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Microbiological and proximate composition of smoked fish samples sold in Benin metropolis

Lucy Ubaka[1*], Barry Omogbai[2], Michael Erhayimwen[3]
[1, 2] Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria
[3] Department of Fishery, University of Benin, Benin City, Edo State, Nigeria

Abstract
Investigation into the microbiological quality, proximate and mineral content of three smoked fish species obtained from three markets in Benin City. The fish samples were identified as *Oreochromis aureus* (Blue tilapia), *Scomber scombrus* (Atlantic mackerel) and *Gymnarchus niloticus* (Trunk fish). Total heterotrophic bacteria and fungi count, coliform count and staphylococcal counts were carried out using standard microbiological procedures. The isolates were identified using both phenotypic and molecular methods. Comparative nutritional estimation of the proximate of moisture, crude protein, fibre, ether extract, dry matter, ash content and gross energy were determined using standard analytical chemical methods. Mineral composition of Fe, Mg, Zn and Ca were determined using atomic absorption spectrophotometer (AAS) while Na and K were by flame photometry. The total heterotrophic bacterial count was in the range of $6.20 \pm 0.27 \times 10^5$ to $7.33 \pm 0.58 \times 10^5$ (cfu/g) while the coliform count was in the range of $3.26 \pm 0.43 \times 10^4$ to $4.26 \pm 0.43 \times 10^4$ (cfu/g). The *Staphylococcal* count was in the range of $2.07 \pm 0.43 \times 10^4$ to $2.76 \pm 0.43 \times 10^4$ (cfu/g). The bacteria isolated were *Escherichia coli* strain NBRC 102203-54, *Serratia marcescens* strain TRL, *Staphylococcus aureus* strain RFI22, *Bacillus cereus* strain PM-2, *Morganella morganii* subsp. sibonii strain ML and *Enterobacter cloacae* PD2. Fungi isolated were *Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces cerevisiae* and *Penicillium commune.* There was a significant difference ($p<0.05$) in gross energy and crude protein levels in all the fish samples. The fish samples showed high magnesium (>59.61mg/g) and potassium (>41.5661mg/g) levels whereas the iron content was low (<7.2361mg/g).

Keywords: smoked fish, Proximate, Mineral content, microbiological quality

Introduction
Fish is an important source of protein, fats and minerals in diet (Ashano and Ajayi, 2003) [22]. Fish is an extremely perishable food immediately after catch and therefore requires immediate and proper handling and good preservation to retain its quality (Adeolu and Enesi, 2013) [8]. Fish smoking is one of the traditional processing methods aimed at preventing or reducing post-harvest losses. Heat application removes water and inhibits bacterial and enzymatic actions of fish (Jeyasanta *et al.,* 2015) [37]. The demand for fish is on the increase due to the increase in world population. It is also relatively cheap unlike the meat protein. Fish has a potential in the relief from malnutrition because of its superior protein quality unlike meat, milk and egg (Adeniji and Okedeyi, 2017) [7]. The presence of Omega -3- unsaturated fatty acids attach medicinal values to fishes in controlling conditions such as arthritis, asthma, coronary heart disease and cancer (Abolagba and Igbinevbo, 2010) [2]. Due to high amount of water and soft tissues, fishes are highly susceptible to microbial contamination and spoilage (Olayemi *et al.,* 2012). Smoking enhances flavour, texture and shelf life of fish. However in most local settings where it is applied, hygienic standards are not to with reports of smoked fishes spoiling as readily as non-smoked fishes adhered to with reports of smoked fishes spoiling as readily as non-smoked fishes (Jeyasanta, *et al.,* 2015) [37]. In preserving fish by smoking, water activity in the fish is lowered to the point where the activity of spoilage microorganisms is inhibited together with the antioxidant and bacteriostatic effects of the smoke which allows smoked products to have extended shelf-life as the wood smoke add some microbial inhibitory substances like formaldehyde and alcohols (Adeyeye *et al.,* 2015, Udochukwu *et al.,* 2016) [15, 53]. The typical smoke flavours result from a number of chemicals found in the smoke, but is mostly attributed to the phenols. Phenolic compounds, which are mainly produced by pyrolysis of lignin, are important for preservation and flavour properties of smoked products. 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) [3]. They are also excellent sources of adequate amounts of lipids, minerals, and vitamins. Fish is also a good source of riboflavin, vitamin A and D. The presence of these nutrients also aids the proliferation of microbes (Olaleye and Abegunde, 2015) [47]. In many communities in Nigeria, smoked fish are usually hawked or sold openly in the markets without taking cognizance of the microbial contamination from the environment (Akinwumi and Adegbehingbe, 2015). The present study aimed at accessing the microbiological quality, proximate and mineral composition of smoked fish samples.

