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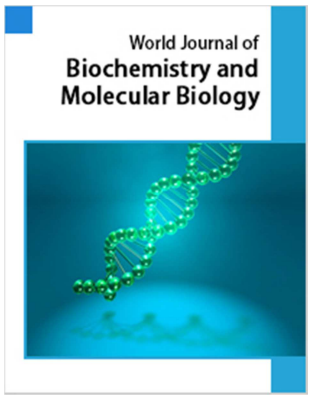

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An Investigation of Nutritional Quality Changes and Microbial Safety of Preserved-Salted Fish of the Benue River

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Abstract

Fresh *Oreochromis niloticus* and *Clarias gariepinus*, obtained from Wadata market, and preserved by sun, oven-baked and smoking to constitute home-preserved samples and those already preserved by sun-drying and smoking obtained from the same market were studied. Microbial analysis to check for viable heterotrophic bacteria was carried out on all the samples. Mean results revealed appreciable levels of contamination in all the samples. Market, sun-dried *C. gariepinus* and *O. nilticus*, had the highest mould counts with mean values of of 2.2×10^4 cfu/g and 2.1×10^4 cfu/g, respectively. Home-smoked *C. gariepinus* and home-baked *O. niloticus* had the least mould count with mean values of 1.1×10^2 and 1.2×10^2 cfu/g respectively. Market sun-dried *C. gariepinus* had the highest bacterial count of 8.9×10^4 cfu/g, and market-smoked *C. gariepinus* 6.8×10^4 cfu/g. Market, sun-dried and smoked *O. niloticus* had counts of 3.6×10^4 and 4.9×10^4 respectively. The least bacteria count was recorded in the home-baked *O. niloticus* and home-smoked *C. gariepinius*, with mean values of 1.2×10^2 and 1.3×10^2 cfu/g respectively. The predominant bacteria isolated were *Staphylococcus aureus* and *Escherichia coli*. Proximal analytical consideration showed that market-smoked *C. gariepinus* and market, sun-dried *C. gariepinus*, had the highest percent moisture, with mean values of 19.40 ± 0.020 and 16.39 ± 0.021, respectively, while home-baked *O. niloticus* and home-smoked *C. gariepinus* had the least moisture content of 4.41 ± 0.025 and 5.41 ± 0.031 respectively. Values for crude protein contents range from (39.00 ± 0.015 – 50.20 ± 0.015 and 42.20 ± 0.020 – 63.70 ± 0.021), lipid (4.54 ± 0.015 – 8.55 ± 0.015 and 5.62 ± 0.021 – 12.40 ± 0.015), ash (5.45 ± 0.025 – 6.42 ± 0.015 and 6.22 ± 0.020 – 10.40 ± 0.015) and crude fibre (1.17 ± 0.020 – 5.42 ± 0.010 and 2.56 ± 0.005 – 8.18 ± 0.005) for market and home samples respectively. This shows that home samples had higher mean nutritional content than market-preserved samples. The results in general show that fish, preserved and sold in the market, are of lower nutritional quality and have higher very significant microbial loads that could be potential deleterious to the health of consumers.

1. Introduction

Fish is one of the most important sources of animal protein and has been widely

accepted as a good protein source that contains other elements, such as vitamins and minerals for the maintenance of a healthy body [1]. Fish, have a rich source of essential nutrients required for supplementing both infant and adult diets [2]. Fish protein is relatively cheaper and richer in lysine and other sulphur amino acids compared to other livestock protein, and thus, suitable for complementing high carbohydrate diets [2]. The national demand of fish resource is put at over 2.6 million metric tonnes, with whole sale value of more than 1.5 billion, while the local production has been estimated to about 700 000 metric tonnes [3]. Nigeria is the largest fish consumer in Africa, with a total consumption of 1.2 million metric tonnes [3].

Fish is a very important source of animal protein in the diets of man. Smoked or dried fish is a traditional part of the diet of a large section of the world's population. However, the gap between the demand and supply of fish is widening due to increase in population, poor postharvest handling, lack of processing and storage facilities and utilization of unconventional fish species [4]. Spoilage is a metabolic process that causes food to be undesirable or unacceptable for human consumption due to changes in sensory and nutritional characteristics. Fish is highly nutritious and easily digestible. It is much sought after by a broad cross-section of the world, particularly in developing countries. Fish is considered a healthier source of protein than beef, chicken, pig and a host of other animal protein sources (National nutrient database for standard reference, September 2011). Fish is rich in beneficial unsaturated fats (Poly Unsaturated Fatty Acids-PUFAs), particularly omega-3 fatty acids, which help protect humans against diabetes, atherosclerosis and other cardiovascular diseases [5]. However, unsaturated fats are readily susceptible to lipid peroxidation. Peroxidation of lipids can instigate loss of nutritional and quality attributes of foods [6] and could predispose consumers to various diseases such as cancer and atherosclerosis [7].

