NISEB JOURNAL Vol. 13, No. 3&4. December, 2013 1595-6938/2013
Printed in Nigeria (2013) Nigerian Society for Experimental Biology
http://www.nisebjournal.org

Effect of Different Preservative Methods on Microbiological, Nutritional and Mineral Contents of Selected Fish from Major Urban Markets in Benin City, Nigeria

Regina R. Sanni, Samuel A. Aziegbemhin, Etinosa O. Igbinosa and Frederick O Ekhaise*

Department of Microbiology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria

ABSTRACT: *The effect of different preservative methods gas dried, wood smoked and frozen on different fish species (Merluccius merluccius, Scomber scombrus and Trachurus trachurus obtained from different markets within Benin metropolis were investigated. Thirty Six fish samples were analyzed using standard culture-based and spectroscopic methods. The wood-smoked method for Merluccius merluccius revealed the highest microbial load of $6.32\times10^{7}\pm0.42$ cfu/g and $2.05\times10^{7}\pm0.14$ cfu/g for bacterial and fungal densities respectively. Frozen and gas-dried methods had the lowest microbial load of $2.2\times10^{6}\pm1.56$ cfu/g and 2.70×10^{7} ±0.17 cfu/g of bacterial densities and 4.3×10^{6} ±0.02 cfu/g and3.3×10^{6} ±0.01cfu/g of fungal densities respectively. The microbial isolates include Bacillus spp., Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus spp., Corynebacteria spp., Escherichia coli, Streptococcus spp., and Proteus spp., The proximate analyses revealed as follows - proteins (12.31 - 23.04%); lipid (7.63 - 37.55%); ash (9.49 - 14.28%), fiber (0.63 - 2.31%) and carbohydrate contents (29.0 - 63.47%). The macro and micro elements constituents revealed that the fish contain significant amount of nitrogen (0.07±0.01-3.69±0.05 mg/100g DWB); phosphorus (0.08±0.03-0.28±0.01mg/100g DWB); potassium (0.09±0.04-0.50±0.01 mg/100g DWB); calcium (0.07±0.01-1.06±0.02 mg/100g DWB); and magnesium (0.05±0.01 - 0.09±0.01 mg/100g DWB). The presence of potential pathogenic microbes from the study revealed that the fish as well as method of processing are of immense public health concerns.*

Keywords: Public health; Contamination; Bony Fish; Microorganism,

Introduction

Fish is a major source of protein and itôs method of harvesting, handling, processing and distribution provide livelihood for millions of people as well as source of foreign exchange to many countries (1). The micro-flora of these products are often complex however, spoilage is mostly caused by microbial activities. These microbes are responsible for spoilage of most fresh and slightly preserved seafood (2). The function of smoking of fish is primarily to provide the desirable colour, aroma and flavour to the fish and fish-products. Undesirable effects are as a result of contamination with toxic components of smoke and some destruction of essential amino acids of food proteins, which are attributed to certain classes of components of smoke especially liquid smoke. However, the preservative properties are not nearly as important as strict hygienic requirements such as modern packaging and continuous refrigeration (3). Improper smoking and drying of fishes may lead to insect infestation, fungal attack, fragmentation and degradation of the product (3, 4). The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species, although the endemic microbial populations of fish can vary significantly (5). According to Akande and Tobor (6), in artisanal fishery, freshly caught fish are covered with damp sacks and at times, they are mixed with wet grass or water weeds to reduce the temperature. Fish treated this way are prone to contamination with micro-organisms. This indicates that spoilage of fish starts right from the source and method of harvesting (7).

