

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the gills, muscle and skin of *Chrysichthys walkeri* after six weeks of storage respectively. There were significant (*P≤0.05*) difference in the mean bacterial count in the three anatomical parts among the fish samples during storage. The predominant bacterial species isolated from the three different anatomical parts of the three fish samples during the six (6) weeks storage period were made up of seven (7) genera of bacteria: *Proteus* (30.1%), *Pseudomonas* (26.5%), *Micrococcus sp* (18.1%) *Bacillus* (9.6%), *Staphylococcus* (9.6%), *Escherichia coli* (4.8%) and *Streptococcus sp* (1.2%)

Conclusion: The study revealed that by the sixth week of storage all tested fish had an overall mean score of acceptability less than 6 (i.e. fair).Therefore, the fish were marginally acceptable based sensory and microbiological evaluation. This is an implication that could pose a serious health concern for consumers.

Keywords: Bacterial load; African red snapper; mullet; catfish; smoke-dried; Hevea brassillensis.

1. 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 [1,2]. In Nigeria, the demand for fish consumption 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) [3].

However, fish is an extremely perishable food after catch and therefore requires immediate and proper handling and good preservation to retain its quality [4]. Some preservation methods used in the tropics include chilling, freezing, drying, salting and smoking. In preserving fish by smoking water activity in the fish is lowered to the point where the activity of spoilage micro organisms is inhibited [4]. 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 [5,6,7].

Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes, Salmonella spp*., and *Clostridium botulinum* [8]. [9] also reported that smoked fish samples from 4 local Markets in Kainji Lake area of Nigeria were dominated by gram-positive bacteria, potential pathogens, coagulase-positive *Staphylococcus*, and *Escherichia coli*. [10,11,12] stated that, bacteria such as *Staphylococcus aureus*, *Proteus, Bacillus, Micrococcus* were the most common micro-organisms associated with smoked fish.

Smoke dried fish is highly relished in Nigerian traditional diets. Dried fish provides an excellent diet in many families. In Delta state of Nigeria, smoke-dried *Lutjanus agennes, Mugil cephalus* and *Chrysichthys walkeri* form part of the requirements in traditional marriages contracted under native law and customs. This study therefore aimed at examining the organoleptic and microbiological qualities of these three fish species smoke-dried using rubber wood (*Hevea brassillensis*). The objectives are to: determine the sensory characteristics of fish samples and provide information on the bacterial flora that harbors the gills, muscle and skin of smoke-dried fish samples.

2. MATERIAL AND METHODS

2.1 Sample Collection

Fifteen freshly caught fish samples, five fish samples per fish species, were purchased from Ogbe-Ijoh market located in Warri, Delta state, Nigeria. The species included; *Lutjanus agennes* (Bleeker, 1863) measuring 23.5 -25cm in length and weighing 150 - 200g, *Mugil cephalus* (Linnaeus, 1758) measuring 29 - 32.5cm in length and weighing 180 - 230g and *Chrysichthys walkeri* (Gunther, 1899) measuring 29 -35cm in length and weighing 160 - 310g were 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, but were thoroughly washed with clean water and placed in a sieve to drain, without salting 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 100^0C, while on the second day smoking lasted for 1hr with a maximum temperature of 45^0C to avoid charring. The smoke was produced by burning rubber wood (*Hevea brassillensis*) to simulate what is practiced by local fish mongers [13]. After smoking, the products were placed in a monolayer 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 ± 2^0C) for an hour and analyzed for sensory and microbiological changes tri-weekly for a period of six (6) weeks.

2.4 Sensory Analysis

Organoleptic assessment of the smoke-dried fish samples was carried out by a trained panel of ten (10) judges. Questionnaires were used by the panelists and scoring was done on a tri-weekly basis. The questionnaires were prepared using 10- point hedonic score described by [14] to evaluate changes in colour, fragmentation, odour, taste and texture. Parameters on the questionnaires were as follows: Dislike definitely (Bad) = 2; Dislike mildly (Poor) = 4; Neither like/Dislike (Fair) = 6; Like mildly (Good) = 8 and Like definitely (Excellent) = 10].