Fish is an extremely perishable commodity and quality loss can occur very rapidly after catch.

To prevent economic losses, the processing and preservation of fresh fish is of utmost importance since fish is highly susceptible to deterioration immediately after harvest. Due to the susceptibility of fresh fish to spoilage, various processing techniques are employed to curb deterioration, add value and maintain product quality. Some of the processing techniques are smoking, chilling, freezing, salting, canning and drying [8]. Among these processing methods, smoking is the most common in many developing countries [9]. Smoking enhances fish flavour, increases utilization, reduces waste, prolongs the shelf life of fish and increases protein availability [10, 11]. Currently, the demand for traceability of food quality and food safety for consumers is increasing [12]. The quality of fish and fish products is an important concern of the industry and consumers. Deterioration of fish products mainly occurs as a result of bacteriological activity and chemical changes during processing and storage.

[13] Gave three main fish processing methods as salting, sun-drying and smoking. Smoking is the removal of most of the moisture content from the fish by wood smoke and the deposition of preservative chemicals from the smoke on the fish flesh. Smoking involves heat application to remove water and inhibit bacterial and enzymatic actions of fish [8, 14]. Smoke-drying, other than lowering the pH of food, also lowers the amount of amino-nitrogen lysine and free sulfhydryl groups. The heat and dryness associated with hot smoking reduces the water activity of the fish thereby limiting growth of microorganisms, a prerequisite for spoilage [14] Drying or dehydration is a term used to describe the removal of water from fish or fish product by evaporation. This is the oldest method of fish preservation known to man. Fish drying in the open sun is usually accomplished by exposing the fish to the open sun and atmospheric air to evaporate the moisture [15]. Considerable losses can occur during open sun drying due to contamination by insects and microorganisms. Also, the quality of the fish is lowered significantly, due to over drying, insufficient drying, pecking on the produce by animals and birds, and contamination by foreign materials as well as discolouration by ultraviolet radiation. Weather changes can drastically affect the quality of the dried produce to such an extent that fungal and bacterial growth can cause decay producing unwanted and very often, offensive odour. Moreover, open sun-dried fish products do not satisfy the nutritional quality standards [16].

Salting is one of the oldest treatments in food preservation. Salted fish products have been shown to be safe for millennia, even in developed countries. It consists of transporting salt into fish structures and consequently, decreasing water activity. It is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes [17].

Fish spoilage in Nigeria is influenced to a large extent by high ambient temperatures, considerable distances of landing ports to points of utilization, poor, as well as inadequate infrastructure for post-harvest processing and landing [18]. Major quality control issues encountered in dried, salted- fish are the variable but often low quality of the final product, high salt content, insect infestation and microbial contamination which induce a rapid rate of deterioration during transport, distribution and storage [18].

The Benue River is known to house several fish species such as *Clarias gariepinus, Oreochromis niloticus, Synodontis budgetti, Tilapia zilli, Tilapia mariae, Clarias lazera,* etc. The preservation methods of smoking, sun drying and salting of fish are all practiced by the fishermen/fishmongers- majority of who rural dwellers, illiterates and poor, along the Benue River. The government provides little or no support in terms of infrastructural provisions, etc. Preservation of the fish catches are done in very unhygienic conditions, leading to microbial contamination and general nutritional deterioration and loss. The present study seeks to investigate and compare the

nutritional quality changes and microbial safety of fishes (*Clarias gariepinus* and *Oreochromis niloticus*) preserved for commercial purposes and those preserved at home for immediate consumption.

2. Materials and Methods

2.1. Sample Collection

Wild fish samples were bought in two phases: fresh fish and dried fish samples.

2.2. Fresh Sample Collection

Here, fresh samples of tilapia fish (*O. niloticus*) and cat fish (*C. gariepinus*) were purchased early in the morning from the fishermen of Wadata area in Makurdi Local Government Area (LGA) of Benue State, Nigeria. 30 fishes, 15 of each species; with *O. niloticus,* weighing about 80-120 g and *C. gariepinus,* weighing about 120-350 g. The samples were handpicked with sterilized gloved hands and taken to the laboratory in separate sterilized polythene bags to avoid contamination from the handling.