During processing like smoke-drying, smoking kilns used in artisanal fishery and the overloading of the fishes on the trays leads to improper processing which in turn encourages microbial attack. Most outbreaks of food poisoning associated with fish and fish-products derived from the consumption of raw or insufficiently heat treated fish are attributed to contamination with bacteria from water environment such as *Vibrio* spp., and *Clostridium botulinum* or terrestrial sources including *Clostridium perfringens*, *Salmonella* spp*., Shigella* spp*., Staphylococcus* spp*.,* and *Vibrio cholera.* The moisture content of the dried products varies between an estimated 40 % in the higher ranges and 10-20 % in lower. The quality of the product after processing is determined by degree of drying, appearance, damage and signs of insect attack. These thus, influence the price of the fish and fish-products (8, 9). This study evaluates the effect of three preservative methods on the microbiological and nutritional qualities on different fish sold in urban markets in Benin City.

Materials and Methods

Sample Collection

Thirty six (36) smoked, gas dried and frozen *Merluccius merluccius* "merluza", *Scomber scombrus* "scumbia" and *Trachurus trachurus* "sese" fishes were randomly selected and purchased between June and November, 2012 from three major markets (Oba, New Benin, and Uselu) in Benin City, Edo State. Samples were transported in clean sterile polythene bags to the laboratory for analysis.

Microbiological Analysis

The fish samples were removed from sterile polythene bags, weighed and measured as whole. With the aid of a sterile forceps, the skin of each sample was removed and 1g was weighed from each. One gram of the weighed sample was added to 10 ml peptone water to make a stock solution. Ten-fold serial dilutions of the homogenates; 0.1ml of 10^{-1}, 10^{-2} and 10^{-3} dilutions was used for pour plate methods to enumerate the microbial population using MacConkey agar and Nutrient agar. The incubated plates were used in observing the cultural and morphological characteristics of representative colonies. Colonies were selected at random and sub cultured to obtain pure isolates on fresh plates containing nutrient agar and incubated at 37 ^{o}C for 24 hours. The pure isolates were stored at 4 ^{o}C and used for Gram reactions and biochemical characterization. Potato Dextrose Agar (PDA) was used to isolate fungi following the same protocol for bacterial isolation. The morphology of isolated fungal isolates was determined using light microscopy techniques. Wet mount of the fungus was prepared and viewed under ×10 and × 40 magnification.

Proximate and mineral analysis

The proximate analysis of carbohydrate, lipid, ash, fibre and protein of the three fish samples was determined using Association of Official Analytical Chemists (AOAC) methods. All the proximate values were reported in percentage. Mineral elements including nitrogen, phosphorus, potassium, calcium and magnesium were determined using the multiple nutrient extraction method (10).

Statistical Analysis

Statistical significance of values obtained was determined using the one-way analysis of variance (ANOVA) and Duncan multiple range significant difference at $P < 0.05$ and $P < 0.01$.

Results

Table 1 reveals the bacterial density obtained from the samples. The highest bacteria population $6.35\times10^7\pm1.33$ cfu/g recorded in smoked fish (*Trachurus trachurus*) from New Benin market. The bacterial count $3.77\times10^7\pm0.55$ cfu/g recorded in frozen *Merluccius merluccius* from Uselu market.