Materials and Method
Sample Collection
Samples were obtained randomly from three different markets in Benin-city using Ziploc freezer bags. A total of twenty-one (21) samples of three types of selected smoked fishes were collected, code-named and identified at the Department of Fisheries, Faculty of Agricultural Sciences, University of Benin, Benin City. The fishes include; *Oreochromis aureus* (Blue tilapia), *Scomber scombrus*

(Atlantic mackerel) and *Gymnarchus niloticus* (Trunk fish). The fish samples were stored in refrigerator at 4 ^{0}C. Sample preparation and Laboratory analysis Before use, the fish samples were blended using an electric blender (Kenwood 1Litre Blender- BL335 350 Watts, China).

Preparation of Culture Media

Nutrient agar (to enumerate bacteria counts), mannitol salt agar (for culture of *Staphylococcus aureus*), MacConkey agar (for the enumeration of total coliform), deoxycolate citrate agar (for determination of *Salmonella* and *Shigella* count) and potato dextrose agar (to enumerate fungal growth). All were prepared according to the manufacturer's instruction Enumeration of Total Viable Bacterial and Fungal Count

Two grams (2 g) of each smoked fish was weighed using a sterile filter paper on a weighing balance. A five-fold serial dilution was made. That is, for each fish sample, five test tubes were used for the serial dilution. The test tubes were filled with 9 ml of distilled water. 1g of these samples were transferred into the assigned test-tube (making it 10 ml) and thoroughly mixed. Further sequential dilutions were made by taking 1 ml of 10 ml mixture to other test-tubes using a sterile pipette. The viable heterotrophic bacterial and fungal counts were done using pour plate method on nutrient agar (NA) and potato dextrose agar (PDA) media respectively. The agar plates were incubated inverted at 37 °C for 24-48 h, while the agar plates for fungi (that is, containing potato dextrose agar) were incubated inverted for 3-5 days (Odu and Imaku, 2013; Dike-Ndudim *et al.,* 2014; Abolagba and Igbinevbo, 2014) [42, 26].

Total coliform count

An aliquot of 0.1 ml of serially diluted sample of smoked fish was aseptically plated on MacConkey agar using a sterile pipette. The plates were incubated at 37 °C for 24 h. The total colonies formed were counted and subjected to further analysis.

Detection of Salmonella

Samples of smoked fish dilutions were inoculated on Salmonella-Shigella agar (Oxoid) and incubated for 24 h. For identification, five suspected colonies were inoculated into tryptone broth for indole test and urea broth. triple sugar iron agar. These were incubated at 37 ^{0}C for 24 h. Salmonella species is indole negative on triple sugar iron agar, it produces acid (yellow) colour and urea negative.

Pour plate method

A measured 0.1 ml of serially diluted sample of smoked fish was aseptically transferred to the center of sterile Petri-dishes in triplicates. These were mixed by a combination of rotational movement clockwise and anticlockwise direction for 5-10 sec. The plates were allowed on the bench to solidify, inverted and properly labeled.

Gram stain

Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95 % alcohol for 30 sec and rinsed with distilled water. The smears were then counter stained with saffranin solution for 60 sec. Finally, the slides were washed off with distilled water, air dried and observed under (x100) oil immersion objective

Morphological characteristics

Isolates were subjected to preliminary microbiological identification based principally on colony morphology, Gram reaction and various biochemical tests. Bacteria isolates were purified and stored in slants for further identification and characterization (Olaleye and Abegunde, 2015) [47].

Identification of fungal isolates

Fungal isolates were examined both macroscopically and microscopically. Macroscopic examination was done by colony shape, colour, size and growing pattern. Using a flamed inoculating wire loop, the edge of each colony was picked and slides of the different colonies were prepared using scotch tape method where transparent scotch tape was lightly pressed to colony and then the tape was fixed to slide that had a drop of lacto phenol cotton blue stain. The slides were observed under microscope in x100 and x400 magnification power and were identified (Abbas *et al.,* 2015; Melaku *et al.,* 2017) [1, 41].

Molecular identification of bacterial isolates

16S rRNA Amplification

The 16SrRNA region of the rRNA genes of the isolates was amplified using 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA). The PCR conditions was as follows: initial denaturation; 94 ^{0}C for 5min, denaturation, 950C for 30 sec; annealing, 50 °C for 60 sec; extension, 72 °C for 30 sec followed for 30 cycles and a final termination at 72 °C for 10 min and chill at 4 0C. The integrity of the amplified gene fragment was checked on a 1% agarose gel ran to confirm amplification at 120 V for 45 min. The product was visualized under an ultraviolet trans-illuminatior and photographed (Frank *et al.,* 2008) [33].