2.3. Dry Sample Collection

Samples of *O. niloticus* and *C. gariepinus* preserved by smoking (most probably by traditional kiln) were purchased early in the morning from Wadata market. 10 fishes, 5 of each species, with *O. niloticus* weighing about 40-120 g and *C. gariepinus* weighing about 100-350 g. Each fish species was obtained from two different sellers in the same market and then mixed together to make a composite of twenty samples. 10 Sun-dried samples, 5 of each species were obtained from the sandy river banks, where they were dried under intense sun rays. The samples were handpicked with sterilized gloved hands and taken to the laboratory in separate sterilized polythene bags to avoid contamination from the handling.

3. Sample Treatment

All the fresh fish samples were thoroughly washed with tap water and treated thus;

3.1. Treatment for Sun Drying

The washed, fresh fishes were first de-scaled, and then eviscerated and opened up to create more surface area for drying. Salt was sprinkled on the samples and placed in a customized sun-dryer, which was placed on an elevated surface for maximum harnessing of sunlight. The customized sun-dryer was constructed with stainless steel material (non-rust metal) and a net of 1.0 mm. It was square shaped, bound by 4 non-rust rods, surrounded by the netting, with a stainless steel perforated tray at the bottom, to give room for water drainage and the non-rust metals serving as conductors of heat.

3.2. Treatment for Smoking

The washed, fresh fish were de-scaled and then eviscerated as above, turned on a sterile stick, salted and placed in a new wire mesh smoker. The smoker was placed on a slightly elevated circle with smoking woods beneath.

3.3. Treatment for Baking

The scales were first removed from the washed fresh samples and the samples eviscerated, salted, and placed in aluminium foil, which were placed in the oven and allowed to bake at 150°C for 2 h.

4. Analyses

The results were analysed statistically by Analysis of Variance (ANOVA) at 95% level of confidence, and Least Significant Difference (LSD).

4.1. Sterilization of Materials and Inoculation of Plates

All the glass wares used were washed, dried and sterilized in a hot air oven at a temperature of 160°C for 1 h according to the method described by [19]. Culture media were prepared and sterilized in an autoclave at a temperature of 121°C for 15 min according to the manufacturer's instruction. Plates of nutrient agar were inoculated with 0.1 ml of the diluted solution (10^{-2} - 10^{-5}) using glass spreader technique. All plates were incubated at a temperature of 37°C for 24 h before colony enumeration and isolation.

4.2. Sample Preparation and Serial Dilution Technique

Sample preparation was made using the method described by [20]. Samples of the eviscerated fish were crushed into small pieces in a sterile mortar and about 10 ml sterile water added. From the crushed samples, 1 ml was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving 1:10 dilutions.

9 ml of sterile water were measured aseptically into 5 tubes and 1 ml of the original crushed fish sample was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. This procedure continued until the fifth test tube. The crushed sample was therefore diluted from 10^{-1} to 10^{-5} for each fish sample.

4.3. Calculation of Bacteria Counts

The method described by [21] for estimating bacteria counts was used to enumerate the total viable counts of the isolates. Countable plates showing colonies were selected and counted. The mean colony count on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (cfu/g) (Equation 1).

$$CFU = \frac{number\ of\ colonies \times dilution\ factor}{volume\ plated\ in\ ml}$$

Where;

$$Dilution\ factor = \frac{volume\ of\ sample}{total\ volume}$$

cfu/g is therefore calculated thus;

$$cfu\ /\ g = \frac{colonies}{ml} \times dilution\ factor \div (gram\ of\ tissue)/(ml\ of\ original\ homogenate) \quad (1)$$

4.4. Biochemical Test

The isolates were identified using morphological and biochemical characteristics described by [22] which includes, Catalase test, coagulase test, urease production, hydrogen sulphide production etc.

4.5. Mould Count

A mortar and pestle was sterilized with 70% ethanol. One gram each of the sample was ground using the mortar and pestle. Serial dilution was done using 9 ml distilled water. The media used was potato dextrose agar which was acidified with 10% tartaric acid to a pH of 3.5 immediately before use. It was then poured into petri dishes and allowed to solidify. The inoculated agar was then incubated for 72 h on a laboratory bench at 25°C after which the colonies were counted.