Table 1: Total population density of bacterial from the three fish samples

Fish samples	**Sampling location**	**Preservative methods**		
		Frozen (cfu/g)	**Gas Dried (cfu/g)**	**Smoked (cfu/g)**
Trachurus trachurus "sese"	Uselu	$3.77\times 10^7 \pm 0.55$	$2.85\times10^7 \pm 0.74$	$5.20\times 10^7 \pm 0.82$
	New Benin	$2.11\times10^7 \pm 1.30$	$1.45\times10^7 \pm 1.06$	$6.35\times 10^7 \pm 1.33$
	Oba Market	$2.04\times10^7 \pm 0.81$	$2.40\times 10^7 \pm 0.45$	$5.45\times10^7 \pm 0.37$
	$\mathbf{X^2}$	72.64	45.75	12.91
	***P*-value**	$P < 0.01$	$P < 0.01$	$P < 0.01$
Scomber scombrus "scumbia"	Uselu	$1.08\times10^7 \pm 0.98$	$2.75\times10^7 \pm 1.25$	$5.10\times10^7 \pm 0.31$
	New Benin	$2.69\times 10^7 \pm 1.04$	$4.05\times10^7 \pm 1.70$	$5.55\times10^7 \pm 1.22$
	Oba Market	$1.77\times10^7 \pm 1.08$	$2.90\times10^7 \pm 1.17$	$4.10\times10^7 \pm 1.26$
	$\mathbf{X^2}$	70.66	30.29	22.41
	***P*-value**	$P < 0.01$	$P < 0.01$	$P < 0.01$
Merluccius merluccius "merluza"	Uselu	$2.2\times 10^6 \pm 1.56$	$4.80\times10^7 \pm 0.31$	$6.32\times10^7 \pm 0.42$
	New Benin	$8.6\times10^6 \pm 0.62$	$5.00\times10^7 \pm 0.06$	$3.52\times10^7 \pm 0.69$
	Oba Market	$7.1\times10^6 \pm 0.83$	$2.70\times10^7 \pm 0.17$	$5.50\times10^7 \pm 1.35$
	$\mathbf{X^2}$	37.55	77.92	81.05
	***P*-value**	$P < 0.01$	$P < 0.01$	$P < 0.01$

Values are means of the count of three independent experiments.

Table 2 shows the total fungi population. The fungal density of $3.3\times10^6\pm0.01$ cfu/g was obtained in gas dried fish (*Merluccius merluccius*) from Oba market while the count $2.00\times10^6\pm0.13$ cfu/g was recurred in gas dried fish (*Trachurus trachurus*) from Uselu market.

Table 2: Total population density of fungi from the three fish samples

Fish samples	**Sampling location**	**Preservative methods**		
		Frozen (cfu/g)	**Gas Dried (cfu/g)**	**Smoked (cfu/g)**
Trachurus trachurus "sese"	Uselu	$5.5\times10^6 \pm 0.14$	$2.0\times10^6 \pm 0.13$	$1.60\times 10^7 \pm 0.15$
	New Benin	$2.3\times10^6 \pm 0.12$	$1.60\times10^7 \pm 0.20$	$6.5\times 10^6 \pm 0.19$
	Oba Market	$2.2\times10^6 \pm 0.11$	$1.45\times10^7 \pm 0.11$	$1.59\times10^7 \pm 0.11$
	$\mathbf{X^2}$	21.14	109.08	46.52
	***P*-value**	$P < 0.01$	$P < 0.01$	$P < 0.01$
Scomber scombrus "scumbia"	Uselu	$3.8\times 10^6 \pm 0.02$	$8.0\times 10^6 \pm 0.05$	$1.20\times 10^7 \pm 0.09$
	New Benin	$3.3\times10^6 \pm 0.20$	$1.25\times10^7 \pm 0.08$	$1.85\times10^7 \pm 0.01$
	Oba Market	$1.9\times10^6 \pm 0.15$	$4.8\times10^6 \pm 0.05$	$1.90\times10^7 \pm 0.10$
	$\mathbf{X^2}$	6.47	35.49	18.49
	***P*-value**	$P < 0.05$	$P < 0.01$	$P < 0.01$
Merluccius merluccius "merluza"	Uselu	$5.4\times10^6 \pm 0.06$	$4.9\times10^6 \pm 0.10$	$2.6\times10^6 \pm 0.23$
	New Benin	$4.3\times 10^6 \pm 0.02$	$5.0\times10^6 \pm 0.21$	$4.1\times10^6 \pm 0.04$
	Oba Market	$5.2\times10^6 \pm 0.01$	$3.3\times10^6 \pm 0.01$	$2.05\times 10^7 \pm 0.14$
	$\mathbf{X^2}$	1.38	4.14	217.51
	***P*-value**	$P > 0.05$	$P > 0.05$	$P < 0.01$

Values are means of the counts of three independent experiments

Eighteen microbial isolates were identified from the different fish samples. This includes 8 bacteria and 10 fungi isolates. The bacterial isolates are *Bacillus* spp*, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus* spp.,*Corynebacteria* spp.*, Eschericha coli, Streptococcus* spp.*,* and *Proteus* spp. While the fungal isolates includes *Aspergillus niger, Aspergillus flavus, Trachoderma* spp.*, Cladosporium* spp.*, Rhizopus* spp.*, Penicilum italicum, Penicilum onalica, Rhodoturola, Yeast* and *Geotrium* spp.