Sequencing

Sequencing was done using that of BigDye terminator v3.1 cycle sequencing kit on a Genetic Analyzer 3130xl sequencer (Applied Biosystem Inc., USA) according to the manufacturer's instruction. Bio- Edit software and MEGA 6 were used for all genetic analysis. The complete genome sequence of all the bacteria isolates have been included in the GenBank whole-Genome Shotgun (WGS) database under various NCBI accession numbers.

Proximate Analysis

The proximate composition were assayed as describe by AOAC (2001) [20]. All chemical used were of analytical grade and supplied by Sigma Co (St. Louis U.S.A). Each analysis was carried out in triplicate.

Determination of minerals

Crude protein, carbohydrate, moisture content, crude fibre, ash and fat content were determined using standard chemical analytical procedures. The mineral content of the samples was determined by the dry ash method. 2.0 g of samples was burnt to ashes in a muffle at 550 ^{0}C to constant weight and dissolving the ash in volumetric flask using

distilled, deionized water and 20 % of 10 ml HCl was added and then filtered and made up to 100 ml in a volumetric flask. The digest so obtained was used for various analyses. The solution was used in the determination of the elements using atomic absorption spectrophotometer (AAS) while the Sodium (Na) and Potassium (K) were by flame photometry using NaCl and KCl to prepare the standards (AOAC, 2001; Oladipo and Jadesimi, 2012) [20].

Statistical Analysis

Data recorded during the study period was summarized. Analysis of variance (ANOVA) was used where applicable for detection of significant differences among sample values. Statistical analyses were carried out using SPSS Version 19.0 (Ogbeibu, 2014) [44].

Results

The result of microbial status of smoked fish samples, *Scomber scombrus*, *Gymnarchus niloticus* and *Oreochromis aureus* sold in Benin metropolis, Edo State are as follows:

Table 1 shows the viable heterotrophic bacteria count of smoked fish samples from the three sources and showed no significant difference at 95% level (P<0.05). The total heterotrophic bacterial count was in the range of 6.20 ± 0.27 $x10^5$ to 7.33 ± 0.58 $x10^5$ (cfu/g).

Table 2 shows the coliform count of the fishes; *Gymnarchus niloticus, Scomber scombrus, Oreochromis aureus* from different location. All the fish samples had high coliform counts which ranged from 3.26 ± 0.43 $x10^4$ cfu/g to 4.26 ± 0.43 $x10^4$ cfu/g. *Oreochromis aureus* from Yanga market had the highest coliform count of 5.00 ± 0.21 while *Gymnarchus niloticus* had the lowest coliform count of 2.40 ± 0.06 $x10^4$ cfu/g.

Staphylococcus sp. was analyzed from the smoked fish samples as shown in Table 3 at range of 2.37 $x10^4$ cfu/g to 2.76 $x10^4$ cfu/g. There was a significant difference (P<0.05) between the scombroid fish samples from Oluku and Aduwawa markets (2.80^b ± 0.17 and 2.00^a ± 0.12) but fish samples from Yanga markets (2.30^{ab} ± 0.17) are not statistically different from each of them.

Table 4 shows the bacteria isolated from the smoked fish samples. They include; *Serratia marcescens, Escherichia coli, Staphylococcus aureus, Morganella morganii, Bacillus cereus* and *Enterobacter cloacae* while Table 5 shows the implicated fungal contaminants of smoked fish samples across the study area which include *Aspergillus niger, Aspergillus fumigatus, Fusarium oxysporum* and *Penicillium commune.*

Plate 1 shows the PCR Product of 16s rRNA gene of bacterial isolates on 1 % Agarose gel electrophoresis. Marker was graduated in 100 but deep graduations are in 500 and 1000base pairs. All the samples had approximately 1500base pairs corresponding to the level on the marker. Table 6 shows the molecular characteristics of the bacterial isolates. Gene sequencing of each sample was carried out following the gel electrophoresis to determine the percentage similarity of each organism using 16S ribosomal RNA gene where all the bacteria isolates recorded 98-99 % homology. The complete genome sequence of all the bacteria isolates have been included in the GenBank whole-Genome Shotgun (WGS) database under various NCBI accession numbers.

The result of the proximate analysis is shown on Table 7. The crude proteins are relatively high indicating that the fishes are good sources of protein. There was a significant difference (p<0.05) in gross energy and crude protein levels. The moisture content in all the samples was relatively low with no significant difference (p>0.05). Crude fibre level was low in the fish samples and was not detected at all among the *Gymnarchus niloticus* (>0.001).

Mineral composition of the fish species are shown on Table 8. There was no significant difference (p>0.05) in the minerals detected (Iron, potassium, magnesium, phosphorus and zinc) across the fish samples. The three fish samples showed relatively high magnesium and potassium content. Magnesium content for *Scomber scombrus, Gymnarchus niloticus* and *Oreochromis aureus* are 59.61±1.36 mg/g, 60.48±0.06 mg/g and 66.48±0.31 mg/g while the potassium content are 41.56±0.53 mg/g, 53.58 ±0.64 mg/g and 59.09±0.54 mg/g.