5. Proximate Analysis

5.1. Determination of Moisture Content

The moisture content was determined using the hot air oven method [23]. Moisture was determined by the reduction in weight when the sample was dried to a constant weight in an oven. The dish with a loose fitting lid was weighed (after drying at 100°C and cooled). 2 g of the sample was transferred into the dish with the lid replaced. The dish and content was weighed and placed in the oven, which was thermostatically controlled at 100°C. The lid was removed to effect drying and the sample allowed to remain in the oven until it was dried to constant weight. The dish was removed from the oven, the lid replaced and transferred to a desiccator for cooling before re-weighing. The dish was allowed to dry for another 1 h, cooled and weighed to ensure that constant weight was achieved. The moisture content was determined as using Equation 2.

$$\%\ Moisture = \frac{Difference\ in\ weight\ before\ and\ after\ drying}{Weight\ of\ sample} \times 100 \quad (2)$$

5.2. Determination of Crude Ash

Ash is the inorganic residue obtained by burning off the organic matter of the samples at 400 – 600°C in a muffle furnace for 4 h. The silica dishes were ignited in the furnace at 600°C for 15 min, cooled in a desiccator and weighed. 2 g of the sample was placed into the pre-heated dishes and weighed. The dishes were placed on a hot plate under a fume cupboard and the temperature was gradually increased until the smoking ceased and samples became thoroughly charred. With the use of tongs, the dishes were placed inside the muffle furnace and ashed at 550°C. This temperature was maintained until a whitish-grey ash remained (between 16-24 h depending on sample peculiarity). The clean whitish ash showed the absence of carbon in the ash. The dish was cooled in a desiccator for 1 h at room temperature and weighed [23]. The crude ash was determined using Equation 3.

$$\%\ Ash = \frac{Weight\ of\ crucible + ash - weight\ of\ crucible}{Weight\ of\ sample} \times 100 \quad (3)$$

5.3. Determination of Crude Protein by Macro Kjedahl Method

The percentage crude protein was determined using the macro kjeldahl method where 2 g of the sample was weighed on a filter paper and both transferred into a 150 ml kjeldahl flask. 3 g of the already mixed catalyst (Na_2SO_4; $CuSO_4$; SeO_2) was added and mixed by swirling the flask. The digestion was effected with concentrated H_2SO_4 in the presence of small amounts of copper sulphate, selenium and sufficient sodium or potassium sulphate with mercury (Hg) as a metal catalyst. Under these conditions, the organic matter was oxidized and the protein nitrogen converted to ammonium sulphate $(NH_4)_2SO_4$. The digestion was followed by the addition of a strong base (NaOH) to liberate ammonia. The ammonia distilled was trapped in 0.5% boric acid indicator and titrated with 0.01 M HCl, until the green colour changed to purple [23]. Almost all organic forms of nitrogen (N) were converted to ammonia by the conditions of the digestion. The result of Kjeldahl analysis is usually expressed as crude protein. The weight of N in a sample can be converted to protein using the appropriate factor based on the percentage of N in the protein sample. To convert a gram of N to a gram of protein, the common factor 6.25 was used. The N value was therefore multiplied by 6.25 to get the weight of protein. The percentage of N was then calculated using Equation 4.

$$\%\ Nitrogen = \frac{Titre \times 0.0014 \times 100}{2(sample\ weight)} \times dilution\ factor \frac{250}{2(sample\ weight)} \quad (4)$$

From Equation 4, the percentage protein can be calculated (Equation 5).

$$\%\ Protein = \%\ Nitrogen \times 6.25 \quad (5)$$

(6.25 is the protein-nitrogen conversion factor for fish and fish by-products).