Table 3 shows the proximate composition of carbohydrate (29.0 - 63.47%); protein (12.31 - 23.04%); ash (9.49 - 14.28%); fibre (0.63 - 2.31%); and lipid (7.63 - 37.55%); contents of the fish samples

Table 3: Proximate analysis of the different fish samples

Parameter (%)	**Fish samples**			***P*-value**
	Trachurus trachurus "sese" (% Dry weight)	*Scomber scombrus* "scumbia" (% Dry weight)	*Merluccius merluccius* "merluza" (% Dry weight)	
Carbohydrate	29.08 ± 0.12^{C}	63.47 ± 0.66^{A}	49.07 ± 1.43^{B}	$P < 0.01$
Lipid	37.55 ± 1.11^{A}	7.63 ± 0.19^{C}	21.77 ± 1.25^{B}	$P < 0.01$
Ash	9.49 ± 0.49^{B}	14.28 ± 0.14^{A}	10.25 ± 0.43^{C}	$P < 0.01$
Fibre	0.63 ± 0.06^{B}	2.31 ± 0.10^{A}	0.70 ± 0.26^{B}	$P < 0.01$
Protein	23.04 ± 0.30^{A}	12.31 ± 0.44^{C}	18.21 ± 0.22^{B}	$P < 0.01$

Values in the same row followed by different letters are highly significantly different $P < 0.01$

The mineral element composition for the following micro and macro elements: nitrogen (0.07±0.01 - 3.69±0.05 mg/100g DWB); phosphorus (0.08±0.03 - 0.28±0.01 mg/100g DWB); potassium (0.09±0.04 - 0.50±0.01 mg/100g DWB); calcium (0.07±0.01 - 1.06±0.02 mg/100g DWB); and magnesium (0.05±0.01 - 0.09±0.01 mg/100g DWB) are presented in Table 1.

Table 4: Mineral content composition of the different fish samples

Fish samples	**Parameter (%)**	**Preservative methods (mg/100g DWB)**			***P*-value**
		Frozen	**Gas Dried**	**Smoked**	
Trachurus trachurus "sese"	N	0.41 ± 0.56^{C}	1.14 ± 0.01^{B}	2.91 ± 0.04^{A}	$P < 0.01$
	P	0.17 ± 0.07^{B}	0.10 ± 0.02^{B}	0.22 ± 0.02^{A}	$P < 0.05$
	K	0.15 ± 0.02^{B}	0.10 ± 0.03^{C}	0.50 ± 0.01^{A}	$P < 0.01$
	Ca	0.10 ± 0.03^{B}	0.09 ± 0.02^{B}	0.63 ± 0.02^{A}	$P < 0.01$
	Mg	0.05 ± 0.01^{A}	0.04 ± 0.01^{B}	0.06 ± 0.01^{A}	$P < 0.05$
Scomber scombrus "scumbia"	N	0.50 ± 0.72^{B}	0.82 ± 0.66^{B}	3.69 ± 0.05^{A}	$P < 0.01$
	P	0.11 ± 0.06^{B}	0.08 ± 0.03^{B}	0.28 ± 0.01^{A}	$P < 0.01$
	K	0.11 ± 0.05^{B}	0.09 ± 0.04^{B}	0.47 ± 0.01^{A}	$P < 0.01$
	Ca	0.10 ± 0.00^{B}	0.10 ± 0.01^{B}	1.05 ± 0.02^{A}	$P < 0.01$
	Mg	0.04 ± 0.03^{A}	0.04 ± 0.02^{A}	0.08 ± 0.01^{B}	$P > 0.05$
Merluccius merluccius "merluza"	N	0.07 ± 0.01^{B}	0.39 ± 0.57^{B}	1.97 ± 0.07^{A}	$P < 0.01$
	P	0.12 ± 0.01^{B}	0.12 ± 0.01^{B}	0.30 ± 0.03^{A}	$P < 0.01$
	K	0.15 ± 0.02^{B}	0.14 ± 0.02^{B}	0.43 ± 0.01^{A}	$P < 0.01$
	Ca	0.07 ± 0.01^{B}	0.07 ± 0.01^{B}	1.06 ± 0.02^{A}	$P < 0.01$
	Mg	0.05 ± 0.01^{B}	0.05 ± 0.01^{B}	0.09 ± 0.01^{A}	$P < 0.01$

