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Comparative study of Bacteriological Contents of Commercially Smoked Fish and Aseptically Smoked Fish sold in Awka and Environs, Anambra State Nigeria

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ABSTRACT

The study was carried out to determine the bacteriological contents of smoked fish sold in Awka markets and environs. A total number of 80 samples of smoked and frozen fishes were purchased from three different markets in Awka and environs. 60 smoked fish and 20 frozen fish of four different species of panla, sardine, mackerel and catfish 15each and 5 each respectively. The samples were collected labeled appropriately and kept in sterile polyethylene bags and were taken to the microbiology laboratory of Nnamdi Azikiwe University, Awka and Anambra State Polytechnic, Mgbakwu for microbial analysis, biochemical and molecular characterization were used for identification of bacteria and fungi isolates. The frozen fishes were taken to fishery department of Anambra State Polytechnic, Mgbakwu for smoking; the fishes were aseptically smoked using traditional kiln and oven drying method. Nutrient agar, Sabouraud dextrose agar, Salmonella shigella agar, Eosin methylene blue agar and Mannitol egg yolk polymyxin (MYP) agar were used for isolation of organisms. The isolated organisms are Bacillus spp, Staphylococcus Saprophiticus, Enterobacter spp., Staphylococcus aureus, Eschericia coli, Klebsiella Aerogenes. Delftia tsuruhatensis, Proteus mirabilis, Comamonas thiooxydans, Stenotrophomonas maltophilia, Crocinitomicaceae bacterium, Klebsiella pneumonia, Micrococcus spp. the fungi isolated are Aspergillus fumigate, Aspergillus oryzae, Fusarium spp. Mucor, Rhizopus sp. Saccharomyces cerevisiae and Penicillum spp. the total viable counts (TVC in CFU/g) of smoked mackerel, sardine, panla and catfish ranges from $1.7x10^2$ to $40.3x10^2$, $0.6x10^2$-$66x10^2$, $11x10^2$-$98x10^2$, $2.5x10^2$ -$54x10^2$ respectively. TVC (CFU/g) of fungi isolates from catfish ranges from $10x10^2$ to $33x10^2$ TVC (CFU/g). In aseptically smoked fish no organism was isolated from fish samples expect in smoked and oven dried panla fish. Their TVC ranges from $0.2x10^2$-$0.8x10^2$. The findings indicates that smoked fish sold in Awka markets and environs are all contaminated, proper awareness should be done to educate the fish vendors on proper and hygienic methods of processing and selling their products.

Keywords: *Aseptically, Kiln, Smoked, Hygienic, Bacteriological.*

1. INTRODUCTION

1.1 Background of the Study

Smoking is the process of flavoring, browning, cooking, or preserving food by exposing it to smoke from burning or smoldering material, most often wood. Meat and fish are often smoked. Smoke is both an antimicrobial and antioxidant, however it is insufficient alone for preserving food as smoke does not penetrate far into meat or fish; it is thus typically combined with salt-curing or drying.

Smoking is especially useful for oily fish, as its antioxidant properties inhibit surface fat rancidification and delay interior fat exposure to degrading oxygen. Some heavily salted, long-smoked fish can keep without refrigeration for weeks or months.

Artificial smoke flavoring (such as liquid smoke) can be purchased to mimic smoking's flavor, but not its preservative qualities.

Fish smoking is one of the traditional fish processing methods aimed at preventing or reducing postharvest losses. Smoking involves heat application to remove water and it inhibits bacterial and enzymatic actions of fish (Abolagba and Melle,2008 ;Kumolu-Johnson *et al.*, 2009,). It also enables the seafood products to get a special aroma, taste, and color.