Table 1: Total viable bacterial counts ($x10^5$ cfu/g) of the fish samples

Locations	Fish Samples		
	Scomber scombrus	***Gymnarchus niloticus***	***Oreochromis aureus***
Oluku	5.20 ± 0.17^a	7.80 ± 0.17^b	7.20 ± 0.11^b
Aduwawa	6.90 ± 0.29^b	8.40 ± 0.12^c	5.40 ± 0.23^a
Yanga	6.50 ± 0.17^b	5.60 ± 0.17^a	9.40 ± 0.23^c
p-value	0.004	0.000	0.000

Key: Mean with the same superscripts down the column are not statistically significant Alpha level (α) = 0.05

Table 2: Total coliform count of ($x10^4$ cfu/g) of the fish samples

Locations	Fish Samples		
	Scomber scombrus	***Gymnarchus niloticus***	***Oreochromis aureus***
Oluku	3.30 ± 0.05^b	2.40 ± 0.06^a	4.60 ± 0.28^b
Aduwawa	2.80 ± 0.17^a	3.60 ± 0.05^b	3.20 ± 0.12^a
Yanga	4.40 ± 0.12^c	3.38 ± 0.06^c	5.00 ± 0.21^b
p-value	0.000	0.000	0.005

Key: Mean with the same superscripts down the column are not statistically significant Alpha level (α) = 0.05

Table 3: *Staphylococcal* Counts of ($x10^4$ cfu/g) of the fish samples

Locations	Fish Samples		
	Scomber scombrus	***Gymnarchus niloticus***	***Oreochromis aureus***
Oluku	2.80 ± 0.17^b	1.90 ± 0.11^a	2.10 ± 0.17^a
Aduwawa	2.00 ± 0.12^a	2.60 ± 0.23^b	3.40 ± 0.13^b
Yanga	2.30 ± 0.17^{ab}	1.70 ± 0.17^a	2.80 ± 0.23^b
p-value	0.030	0.028	0.009

Key: Mean with the same superscripts down the column are not statistically significant Alpha level (α) = 0.05

Table 4: Cultural, morphological and biochemical characteristics of bacterial isolates

Characteristics	A	B	C	D	E	F
Shape	Circular	Circular	Circular	Irregular	Flat	Circular
Colour	Creamy	Milky	White	Pale green	Milky	Milky
Margin	Entire	Entire	Entire	Entire	Rhizoid	Entire
Opacity	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat	elevated
Gram stain	-	-	-	+	-	+
Shape	Rod	rod	Rod	rod	Rod	Cocci
Arrangement	single	single	Pair	Single	Single	Cluster
Coagulase	-	-	-	-	-	+
Catalase	+	+	+	-	+	+
Oxidase	-	-	-	-	-	-
Urease	+	-	-	+	+	+
Citrate	+	-	+	+	-	+
Lactose	-	-	-	-	-	-
Sucrose	+	-	+	+	+	+
Glucose	+	-	+	+	+	+
Spore	-	-	-	+	-	-
Identity	*Serratia* spp.	*Enterobacter* spp.	*Morganella* spp.	*Bacillus* spp.	*Escherichia coli*	*Staphylococcus aureus*

Table 5: Cultural, morphological and microscopic characteristics of the implicated fungi in the study samples.

Characteristics	F1	F2	F3	F4
Morphology				
Cultural characteristics	Black, fluffy and woolly colonies with profuse growth	Green, non-luxuriant with concentric rings	White, cottony fast growing colonies	Cream, mucoid,entire edge and raised
Colour of reverse plates	Dark	Faded white	Violet	Creamy
Microscopy				
Hyphae	Septate	Septate	Septate	Non- Septate
Conidiophore	Non-septate, upright,terminating in globose swelling	Septate arise from a mycelium, singly branched near apex	Septate, short, arise from aerial mycelium with almost straight globose	Non
Conidia	Present, one-celled globose in dry basipetal chains	Present, one-celled hyaline globose bright colour produced basipetally	Present, one-celled ellipsoidal or reniform with hyaline arranged in false heads	Ellipsoid cells with buds on the sides
Rhizoid	Absent	Absent	Present	Absent
Spore colour	Dark	Green	Pinkish	Whitish
Spore Attachment	Bear phailides at the apex with conidia at the tip	Phialids which pinch off conidia in dry chains at the tips	Monophialidic conidiogenous cells.	Buds growing on the side
Identity	*Aspergillus niger*	*Penicillium commune*	*Fusarium oxysporum*	*Saccharomyces cerevisiae*