Values in the same row followed by different letters are significantly different $P < 0.05$ and $P < 0.01$

Legend: N-nitrogen, P- phosphorus, K- potassium, Ca- calcium, Mg- magnesium, DWB - dry weight basis

Discussion

The environments in which fishes are displayed in the market are not always hygienic and this plays significant roles in the microbial contamination of the stored fishes (11). The retailers often display smoke and dried fish in open trays in unhygienic condition this encourages microbial attack and subsequent production of toxins (12). It is assume that with fire heat, most microorganisms could fail to flourish as stated by Oyewole *et al*. (13), who reported that heat from fire dries fish; reducing the moisture content to a level that prevents growth of microorganisms. Asita and Campell (14), also reported that heat and dry air associated with smoking reduces the water activity of food, thereby depriving organisms of a prerequisite for growth. These reports however do not agree with this study as pathogenic food bacteria and molds were present in smoked fish samples, which passed through heat and its moisture content reduced. The level of growth of microorganisms on smoked fish depends on the amount of water which has been expelled from the fish (13).

The variations in microbial counts of fish samples from different markets and preservative methods in which some have higher microbial counts may be likely due to a lack of proper preservation on the side of the fish processors or/and improper hygienic and handling procedures. This is in agreement with the findings of Abolagba and Iyeru (15), who reported that lack of proper smoking and proper hygienic handling of smoked fish products, would result in a very high microbial load.

Studies by Nketsia-Tabiri and Sefa-Dedeh (16), showed the presence of *Staphylococcus* spp*., Enterobacter sakazaki, Klebsiella* spp., *Pneumoniae ozaen*ae, *Bacillus* spp., *Aspergillus* spp*.*, and *Penicillium* spp., in smoked sardines. Both *Aspergillus* spp*.,* and *Penicillium* spp., have been reported to grow rapidly at chill temperature, and may subsequently develop in the stored final product (17). Omojowo and Ihuahi (18), reported that smoked fish samples from four local markets in Kainji Lake area of Nigeria were dominated by coagulase-positive *Staphylococcus* and *Escherichia coli*. This is in agreement with the results obtained from this study. The results indicated that smoked fish from the markets had the highest microbial load (bacteria and fungi) (Tables 1 and 2) when compared with gas dried fish. The occurrence of microorganisms such as *Staphylococcus aureus*, yeast *Saccharomyces* spp., and moulds *Penicillium* spp., and *Aspergillus niger* in the smoked-dried fish samples were in accordance with Martin (19) the author stated that these organisms were the commonest microorganisms associated with smoked fish and these microorganisms were also reported by Abolagba and Igbinevbo (20) in smoked fish (*Clarias* spp.,) sold in Benin metropolis.