Fish is a good source of animal protein and minerals (Tidwell and Allan, 2001). Fish is widely consumed in many parts of the world because it has high protein content. Fish makes up about 60% of world protein supply and developing countries derive more

than 30% of their annual protein from fish (FAO, 1994). The quality of fish protein is very high because of its low saturated fat, its riches with essential amino acids and also it's containing omega3 and omega 6 fatty acids that are known to support good health. According to FAO. (2008) and Gandotra *et al.* (2012), fish provides 20% of animal protein intake to about 2.6 billion people globally and at least 50% of animal protein intake for over 400 million in Asia and Africa. Fish provides not only animal protein but also serves as a major means of livelihood to humans. In Nigeria, there is an increasing demand for fish because it is a cheaper source of animal protein; it is also a delicacy with demands cutting across socio-economic, religious, educational or age groups (Adebayo-Tayo *et al*., 2008). Fish is eaten fresh, processed or preserved and fish protein makes up 40-80% of the optimal protein consumed (Adebayo-Tayo *et al* 2008). Fish is an extremely perishable food. Spoilage proceeds as a series of complex enzymatic, microbial and chemical changes that begin as soon as the fish dies (Junaid *et al.*, 2010). This study is carried out to compare the commercial smoked fish sold in Awka markets and environs and aseptically smoked fish.

2.0 STUDY AREA

The study was conducted in NnamdiAzikiwe University, Awka and Anambra State Polytechnic, Mgbakwu, Anambra State Nigeria.

3.0 MATERIALS AND METHODS

3.1 Sample Collection

A total of eighty (80) samples of smoked fishes and twenty (20) samples of frozen fish sold in the market were purchased. 6 replicates of 4 different smoke dried fish including Mackerel (*Scomber scombrus*), Sardine (*Sardinela eba*), Panla (*Gadus morhua*) and Cat fish (*Clarias gariepinus*) were randomly purchased from 5 different markets; in Awka and environs Anambra state. The fish samples were collected labeled appropriately and kept in sterile polythene bags for microbial analysis.

3.2 Preparation of Materials:

The working tables were swabbed with 70% ethanol to disinfect them. All the wares were washed and air-dried after which they were sterilized in hot air oven at 60^0C for 1hour. The 60 smoked fish samples were taken to Microbiology Laboratory of Nnamdi Azikiwe University, Awka and Anambra State Polytechnic, Mgbakwu for microbial analysis and fishery department of Anambra State Polytechnic, Mgbakwu was used for smoking of fish.

3.3 Sample Preparation:

a) **Smoking of fish: Two methods were used; smoking with traditional kiln and smoking with oven.**
b) **Smoking of fish with traditional kiln:**The collected fish samples were washed cleaned smoked for 4h under monitored ambient conditions. The fish smoking kiln was operated by first loading charcoal into the heat chamber, preheating for some minutes, and then loading the fish onto the trays in its central chamber, and then was closed for some time to allow the smoking to take place. The smoking time, temperature and ambient conditions was monitored during the smoking operations. The smoking was terminated when the fish were properly brown (Olayemi *et al.*, 2011).
c) **Oven drying:** some foil was placed on the oven rack. The bent and pinned fish was placed on the foil lined oven rack and covered with another sheet of aluminum foil. The oven was set to 250°C / 480F and top and down heating (bake). The bake setting cooks the fish before the drying starts. The fish was baked for 15 minutes. All the foil sheets were removed and oven setting changed to Grill/Broil and grilled for 10 more minutes or till the fish browns (Owan,2020). This served as control.

3.4 Serial dilution:

Ten gram (10g) of each fish sample were selected at randomly which represented whole body of the fish both bone and skin were weighed aseptically and homogenized in 90ml sterile peptone water using electric blender. Then, serial dilutions were made by mixing I.0ml of the suspension in 9.0mlsterile peptone water to obtain 10^1 dilutions. Analysis was done for 30days.

3.5 Media preparation:

Two media were used for isolation of fungi, Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). Nutrient agar was used for isolation of bacteria, Mannitol salt agar (for *Staphylococcus* spp); MacConkey agar (for *E. coli* and other enteric bacteria); Robertson cooked meat medium (for *Clostridium botulinum*) and Eosin Methylene blue agar (for enteric bacteria). Incubation was in an aerobic incubator for 24 hours at 37°C. After 24 hours, the bacteria colonies that appeared on plates were counted using a digital colony counter. The average colony counts from duplicate plates was obtained and expressed as colony forming units (c.f.u.) per gram of sample. Colonies on the plates were sub-cultured on nutrient agar plates to ensure purity of cultures. The different pure cultures were cultured in nutrient agar slant for identification and storage.