2.5 Moisture Content

The moisture content of smoke-dried fish samples was determined tri-weekly for six (6) weeks using [15] methods.

2.6 Microbiological Analysis

2.6.1 Isolation of bacterial isolates from fish samples

Samples of the three fish species were drawn tri-weekly during six (6) weeks of storage. One gram of each of the anatomical parts used (gills, muscles and skin) was cut out and weighed using a top loading balance (Metler, PM4800). Each weighed sample was transferred into a blender containing 9.0 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 nine (9.0) ml diluent, one at a time in repeated

succession up to the sixth test tube, to obtain 10^{-7} dilution [16]. Dilutions of 10^{-2}, 10^{-4} and 10^{-6} were selected for each of the fish treatments. A pour plate of each of the selected serially diluted samples was prepared by using approximately 20.0ml of nutrient agar, amended with an antifungal agent, Fulcin (500 mg in 200 ml of H_20 used at the ratio of 2-3 drops in 20 ml of agar) and 0.5ml of dilution suspension from each of the fish samples were duplicated and incubated at room temperature (28 ± 2^0C) for 24hrs [17].

2.6.2 Identification of bacterial isolates

All isolates were characterized and identified using morphological and biochemical tests such as Gram stain, Methyl red, Voges-proskaur, Indole, Oxidase, Catalase, Coagulase, Hydrogen sulphide, motility, Gelatin liquefaction, Citrate and sugar fermentation test as described by [18].

2.7 Statistical Analysis

The data were subjected to 3 (fish species) x 3 (fish parts) x 3 (sampling period) factorial arrangement laid in a 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. RESULTS AND DISCUSSION

3.1 Organoleptic (Sensory) Quality Changes

The results of organoleptic assessment for colour, fragmentation, odour, texture and taste of fish samples examined on a tri-weekly basis 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 [19] and crustaceans (Oyster and shrimps) [20,21] which revealed quality loss during storage both at ambient temperature and chilling.

There were significant differences ($P \leq 0.05$) in the overall acceptability of fish samples during the sampling period. All fish samples studied had a below average score at the end of six (6) weeks of storage, suggesting that they were not organoleptically acceptable.

3.2 Moisture Content

The moisture content of fish samples during storage are presented in Table 2. The moisture content ranged from 19 - 25.8 % and decreased sharply after smoking. This decrease was due to loss of water during smoking [22]. 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 *Chrysichthys walkeri* increased during storage. Hence, it may be assumed that *Chrysichthys walkeri* absorbed moisture during storage whereas *L. agennes* and *M. cephalus* lost moisture as a result of evaporation due to storage conditions to some extent because the fish were kept in open laboratory benches unprotected [23,24]. However, no significant ($P \geq 0.05$) difference was observed in moisture content with storage time in all fish species.

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

Fish species	Storage time (weeks)	Parameters					
		Colour	Fragmentation	Odour	Texture	Taste	Overall acceptability
L. agennes	After smoking	8.0[a]	6.6[a]	6.6[a]	7.2[a]	8.0[a]	7.28[a]
	3	6.4[b]	6.0[a]	5.6[ab]	6.6[a]	6.6[ab]	6.24[ab]
	6	4.6[c]	4.4[b]	5.4[b]	5.2[b]	6.2[b]	5.16[b]
M. cephalus	After smoking	9.0[a]	6.8[a]	7.4[a]	7.4[a]	6.6[a]	7.44[a]
	3	6.6[b]	6.0[ab]	6.6[a]	6.8[a]	6.2[a]	6.44[a]
	6	4.8[c]	4.8[b]	3.6[b]	5.2[b]	5.8[a]	4.84[b]
C. walker	After smoking	6.4[a]	8.0[a]	7.8[a]	7.4[a]	8.0[a]	7.52[a]
	3	5.2[a]	5.4[b]	5.2[b]	6.8[ab]	6.6[b]	5.96[b]
	6	3.0[b]	4.8[b]	4.2[b]	5.8[b]	6.6[b]	4.88[b]