5.4. Determination of Crude Fat

The percentage fat content was determined using Soxhlet extraction method [23]. Soxhlet apparatus is the equipment used for the determination of ether extract. About 150 ml of

anhydrous diethyl ether (petroleum ether) (boiling point of 40°C - 60°C) was placed in the flask. 2 g of the sample was weighed into a thimble of known weight and the thimble plugged with cotton wool. The thimble with content was placed into the extractor; a reflex condenser (Davis double surface condenser), was connected to the extractor. Extraction was then carried out under reflux on the heating mantle for 6 h. The thimble was removed and most of the solvent from the flask was distilled into the extractor, recovering each fraction. The ether in the flask was then heated. As the ether vapour reached the condenser through the side arm of the extractor, it condensed to liquid form and dropped back into the sample in the thimble; the other soluble substances were dissolved and carried into solution through the siphon tube back into the flask. The thimble was removed and most of the solvent was distilled from the flask into the extractor. After extraction, the solvent and residual water content in the thimble was evaporated by drying in the oven for 2 h at 100°C after which they were removed, cooled in desiccators and weighed. The percentage fat was determined using the Equation 6 below;

$$\%\ Oil/fat = \frac{Weight\ of\ extracted\ oil}{Weight\ of\ sample} \times 100 \qquad (6)$$

5.5. Determination of Crude Fiber by Trichloroacetic Acid

The organic residue left after sequential extraction of sample with ether was used to determine the crude fibre of the dry samples. However, for the fresh samples, the fat in them was extracted by adding petroleum ether, stirred and allowed to settle and was afterwards decanted. This was done three times. The fat-free material was then transferred into a flask and 200 ml of pre-heated 1.25% H_2SO_4 was added, and the solution gently boiled for about 30 min, maintaining constant volume of acid by the addition of hot water. The Buckner flask funnel fitted with Whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under sufficient suction. The residue was then washed several times with boiling water (until the residue was neutral to litmus paper) and transferred back into the beaker. 200 ml of pre-heated 1.25% Na_2SO_4 was then added and boiled for another 30 min, filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65°C for about 24 h and weighed; and then transferred into a crucible and placed in muffle furnace (400-600°C) and ashed for 4 h. It was then allowed to cool in a desiccator and weighed. The crude fibre was determined using the equation 7 below.

$$\%\ Crude\ fiber = \frac{weight\ of\ insoluble\ matter(before\ ashing)}{weight\ of\ sample} - \frac{weight\ of\ residue(after\ ashing)}{weight\ of\ sample} \times 100 \qquad (7)$$

6. Results

6.1. Microbial Identification and Enumeration

A total of 5 different bacteria and 2 moulds were isolated from the different fish samples, with varying degree of concentrations, as shown in Table 1. The bacteria identified included; *Escherichia coli, Staphylococcus aureus, Bacillus spp, Salmonella spp, and Streptococcus spp*. While the moulds included were; *Aspergillus flavus and Aspergillus niger*. The major characteristics used to identify the different fungi and bacteria are shown in Tables 2 and 3 respectively.

Market, sun-dried *C. gariepinus* and *O. nilticus,* had the highest mould counts with mean values of of 2.2 x 10^4 cfu/g and 2.1 x 10^4 cfu/g, respectively. Home-smoked *C. gariepinus* and home-baked *O. niloticus* had the least mould count with mean values of 1.1 x 10^2 and 1.2 x 10^2 cfu/g, respectively. Fresh *O. niloticus* and *C. gariepinus* had a mean mould count of 1.1 x 10^3 and 1.0 x 10^3 respectively. Market sun-dried *C. gariepinus* had the highest bacterial count of 8.9 x 10^4 cfu/g, with fresh *C. gariepinus* and market- smoked *C. gariepinus* coming second, with an identical value of 6.8 x 10^4 cfu/g. Market, sun-dried and smoked *O. niloticus* had counts of 3.6 x 10^4 and 4.9 x 10^4 respectively. The differences in the mean values in both the bacteria and mould counts were statistically significant ($p < 0.05$) using the one-way ANOVA.