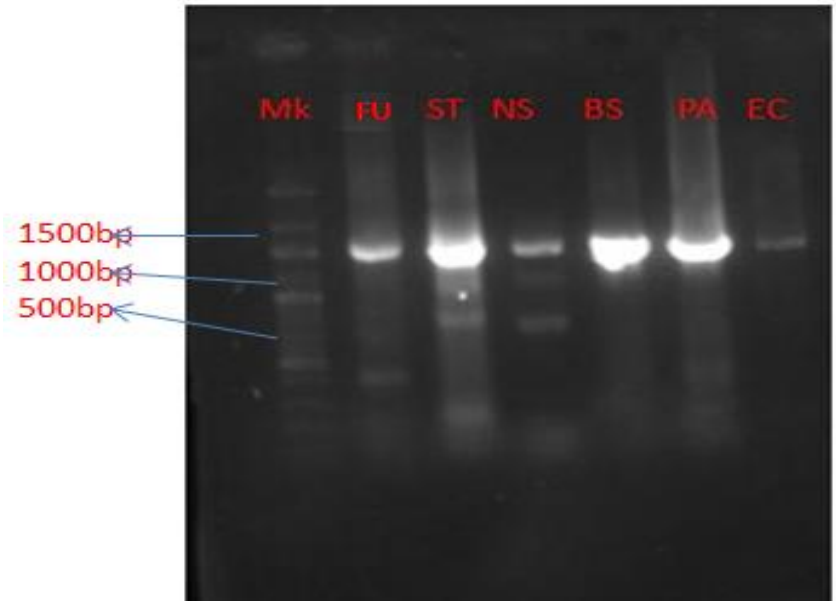

Fig 1: Gel electrophoresis of amplified 16s rRNA gene of bacterial isolates on 1% Agarose Gel Electrophoresis. Lane Mk-Molecular weight marker, (1500bp); Lane FU-*Serratia marcescens*; Lane St-*Staphylococcus aureus*; Lane Ec- *Escherichia coli*; Lane Ns- *Bacillus cereus*; Lane Pa-*Morganella morganii*; Lane Bs-*Enterobac ter cloacae*

Table 6: Molecular characterization of bacterial isolates.

Bacterial Isolates	Blast Results	% Homology	NCBI Accession Number
FU	*Serratia marcescens* strain TRL	99	KT_438849.1
NS	*Bacillus cereus* strain PM-2	98	NR_023154.1
EC	*Escherichia coli* strain NBRC 102203-54	99	HM_576813.1
BS	*Enterobacter cloacae* strain PD2	99	CP_003739.1
ST	*Staphylococcus aureus* subsp. aureus strain RF122	98	NC_007628.1
PA	*Morganella morganii* subsp. sibonii strain ML	99	KR_150991.1

Table 7: Proximate analysis of the smoked fish samples Proximate

Fish Samples	MC	CF	CP	ASH	EE	GE	DM
Scomber scombrus	31.13±1.2^a	0.11±0.03^a	50.13±0.59^a	10.00±1.1^a	7.57±0.23^a	3576.03 ±47.92^a	68.87± 1.25^a
Gymnarchus niloticus	36.50±2.2^a	>0.001^c	44.56±0.83^b	12.00±1.0^a	7.17±0.12^a	3229.67 ±37.97^b	63.50± 2.25^a
Oreochromis aureus	31.80±0.6^a	0.04±0.0^b	50.18±0.51^a	19.70±0.4^b	6.80±0.47^a	3506.26 ± 73.97^a	68.20± 0.65^a
P-value	0.093	----	0.001	0.233	0.294	0.010	0.093

Values are means of three replicates ±SD

Key: MC: Moisture content. CF: Crude fibre. CP: Crude protein. EE: Ether extract GE: Gross energy.

DM: Dry matter Mean with the same superscripts down the column are not statistically significant Alpha level (α) = 0.05

Table 8: Elemental composition of the smoked fishes in (mg /100g)

Parameters	Fe	Zn	Mg	Ca	K	Na
Fish Samples						
Scomber scombrus	7.23±0.04^a	2.14±0.03^b	59.61±1.36^b	18.84±0.15^c	41.56±0.53^c	10.22±0.07^c
Gymnarchus niloticus	4.11±0.02^b	9.67±0.13^a	60.48±0.06^b	31.50±1.03^a	53.58±0.64^b	75.31±0.65^a
Oreochromis aureus	3.63±0.32^b	19.86±0.08^a	66.48±0.31^a	21.20±0.20^b	59.12±0.54^a	55.77±0.16^b

Key: Mean with the same superscripts down the column are not statistically significant Alpha level (α) = 0.05