[*]Storage time and Parameters; [*]Mean of duplicates values

[*]Different letters in the same column for each fish species indicate significant difference (P≤0.05)

Table 2. Moisture content in the three fish samples during storage

Fish species	Fresh fish samples	Smoke-dried fish samples/ Storage time (weeks)		
		After smoking	3	6
Lutjanus agennes	71.7	22[a]	25.8[a]	24[a]
Mugil cephalus	78.4	21[a]	24[a]	23[a]
Chrysichthys walker	77.7	19[a]	25.3[a]	25.8[a]

[*]Mean of duplicates values

[*]Different letters in the row indicate significant difference (P≤0.05)

3.3 Bacterial Population of Fish Samples

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 (8.0×10^5 to 3.9×10^6) cfu/g, (3.8×10^5 to 1.4×10^6) cfu/g and (2.6×10^5 to 2.4×10^6) cfu/g respectively. 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.

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 fluctuations in bacterial counts 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 [25]. 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 [26]. There were significant differences ($P \leq 0.05$) in the three anatomical parts among the fish species during storage. The highest mean bacterial count of 3.9×10^6, 1.4×10^6 and 2.4×10^6cfu/g recorded in the gills, muscle and skin of *Chrysichthys walkeri* after six weeks of storage respectively, suggest that bacterial fish spoilage may have commenced at the point of higher bacterial counts.

Table 3. Bacteria count in cfu/g x 10^4 for gills, muscle and skin of three fish species during storage

Fish parts	Fish species	Storage time (weeks)		
		After smoking	3	6
Gills	*Lutjanus agennes*	90^a	100^a	80^b
	Mugil cephalus	108^b	184^a	138^b
	Chrysichthys walkeri	160^c	192^b	392^a
Muscle	*Lutjanus agennes*	38^c	74^a	56^b
	Mugil cephalus	58^c	84^b	100^a
	Chrysichthys walkeri	60^c	80^b	144^a
Skin	*Lutjanus agennes*	78^b	160^a	26^c
	Mugil cephalus	70^c	104^a	90^b
	Chrysichthys walkeri	64^c	156^b	240^a

** Mean of duplicates values*

** Different letters in the row indicate significant difference (P≤0.05)*

3.4 Bacterial Flora of Fish Samples

All the fish samples examined in this study contained bacterial isolates of diverse spp. Fig 1 shows the comparative frequency of occurrence of bacteria isolates from smoke-dried fish samples during storage. In smoke-dried *Lutjanus agennes*, the most frequently isolated bacteria was *Pseudomonas sp* 8(27.6%) followed by *Proteus sp.* 7(24.1%), *Micrococcus sp.* 5(17.2%), *Bacillus sp* 3(10.3%), *Staphylococcus epidermidis* 2(6.9%), *Escherichia coli* 2(6.9%), *Staphylococcus aureus* and *Streptococcus sp* with 1(3.5%) respectively. In smoke-dried *Mugil cephalus,* the most frequently isolated bacteria was *Proteus sp.* 9(34.6%) followed by *Pseudomonas sp* 8(30.8%), *Micrococcus sp.* 4(15.4%), *Bacillus sp* 3 (11.5%) and *Staphylococcus aureus* 2(7.7%) respectively. Whereas in smoke-dried *Chrysichthys walkeri,* the most frequently isolated bacteria was *Proteus sp.* 9(32.1%), followed by *Micrococcus sp.* and *Pseudomonas sp* with 6(21.4%), *Staphylococcus aureus* 3(10.7%), *Bacillus sp* and *Escherichia coli* with 2(7.1%) respectively.