Table 1. *Bacteria count and isolates identified from the samples.*

Samples	Bacteria count (cfu/g)*	Bacteria	Fungal count (cfu/g)*	Fungi
1^A	3.6 x $10^4 \pm 0.001$	*E. coli, S. aureus,*	1.9 x $10^4 \pm 0.002$	*A. flavus, A. niger*
1^B	8.9 x $10^4 \pm 0.000$	*S. aureus, E. coli, Bacillus spp*	2.1 x $10^4 \pm 0.001$	*A. flavus, A. niger*
2^A	4.9 x $10^4 \pm 0.001$	*Salmonella spp, S. aureus,*	1.7 x $10^4 \pm 0.001$	*A. flavus, A. niger*
2^B	6.8 x $10^4 \pm 0.100$	*S. aureus, Streptococcus spp.*	2.1 x $10^4 \pm 0.000$	*A. flavus, A. niger*
3^A	3.5 x $10^2 \pm 0.100$	*S. aureus, E. coli*	2.4 x $10^2 \pm 0.100$	*A. niger*
3^B	3.8 x $10^2 \pm 0.100$	*S. aureus, E. coli*	2.8 x $10^2 \pm 0.100$	*A. niger*
4^A	2.8 x $10^2 \pm 0.100$	*S. aureus*	2.8 x $10^2 \pm 0.280$	*A. niger*
4^B	1.3 x $10^2 \pm 0.100$	*S. aureus*	1.1 x $10^2 \pm 0.110$	*A. niger*
5^A	1.2 x $10^2 \pm 0.010$	*S. aureus*	1.2 x $10^2 \pm 0.100$	*A. niger*
5^B	1.5 x $10^2 \pm 0.020$	*S. aureus, Streptococcus spp,*	1.4 x $10^2 \pm 0.020$	*A. niger*
6^A	6.3 x $10^4 \pm 0.000$	*S. aureus, E. coli*	1.1 x $10^3 \pm 0.000$	*A. niger*
6^B	6.8 x $10^4 \pm 0.100$	*S. aureus, E. coli*	1.0 x $10^3 \pm 0.000$	*A. niger*

*Mean counts ± S.D. Key; 1^A: Market, sun- dried *O. niloticus;* 1^B: Market, sun-dried *C. gariepinus;* 2^A: Market- smoked *O. niloticus;* 2^B: Market- smoked *C. gariepinus;* 3^A: Home, sun- dried *O. niloticus;* 3^B: Home, sun- dried *C. gariepinus;* 4^A: Home-smoked *O. niloticus;* 4^B: Home-smoked *C. gariepinus;* 5^A: Home-baked *O. niloticus;* 5^B: Home-baked *C. gariepinus;* 6^A: Fresh *O. niloticus;* 6^B: Fresh *C. gariepinus*

Table 2. *Biochemical reactions and inoculation results of the different fungal isolates.*

Isolates	PDA	LAC	GLU	SUC	FRUC	CAT
Aspergillus flavus	Black	+	+	-	+	-
Aspergillus niger	White	+	+	+	-	-

Key: + = positive; - = negative; PDA = Potatoes Dextrose Agar; Lac = lactose; Glu = glucose, SUC = sucrose; FRUC = Fructose, CAT = catalase.

Table 3. *Morphological, Grams reaction and Biochemical Characteristics of bacteria isolates.*

Isolates	Colony Morphology on NA		Mac conkey	SSA	EMBA	MSA	Shape
	Colour	Elevation					
A	Cream	Flat	LF	Nil	Blue black	Nil	Rods
B	Golden yellow	Convex	Pink colonies	Nil	Nil	Yellow	Clusters
C	Gray	Flat	Pale colonies	Colourless with black spots	Nil	Nil	Rods
D	Creamy	Flat	Pale	Nil	Nil	Nil	Cocci in chains

Table 3. *Continue.*

Isolates	Gram reaction	Catalase test	Indole production	Coagulase test	Motility	Gas	Suspected organism
A	-	-	+	-	+	+	*E. coli*
B	+	+	-	+	-	ND	*S. aureus*
C	-	+	-	ND	+	-	*Salmonella spp*
D	+	-	-	-	-	ND	*Streptococcus spp*

Key: NA = Nutrient Agar; SSA = salmonella Shigella Agar; EMBA = Eosin Methylene Agar; MSA = Manitol Salt Agar; + = Positive; - = Negative ND = Not Determined; LF = Lactose Fermenter

6.2. Proximate Fractions

Table 4 shows the results of the proximate fractions evaluations conducted on the fish samples. The values represent the mean of three replications ± S.D.

6.3. Moisture Content

The mean moisture content of each of the samples indicated that fresh *O. niloticus and C. gariepinus* had the highest percent moisture, with mean values of 70.36 ± 0.025 and 67.20 ± 0.015, respectively. Market-smoked *C. gariepinus* and market, sun-dried *C.* gariepinus, had the highest percent moisture, with mean values of 19.40 ± 0.020 and 16.39 ± 0.021, respectively. Home-baked *O. niloticus* and home-smoked *C. gariepinus* had the least moisture content of 4.41 ± 0.025 and 5.41 ± 0.031 respectively. Statistical analyses of the moisture contents of the samples using a one-way ANOVA ($p < 0.05$) showed that there was significant differences between the sample means as $p = 0.0$.