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3.5.1 Sub-culture

After incubation period, discrete colonies from bacteria plates were picked with a flamed wire loop and sub-cultured onto a newly prepared nutrient agar plates. Also, a flamed knife was used to sub-culture different colour of mycelia growth from Sabrouraud agar. All plates were incubated appropriately. All nutrient plates were transferred into an incubator at 37^{o}C for 24hrs while all the sabrouraud agar plates incubated at room temperature for 48 hrs. Purified colonies and mycelia were transferred into agar slants and stored properly for further characterization.

3.5.2 Identification of isolates

Isolates were identified with the aid of keys and diagrams presented by Frazier and Westhoff (2004), Barnett and Hunter (2000); the following test were carried out: Gram staining, catalase test, citrate test, methyl red test, indole test urea test, coagulase test, sugar fermentation test, oxidase test, lactose test, glucose test. Mannitol test and motility test.

3.5.3 Isolation of Fungal Flora

Ten gram (10g) of each fish sample was taken and crushed in a sterile mortar with pestle under laboratory condition. Nine milliliters (9ml) sterile distilled water was added and serially diluted up to 10^6 fold as described by Syllabi and Façade (Ayolabi and Fagade, 2010). 0.1ml aliquots aseptically removed separately with a sterile pipette and transferred into labeled sterile Petri dishes and 20ml melted Potato Dextrose Agar (PDA) was added by pour plate method. The PDA (Biotech, USA) was prepared according to manufactures instruction. After rotating gently, the plates were incubated at 27°C for 72 hours. Pure colonies was isolated from mixed culture and inoculated onto the surface of freshly prepared PDA which was supplemented with 30mg/ml of Chloramphenicol (Micro Lab Limited) to inhibit bacterial growth. The plates were incubated at 27°C for 72 hours.

3.5.4 Identification of fungi

Fungal isolates was transferred to sterilized plates for purification and identification. The grown fungi was placed on a slide, stained with gram stain for yeast identification and lacto phenol cotton blue to detect fungal structures covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Cheesbrough, 2000).
Macroscopic and microscopic observations were carried out on the cultures. The physical characteristics of the mycelia such as the colour and structure were noted as well as the microscopic characteristics (Barnett and Hunter, 2000).

3.6 Bacterial counting

The Petri dishes containing the 24hrs medium that was obtained from serially dilution was placed on colony counter and the colony was counted. The number of colonies counted on the plates was recorded taking into consideration the dilution factor and used to calculate colony forming units (cfu) per ml.

$$\text{CFU/ml} = \frac{No.of\ colonies\ x\ dilution\ factor}{Volume\ of\ culture\ plate}$$

4.0 RESULTS

Table 1 presents total viable counts of bacteria isolated from smoked mackerel fish bought from different markets. The total number of fifteen (15) smoked mackerel fish was purchased from 15 different fish monger in three markets as stated on the table3 below. The total viable counts ranges from $1.7x10^2$ to $40.3x10^2$ in which Nwko Mgbakwu market has the lowest total viable count and Eke Awka has the highest viable count respectively.

Table 1: Total viable count of bacteria isolated from smoked mackerel fish bought from different markets

Samples	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	$2.9\ x10^2$	$13.5\ x10^2$	$9.6\ x10^2$
2	$11.3\ x\ 10^2$	$10.8\ x10^2$	$4.1\ x10^2$
3	$3.2\ x10^2$	$2.2\ x10^2$	$10.7\ x10^2$
4	$2.4\ x\ 10^2$	$1.7\ x10^2$	$17.1x10^2$
5	$40.3x10^2$	$5.8x10^2$	$3.6x10^2$

Table 2 presents total viable counts of bacteria isolated from smoked sardine fish bought from three different markets in Awka and environments. A total number of 15 fishes were purchased from 15 different fish mongers in three different markets as stated on the table 6 below, the total viable counts obtained ranges from $0.6x10^2$-$66x10^2$ in which Eke Awka market scores the lowest and Amaenyi Awka Market scores highest respectively.