Discussion

From the results obtained from this study, smoked fish samples were highly contaminated with microorganisms. The mean total heterotrophic bacterial count was 6.20±0.27 x10^5, 7.26±0.43 x10^5 and 7.33±0.58 x10^5 (cfu/g) for *Scomber scombrus*, *Gymnarchus niloticus* and *Oreochromis aureus* respectively. The mean coliform count was 3.26±0.43 x10^4, 3.50±0.24 x10^4 and 4.26 ±0.43 x10^4 (cfu/g) for *Gymnarchus niloticus*, *Scomber scombrus* and *Oreochromis aureus* respectively. The mean *Staphylococcal* count was 2.07±0.43 x10^4, 2.36±0.43 x10^4 and 2.76±0.43 x10^4 for *Gymnarchus niloticus*, *Scombrus scombrus* and *Oreochromis aureus*. There was also a significant difference (p<0.05) in microbial load of the fishes from different locations. The total heterotrophic count obtained for the smoked samples were above the range of specified microbiological limits recommended by International Commission on Microbiological Specification for Food (ICMSF, 1986) [34], ICMSF (1986) [34] for fish and fishery products, the maximum recommended bacterial counts for good quality products is 5 x 10^5 (5.7 log10 cfu/g) (Adelaja *et al.,* 2013; Adeyeye *et al.,* 2015) [6, 15]. The moisture content levels in the fish samples were high, which encourages the growth of microorganisms. Among the tilapia species (*Oreochromis aureus*), the highest bacterial count (9.4±0.23x10^5cfu/g) was found in the samples obtained from Yanga market while the lowest count (5.20±0.17 x 10^5cfu/g) was observed in the *Scomber scombrus* gotten from smoking kiln in Oluku. The high bacterial count from Yanga market may be due to the environmental condition and also due to the influx of dust resulting from the heavy traffic from which the fishes may be contaminated. Smoking kiln used in Oluku markets help in the reduction of microbial load and infestation of flies unlike local cut drums when the fishes were smoked in open air. The diversity of microorganisms associated with these smoked fishes could be attributed to some features such as exposure at marketplace as the tissues of fish is capable of reabsorbing moisture from the atmosphere. This is corroborated by Eyo (2001) [32] who stated that smoked fish samples may have a relatively high water activity level which is a prerequisite for microbial growth. Secondly, is the introduction of the organisms into foods from water used for washing, utensil or wrapping materials? This is supported by the observation of Eklund *et al.* (1993) [30], who stated that any handling of fish and the associated sanitary practice from the point of harvesting can potentially contribute to the micro flora on the final product. *Escherichia coli* and *Staphylococcus aureus* were encountered on the fish samples in all the locations. *Bacillus subtilis, Morganella morganii, Enterobacter cloacae and Serratia marcescens* were also present featured in the fishes. A similar trend was also observed in the coliform count of the tilapia species from Yanga market with the highest value (5.00± 0.21 x 10^5cfu/g). The staphylococcal counts obtained for the smoked fishes were below the specified recommended value for all fish (Adeyeye *et al.,* 2015) [15]. Similar results were reported by Adebayo-Tayo *et al.* (2008) [5] and Abolagba and Igbinevbo (2010) [2] in smoked fish sold in Benin and Uyo metropolis respectively. Furthermore, Adelaja *et al.,* (2013) [6] stated that *Staphylococcus aureus* and *Escherichia coli* were the commonest microorganisms associated with smoked fish. Okareh and Erhahon (2015) [45] reported that *Escherichia coli* caused diarrhoea and kidney damage as well as community-acquired urinary tract infections. *Enterobacter* sp. could be indicative of fecal contamination. *Bacillus* sp. produces toxins that withstand high temperatures and are spore forming which germinate and release enterotoxins (Odu and Imaku, 2013) [42]. Some *Bacillus* species are pathogenic and can cause food poisoning, their presence in some of the fresh and smoked fish samples may be because they are sold openly in the market and are exposed to the spores of the organism which are dormant in that

environment and are highly resistant to the lethal effects of heat drying and ultraviolet radiation (Udochukwu *et al.*, 2016) [53]. *Bacillus cereus* and to a lesser extent *Bacillus subtilis* and *Bacillus licheniformis* are intermittently allied with bacteremia, meningitis, and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract (Ineyougha *et al.*, 2015) [35]. Adelaja *et al.* (2013) [6] and Ehiri *et al.* (2001) [29] reported that *Staphylococcus* sp. has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus* enterotoxin which is resistant to gastrointestinal enzymes. *Staphylococcus aureus*, a normal flora of human skin and mucous membrane, is one of the most common causes of boils, impetigo and folliculitis and in some cases, infections of the bones and wounds (Akinwunmi and Adegbehingbe, 2015). Again the isolation of *Staphylococcus aureus* and *Bacillus cereus* is an indication of poor handling or cross contamination of smoked fish products, since the two organisms have been indicted in food poisoning (Dike-Ndudim *et al.*, 2014) [26]. High level of *Bacillus* sp., coliforms and other pathogenic bacteria in food should not be dismissed as mere contamination, since they are capable of causing serious infections (Olaleye and Abegunde, 2015) [47] Abolagba *et al.* (2011) [4] stated that bacteria such as *Staphylococcus aureus*, *Proteus*, *Bacillus*, *Micrococcus*, were the most common microorganism associated with smoked fish. *Salmonella typhi* and *Salmonella paratyphi* was not detected among the fish samples and this result conformed to the specified microbiological limits recommended by ICMSF (1986) [34] for fish and fishery products, that is, zero tolerance. *Salmonella paratyphi* and *Escherichia coli* serve as indicator serve as indicator organism for fecal contamination of food (Adeyeye *et al.*, 2015) [15].