Table 4. *Proximate fractions of the samples.**

Samples	Moisture	Crude protein	Lipid	Ash	Crude fibre
1^A	15.49 ± 0.015	47.40 ± 0.020	5.73 ± 0.015	6.15 ± 0.015	1.17 ± 0.020
1^B	16.39 ± 0.021	39.00 ± 0.015	4.54 ± 0.015	6.42 ± 0.015	5.14 ± 0.015
2^A	16.29 ± 0.020	50.20 ± 0.015	8.55 ± 0.015	6.38 ± 0.026	2.61 ± 0.292
2^B	19.40 ± 0.020	49.20 ± 0.020	7.11 ± 0.010	5.45 ± 0.025	5.42 ± 0.010
3^A	8.45 ± 0.015	49.20 ± 0.010	7.55 ± 0.010	8.84 ± 0.021	2.56 ± 0.005
3^B	10.40 ± 0.015	42.20 ± 0.020	5.62 ± 0.021	10.40 ± 0.015	7.55 ± 0.035
4^A	6.30 ± 0.020	63.70 ± 0.021	12.40 ± 0.015	7.15 ± 0.015	5.54 ± 0.020
4^B	5.41 ± 0.031	56.30 ± 0.015	9.58 ± 0.025	6.22 ± 0.020	6.44 ± 0.015
5^A	4.41 ± 0.025	60.90 ± 0.015	11.40 ± 0.015	7.86 ± 0.245	8.18 ± 0.005
5^B	14.98 ± 0.015	52.00 ± 0.015	9.05 ± 0.015	9.53 ± 0.020	7.77 ± 0.015
6^A	70.36 ± 0.025	18.80 ± 0.021	4.54 ± 0.015	3.34 ± 0.020	1.14 ± 0.015
6^B	67.20 ± 0.015	19.60 ± 0.015	6.42 ± 0.020	2.91 ± 0.010	3.16 ± 0.015

*Percentage mean values of replicate readings ± S.D ($P < 0.05$).
Key; 1^A: Market sun- dried *O. niloticus*; 1^B: Market sun-dried *C. gariepinus*; 2^A: Market- smoked *O. niloticus*; 2^B: Market- smoked *C. gariepinus*; 3^A: Home, sun- dried *O. niloticus*; 3^B: Home, sun- dried *C. gariepinus*; 4^A: Home- smoked *O. niloticus*; Sample 4^B: Home- smoked *C. gariepinus*; 5^A: Home-baked *O. niloticus*; 5^B: Home-baked *C. gariepinus*; 6^A: Fresh *O. niloticus*; 6^B: Fresh *C. gariepinus*

6.4. Crude Protein

From Table 4, the percent mean crude protein content of each of the samples showed that home- smoked *O. niloticus and* home-baked *O. niloticus* had the highest percent crude protein, with mean values of 63.70 ± 0.021 and 60.90 ± 0.015 respectively. Fresh *O. niloticus* and *C. gariepinus* had the least crude protein content with means of 18.80 ± 0.021 and 19.60 ± 0.015 respectively while market, sun-dried *C. gariepinus* and home, sun-dried *C. gariepinus,* had the least with means of 39.00 ± 0.015 and 42.20 ± 0.020 respectively. Statistical evaluation using one-way ANOVA ($p < 0.05$) revealed significant differences between the means as $p = 0.0012$.

6.5. Lipid Content

Table 4 also presents the percent lipid content of each of the samples. Home-smoked and home-baked *O. niloticus* had the highest percent lipid with a mean of 12.40 ± 0.015 and 11.40 ± 0.015 respectively. Fresh *O. niloticus* and market, sun-dried *C. gariepinus* had the least lipid content, with means of 4.54 ± 0.015 each. Statistical evaluation using one-way ANOVA ($p < 0.05$) revealed significant differences between the means as $p = 0.0255$

6.6. Ash Content

The percent mean ash content of the samples can also be seen in Table 4. Home, sun-dried and home-baked *C. gariepinus* had the highest ash content with means of 10.40 ± 0.015 and 9.53 ± 0.020 respectively. Fresh *C. gariepinus* and *O. niloticus* had the least ash content with means of 2.91 ± 0.010 and 3.34 ± 0.020 respectively. Amongst the preserved samples, market-smoked *C. gariepinus* and market, sun-dried *O. niloticus*, had the least ash content, with a mean of 5.45 ± 0.025 and 6.15 ± 0.015 respectively. Statistical analyses using one-way ANOVA ($p < 0.05$) showed that the sample means were significantly different as $p = 0.0016$.