The pathogens isolated in this present study are similar to the microorganisms reported by Abolagba and Uwagbai (21) 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. *Proteus* sp. is an opportunistic human pathogen and has simple nutritional requirements. They are important causative agents in community-acquired and nosocomial urinary tract infections (22). The occurrence of *Bacillu*s sp. is suggestive as a result of prevalence of their spores in the environment most especially in the soil (23). *Bacillus* sp causes a toxin-

medicated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (23). The infectious dose has been estimated to be 10^5/g (23). The occurrence of *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp., could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the fungi in addition to the contamination during processing, handling and display on the market stalls (24)

The presence of *Staphylococcus* in smoked fish samples is in confirmation with Okonta and Ekelemu who reported *Staphylococcus* as one of the predominant microorganisms affecting smoked fish and causing their spoilage. Fafioye *et al.* (25) studied the fungal infestation of five traditionally smoked dried freshwater fish in Ago-Iwoye, Nigeria and isolated and identified eleven different fungal species of which *Aspergillus flavus* was the most frequently encountered fungi on the fish species, which also is in agreement with this study as *Aspergillus flavus* and *Aspergillus niger* were identified as the most occurring organism. The smoking of fish is supposed to be effective in reducing the microbial load in the fish but this study has shown that fish smoking from these three markets does not help to improve the microbiological quality of smoked fish. Considering the public health implications of the poor microbiological state of the smoked fish, particular attention should be make available for safety through proper processing, storage and handling procedures. All the pathogens isolated are of public health implication and hence unsafe to human health if consumed (26).

The fish provides dietary supplements and may promote bowel regularity and enhance frequent waste elimination including bile acid. Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (27). The relatively high level of ash, lipid, protein, and carbohydrate is suggestive of its considerable nutritive value.

The macro and micro elements of fish contained very important nutrients relevant to the well being of humans (Table 4). Potassium is necessary for the function of all living cells and is thus present in all animal tissues (27). Calcium, potassium, magnesium, nitrogen observed in the fish are required for repairs of worn out cell, strong bones and teeth in human, building of red blood cells and for body mechanisms (28).

Conclusion

The high microbial load obtained for the smoked fish purchased from the markets could be attributed to the poor fish handling and improper smoking processes adopted by fish handlers. The presence of *Escherichia coli*, *Staphylococcus aureus*, fecal streptococci and moulds at high population density revealed that the fish samples are of great public health concerns.