A variety of filamentous fungal species were also observed in the fish samples across the study area. The presence of *Aspergillus niger* in some of the samples is in agreement with the findings of Adebayo-Tayo *et al.* (2006) who suggested that the presence of *Aspergillus* makes the consumption of fish hazardous to man. *Aspergillus* species produce aflatoxins. Aflatoxins are primarily hepatoxic toxins targeting majorly the liver with aflatoxin B1(AFB1) being the most potent and classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (Egbuta *et al.*, 2017) [28]. The occurrence of *Aspergillus* sp., *Penicillium* and *Fusarium* in the tilapia 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 possible contamination during processing, handling and display on the market (Christinah *et al.*, 2010). *Fusarium* infections in farmed fish have only been described in marine fishes and are considered important fish pathogens affecting marine fishes. Fusarial infections in marine fishes include skin lesions, fatal ulceration, keratitis, localized necrotic diseases and necrohemorrhagic dermatitis (Yanong, 2003; Egbuta *et al.*, 2017) [28, 54]. Cutuli *et al.* (2015) [24] reported the presence of *Fusarium oxysporum* in subcutaneous lesions of Nile tilapia (*Oreochromis niloticus*). *Penicillium* species produce ochratoxin A, rubratoxin, rugulosin, patulin, citrinin and viopurpurin which have been implicated in kidney and liver infections which are the primary target organs (Sweeny and Dobson, 1998; Akinyemi *et al.*, 2011) [52]. The isolation of *Saccharomyces cerevisiae* (yeast) in the study is in agreement with the findings of Abbas *et al.* (2015) [1] that most yeast has been used as aqua feeds for supplementation based diets with beneficial effect on fish growth.

From the proximate composition of the experimental fish samples, the moisture content was relatively low with no significant difference ($p>0.05$). Ahmed *et al.* (2011) [16] reported similar result and attributed this to the fact that during smoke-drying, the fish flesh loses water in the initial phase that could be compared to cooking (first 3 h at 80 ^{0}C) in addition to a protective coating formed due to partial carbonization of tissue and other components by wood smoke. The relatively moderate to high percentage crude protein may be attributed to the fact that fishes are good source of pure protein, but the observed difference in value could be due to absorption capability and conversion potentials of essential nutrients from their diets or their local environment (Adewoye and Omotosho, 1997) [10]. The relatively high protein levels in smoked fish samples suggested that protein nitrogen was not lost during hot-smoking. This is in agreement with the work of Adeosun *et al.* (2015) [9] and Ogbonaya and Ibrahim (2009) [44]. There is a significant difference ($P<0.05$) among the gross energy (GE) values of the fishes. The lowest energy value (3229.67) is found among the *Gymnarchus niloticus*, however, the daily energy requirement for an adult range between 2500 – 3000 kcal (10455 – 12548 kJ) depending on his physiological state while that of infants is 740 kcal (3094.68 kJ) (Adeyeye and Adamu, 2005) [14]. This definitely shows that the protein concentration in *Gymnarchus niloticus* in terms of energy would be more than enough to prevent malnutrition in children and adult fed solely on *Gymnarchus niloticus* as a main source of protein (Adeyeye and Adamu, 2005) [14]. *Scomber scombrus* had the highest energy value of 3576.03. This shows that *Scomber scombrus* favourably compares to some plant foods in terms of its energy content Aremu *et al.* (2014) [21]. This undoubtedly indicates that the protein concentration in *Scomber scombrus* in terms of energy would be more than enough to avert a disease known as malnutrition in children and adult who feed solely on *Scomber scombrus* as a main source of protein (Adeyeye and Adamu, 2005) [14]. Tilapia had the highest ash content of 19.70±0.40. This is in agreement with Katola and Kapute (2017) [39] who suggested that high ash content is consistent with bony fish such as tilapia species. Ash content is generally influenced by size of fish. Also, smaller sized fish species tend to have higher ash content due to the higher bone to flesh ratio (Daramola *et al.*, 2007 and Kapute *et al.*, 2013) [25, 39]. Fat content was generally low in all the fish samples; however, the fat concentration in this report is higher than the value (5.2±0.01) of *Gymnarchus niloticus* reported by Adeyeye, and Adamu (2005) [14]. This shows that the fish samples might be very good sources of fish oil (Kefas *et al.*, 2014) [40]. Reduced fat content could be explained by oxidation and break down of crude fat into other components due to oxidation of poly-unsaturated fatty acids contained in the fish tissue to products such as peroxides, aldehydes, ketones and free fatty acids (Daramola *et al.*, 2007) [25]. Crude fibre was generally low in the experimental fishes. Fibre content for *Oreochromis aureus* was 0.04±0.01, 0.11±0.03 for *Scomber scombrus* but was not detected at all among the *Gymnarchus niloticus* species. The absence of fibre in *Gymnarchus niloticus* species was also reported by Adeyeye