6.7. Crude Fibre

Percentage crude fiber levels of the samples are also presented in Table 4. Home-baked and home, sun-dried *C. gariepinus* had the highest crude fiber content with mean values of 7.77 0.015 ± and 7.55 ± 0.035 respectively. Fresh *O. niloticus* and market, sun-dried *O. niloticus* had the least crude fiber content, with means of 1.14 0.015 ± and 1.17 ± 0.020 respectively. Furthermore, market, sun-dried and market-smoked *O. niloticus*, had the least crude fiber content, with mean values of 1.17 ± 0.015 and 2.61 ± 0.292 respectively. Statistical analyses using one-way ANOVA ($p < 0.05$) however, showed that the sample means were not significantly different as $p = 0.2185$; hence, accepting the null hypothesis.

7. Discussion

Microbial presence in fish is not new.[24] had previously reported that microbes present in fishes are normally associated with those found in their natural environment and are influenced by the season and the harvesting conditions. Market preserved samples had the highest levels of bacterial (6.3×10^4 - 6.9×10^4 cfu/g) and fungal (1.6×10^4 - 2.2×10^4 cfu/g) contamination compared to the home-prepared ones with values ranging from 1.2×10^2 - 3.8×10^2 cfu/g and 1.1×10^2 - 2.8×10^2 cfu/g respectively. Many potentially pathogenic bacteria and fungi isolated from the fish samples including, *E. coli, S. aureus, Salmonella spp, Bacillus spp, Streptococcus spp, A. flavus* and *A. niger we*re similar to those previously reported in Nigeria [25, 26, 27]. *E. coli* has been associated with symptoms of food poisoning such as acute stomach pain, severe, haemorrhagic diarrhoea, vomiting, haemolytic-uremic syndrome (characterised by renal failure and haemolytic anaemia), persistent and watery diarrhoea in infants [28]. *Salmonella* causes a wide range of human diseases, such as enteric fever, gastroenteritis, endocarditis and bacteraemia [29]. *Aspergillus spp,* has been implicated in causing mycetoma in humans [30] Similarly, *A. flavus* is involved in allergic aspergillosis (pulmonary aspergillo-sis) and also produces highly carcinogenic aflatoxins [31, 30]. The presence of *Aspergillus spp,* could be attributed to the prevalence of their spores in the atmosphere [30].

Poor handling, especially unacceptable hygienic practices during post-harvest processing of fish is largely responsible for the high prevalence of potentially pathogenic microbes in market samples. In addition to the very low level of education of the fish processors, crude transportation means and open display of fish in the market further compound the already bad situation [32, 33, 34, 35]. There is also the practise of would-be-buyers handling the processed fish with uncleaned hands and making contacts that could potentially introduce pathogens on the products even before purchasing them.

Microbial contamination also impacts negatively on the nutritional quality of fish. Although fresh fish samples had the highest moisture content, market preserved samples had higher moisture content than home-preserved samples, a factor partly responsible for the high level of microbial growth on market-preserved samples. Baking and smoking best preserve the nutritional content of *O. niloticus* and *C. gariepinus* compared to sun-drying. Expectedly, home-baked and home-smoked samples accounted for the highest crude fibre, lipid and ash contents. However, it was unclear why home-smoked samples had more lipid than home-baked samples.

Ash content is a measure of the amount of minerals present in the sample. The higher ash content found in home-smoked as opposed to home-baked samples was attributable to the deposition of potentially toxic minerals like Polycyclic aromatic hydrocarbons (PAHs) on the fish during smoking as previously reported [36, 37, 38]. Consequently, home-baked fish probably constitutes the most nutritive and safe type of preserved fish.

8. Conclusion

The level of microbial contamination is almost directly connected to the nutritional quality of the fish. Market-preserved samples had higher moisture content and unsurprisingly higher levels of microbial contamination. Consequently, they had lower levels of lower crude protein, crude fiber, ash and lipid content compared to home-preserved samples- even those preserved by same methods. The poor quality of market samples may also not be unconnected with market pressure and the quest to keep up with market demands. Preserved fish sold in the market are of poor nutritional quality and microbially-unsafe. Home-preserved fish are healthier and have better nutritional content. Oven-baked fish are the most nutritive compared to

other drying methods.

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