References

1. Al-Jufaili MS, Opara IU: Status of fisheries postharvest in the Sultanate of Oman: Part 1: handling and marketing system of fresh fish. J Fisheries Intõl 1:144-149, 2006.
2. Lund BM, Baird-Parker AC, Gould GW: The microbiological safety and quality of foods. Aspen Publishers, Inc. Maryland, USA, 1885pp. 2000.
3. Eyo AA: Traditional and improved fish handling, preservation and processing techniques. NAERLS/NIFER national workshop on fish processing, storage, marketing and utilization, 15pp. 1992.
4. Banwart CI: Basic Food Microbiology. 1st Ed. Publications. S. K. New Delhi.78pp. 1989.
5. Edris AM: Microbial evaluation of some marketed smoked fish. Zagazig Veter J 24: 76-81, 1996.
6. Akande GR, Tobor JG: Conservation needs of fisheries resources and re-orientation for sustainable captive and cultural practices. Proceedings of the 10th Annual Conference of Fisheries Society of Nigeria, Port Harcourt. pp. 230-234, 1992.
7. Akande GR: The concept of HACCP and artisanal fishery to improve quality. Food and Agriculture Organization (FAO) Fisheries Report 574: pp. 198-202, 1998.
8. Ito S: The distribution effect of compliance with food safety and agricultural health standards on small producers in developing countries. Proceedings for the Japan Society for International Development, pp. 113-116, 2005.
9. Oyelese OA: Quality assessment of cold smoked, hot smoked and oven dried Tilapia nilotica under cold storage temperature conditions. J Fisheries Intõl 1: 92-97, 2006.
10. AOAC: Official Method of Analysis (18^{th} edn.). Association of Official Analytical Chemists International, Maryland, USA, 96pp. 2005.
11. Abolagba OJ, Nuntah JN: Processing and distribution of smoked *Clarias* spp., in Benin City, Edo State. Intõl Res J Biotech 2: 213-219, 2011.
12. Akande GR, Tobor JG: Improved utilization and increased availability of fishing products as an effective control of aggravated animal protein deficiency induced malnutrition in Nigeria. Proceedings of the 10th Annual Conference of the Fisheries Society of Nigeria, Port Harcourt. pp. 18-31, 1992.
13. Oyewole BA, Agun BJ, Omotayo KF: Effects of different sources of heat on the quality of smoked fish. J Food, Agric Environ 4: 95-97. 2006.
14. Asita AO, Campbell IA: Anti-microbial activity of smoke from different woods. Lett Appl Microbiol 10: 93-95, 1990.
15. Abolagba OJ, Iyeru OA: Study of insect pest infecting traditionally processed fish sold in Benin City Metropolis, Nigeria. Nig J Appl Sci 16: 25-29, 1998.
16. Nketsia-Tabiri J, Sefa-Dedeh S: Quality attributes and utilization of cured fish in Ghana. J Appl Sci Tech 5:148-155, 2000.
17. Mounir M, Salem-Bekhet MW, Al-Azeem ABD, Hashim ESY: Mycological aspect of smoked fish at retail outlet at the Delta Province of Egypt. J Appl Environ Biol Sci 1: 26-31, 2011.
18. Omojowo FS, Ihuahi JA: Microbiological quality and safety of smoked fish from Kainji Lake area. African Scientist 7(4), Dec 31, 2006.
19. Martins AM: Fisheries Processing: Biochemical Applications. Published by Chapman and Hall, London. 88pp. 1994.
20. Abolagba OJ, Igbinevbo EE: Microbial load of fresh and smoked fish marketed in Benin Metropolis, Nigeria. Res J Fisheries Hydrobiol 5: 99-104, 2010.
21. Abolagba OJ, Uwagbai EC: A comparative analysis of the microbial load of smoke dried fishes (*Ethmalosa fimbriata* and *Pseudolithus Elongatus*) sold in Oba and Koko Markets in Edo and Delta States, Nigeria at Different seasons. Aus J Basic Appl Sci **5:** 544-550, 2011.
22. Abbott SL: (2007). *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas* and other Enterobacteriaceae. In: Manual of Clinical Microbiology. PR Murray, EJ, Barron, JH Jorgensen, ML Landry, MA Pfaller, (eds.) 9th edition. Washington, USA. ASM Press. pp. 1-70, 2007.

23. Adebayo-Tayo BC, Adegoke AA, Akinjogunla OJ: Microbial and physico-chemical quality of powdered soymilk sample in Akwa-Ibom, South-southern, Nigeria. Afri J of Biotech 8: 3066-3071, 2009.
24. Christianah I, Ayolabi, Fagade OE: Mycological evaluation of smoked fish from the retail outlets in Ago-Iwoye, Ogun State, Nigeria. J Life and Phys Sci Acta SATECH 3: 65-66, 2010.
25. Fafioye OO, Efuntoye MO, Osho A: Studies on the fungal infestation of five traditionally smoke dried fresh water fish in Ago-Iwoye, Nigeria. Mycopathol 154: 177-179, 2002.
26. Okonko IO, Adejoye OD, Ogun AA, Ogunjobi AA, Nkang AO, Adebayo-Tayo BC: Hazards analysis critical control points (HACCP) and microbiology qualities of sea-foods as affected by handlerôs hygiene in Ibadan and Lagos, Nigeria. Afri J Food Sci 3: 035-050, 2009.
27. FND: Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. Dietary reference intake for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acid (micro-nutrients). pp. 1-75, 2002.
28. WHO: World Health Organization Technical Series: Trace elements in Human Nutrition and Health. World Health Organization Geneva. pp.199-205, 1996.