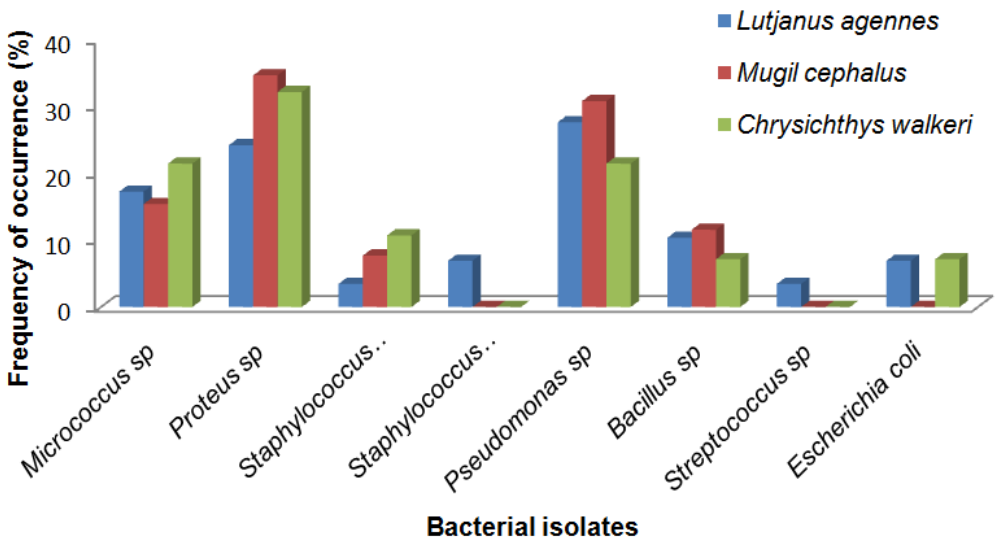

Fig. 1. Comparative frequency of occurrence of bacterial isolates from three smoke-dried fish samples during storage

The predominant bacterial isolates from the three different anatomical parts of the three fish samples during the period of storage was made up of seven (7) genera of bacteria *Proteus* (30.1%), *Pseudomonas* (26.5%), *Micrococcus sp* (18.1%) *Bacillus* (9.6%), *Staphylococcus* (9.6%), *Escherichia coli* (4.8%) and *Streptococcus sp* (1.2%).

The highest frequency of occurrence of isolates from the fish samples was obtained from *Lutjanus agennes* which constituted 34.9% while *Chrysichthys walkeri* and *Mugil cephalus* had 33.7% and 31.3% respectively. The pathogens isolated in this present study are similar to the microorganisms reported by [11] who reported the presence of *Proteus, Micrococcus, Staphylococcus aureus, Bacillus,* among other organisms.

The occurrence of *Proteus sp* may also be due to contamination of soil and water [27]. *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 [28]. The occurrence of *Bacillus* sp. can be said to be as a result of prevalence of their spores in the environment most especially in the soil [29,30]. *Bacillus* species are spore formers whose spores could survive high temperatures of fish processing. *Bacillus sp* causes a toxin-medicated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting [29,31,32]. The infectious dose has been estimated to be 10^5/g [29,33]. *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 [31].

An organism like *Escherichia coli* in *L. agennes* and *C. walkeri* 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 [34,35] stated that they are able to grow in concentrations of sodium chloride up to 15%. The presence of *Staphylococcus aureus* in the three fish samples according to [36] might have been through handling as it is a normal flora of the skin. 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.

4. CONCLUSION

The study has shown that by the sixth week of storage all tested fish had an overall mean score of acceptability less than 6 (i.e. fair).Therefore, the fish were marginally acceptable based sensory and microbiological evaluation. This is an implication that could pose a serious health concern for consumers.

COMPETING INTEREST

The authors have declared that no competing interests exist.

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