and Adamu, (2005) [14]. The significant reduction of fibre observed in this study posed no threat because fish is usually consumed as adjuncts or additives to other food. This observation concurs with the report of Adeyemi *et al.* (2013) [39] who reported that high fibre is poorly digested by animals. Dry matter is the actual dry weight of the fish when moisture content has been eliminated. Dry matter values observed in the fish species are at variance with Kefas *et al.* (2014) [40] who recorded high values (88.80±0.73-94.30±0.86) in tilapia. The variations in the concentration of the different nutritional components in the fish species could have been as a result of the rate in which these components are available in the water body (Emurotu *et al.,* 2014) [31].

The result of the mineral composition shows that magnesium had the highest value among the fish samples. *Oreochromis aureus* had a high value of 60.48 mg/g. Magnesium is an important mineral element in connection with circulating diseases, such as ischaemic heart disease and calcium metabolism in bone (Ishida *et al.,* 2000). Concentrations of potassium (41.56 ± 0.53, 53.58 ± 0.64 and 59.09 ± 0.54) were equally high in the fish samples. Onyia *et al.* (2010) [50] reported similar findings and attributed this to the dominance of the element in the water body where the fish lives. Adewoye and Omotosho (1997) [10] suggested that high level of Mg and K could be due to the ability of the fish to absorb these organic elements from their diet and the environment where they live. Sodium was relatively high in the fish samples. Sodium is an activator of transport ATP-ases in animals and possibly also in plants (Adeyeye, 2005) [14]. Zinc plays important role in the management of diabetes, which results from insulin malfunction (Emurotu *et al.,* 2014) [31]. The higher level of potassium than sodium in the studied samples is in agreement with what was observed in vegetable material by Adeyeye and Fagbohun (2005) [14]. Both sodium and potassium are required to maintain osmotic balance of the body fluid and the pH of the body; regulate muscles and nerve irritability, control glucose adsorption and enhance normal retention of protein during growth (Aremu *et al.,* 2014) [21]. The Zinc content (9.67mg/100g) met the adequate zinc intake of 8mg in females but fell below the recommended daily allowance (RDA) of 11mg in adult male (Yates *et al.,* 2001) [55], but will still be available for biological functions. However, the Zinc value in the current report was similar to the result (9.20mg/100g) obtained by Adeyeye and Adamu (2005) [14]. *Scomber scombrus* also recorded the highest Iron (Fe) value of 7.23. The variation in the iron concentration is attributed to the chemical forms of the element and concentration in the environment (Kefas *et al.,* 2014) [40]. The daily iron requirements by humans are 10 to 15 mg for children, 18 mg for women and 12 mg for men. Fe is an essential trace element for hemoglobin formation, normal functioning of the central nervous system and in the enzyme cytochrome oxidase involved in energy metabolism (Adeyeye and Adamu, 2005; Alfa *et al.,* 2014) [14, 19]. Iron and copper are present in the enzymes cytochrome oxidase involved in energy metabolism (Aremu *et al.,* 2014) [21]. *Gymnarchus niloticus* had the highest calcium composition value of 31.50mg/100g. This is comparable with the work of Adeyeye and Adamu (2005) [14] who recorded Ca value of 31.34mg/100g. High Calcium composition is an indication that the sample may serve as good source of minerals for bone and teeth formation as well as body structure and blood clotting (Osborne *et al.,* 1996) [51]. Many studies have shown that the concentration in minerals is greatly affected by different environmental (food availability, salinity, temperature) and intrinsic factors such as species, age, sex as well as physiological factors (Olopade *et al.,* 2016) [49]. The variations in the mineral composition may also be attributed to the ability of the fish to absorb and convert the essential nutrients from diet or the water bodies where they live. Thus, it is right to say that, the mineral elemental contents of each species is a function of the availability of these elements in their local environment, diet absorption capability and as well as their preferential accumulation (Adewoye and Omotosho, 1997; Effiong and Fakunle, 2012; Alfa *et al.,* 2014; Emurotu *et al.,* 2014) [10, 31, 19, 33].

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