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Table 2: Total Viable Counts of Bacteria Isolated from Smoked Sardine Fish Bought from Different Markets

Samples	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	4.5×10^2	21.4×10^2	2.4×10^2
2	0.6×10^2	3.4×10^2	3.3×10^2
3	2.2×10^2	11.5×10^2	13.8×10^2
4	$13x10^2$	$20x10^2$	$3.9x10^2$
5	$10x10^2$	$6.2x10^2$	$66x10^2$

Table 3: presents total viable counts of bacterial isolates from smoked panla fish bought from three different markets in Awka and environments. A total number of 15 fishes were purchased from 15 different fish mongers in three different markets as stated on the table 3 below, the total viable counts obtained ranges from $11x10^2$-$98x10^2$ in which Nkwo Mgbakwu market recorded the lowest and Eke Awka Market recorded highest respectively.

Table 3: Total viable count of bacterial isolates from smoked panla fish bought from different markets

Samples	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	11.6×10^2	13.3×10^2	2.0×10^2
2	5.0×10^2	2.9×10^2	2.2×10^2
3	23.5×10^2	1.1×10^2	3.6×10^2
4	2.9×10^2	1.7×10^2	2.7×10^2
5	$98.1x10^2$	$8.2x10^2$	$44x10^2$

Table 4: presents total viable count of bacterial isolated from smoked catfish purchased from different markets, the TVC ranges from $2.5x10^2$ -$54x10^2$ in which Eke Awka market has the lowest TVC and Nkwo Mgbakwu market has the highest TVC respectively.

Table 4: Total viable count of bacterial isolates from smoked catfish purchased from different markets CFU/g.

Samples	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	$16. \times 10^2$	4.7×10^2	3.0×10^2
2	2.5×10^2	3.4×10^2	14.1×10^2
3	5.8×10^2	3.0×10^2	3.3×10^2
4	12.6×10^2	4.4×10^2	11.0×10^2
5	$3.2x10^2$	$54x10^2$	$5.4x10^2$

Table 5 presents bacteria species isolated from mackerel fish bought from different markets, most of the listed bacteria were isolated in all the samples expect *Stenotrophomas maltophils*, *Crocinitomicaceae bacterium*, *Comamonas thiooxydans*, which were not isolated from any of the samples purchased *Klebsella aerogene* was isolated from sample purchased from Amaenyi Awka market and *Delftia tsuruhatensis* which was only isolated from sample purchased from Eke Awka market, *Salmonella enteric* was also isolated from sample purchased from Nkwo Mgbakwu market.

Table 5: Bacteria species isolated from smoked mackerel fish bought from different markets

Samples	Bacterial isolates	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	*Staphylococcus aureus*	+	+	+
2	*Bacillus sp*	+	+	+
3	*Kelbsella Pneumonia*	+	+	+
4	*Enterobacter sp*	+	+	+
5	*Stenotrophomonas maltophils*	-	-	-
6	*Proteus mirabilis*	+	+	+
7	*Crocinitomicaceae bacterium*	-	-	-
8	*Micrococcus luteus*	+	+	+
9	*Comamonas thiooxydans*	-	-	-

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10	*Klebsiella aerogenes*	-	-	+
11	*Delftia tsuruhatensis*	+	-	-
12	*Salmonella enteric*	-	+	-
13	*Staphylococcus saprophiticus*	+	+	-

Table 6 presents bacteria species isolated from smoked sardine bought from different markets, almost all the listed bacteria were isolated expect *Crocinitomicaceae bacterium* was only isolated from sample bought from Amaenyi market, *Stenotrophomonas maltophils*, *Comamonas thiooxydans*, *Delftia tsuruhatensis* were not isolate from any of the samples.

Table 6: The bacteria isolated from smoked sardine fish bought from different markets

Samples	Bacterial isolates	Eke Awka	Nwko Mgbakwu	Amaenyi Awka
1	*Staphylococcus aureus*	+	+	+
2	*Bacillus sp*	+	+	+
3	*Kelbsella pnemoniea*	+	+	+
4	*Enterobacter sp*	-	+	+
5	*Stenotrophomonas maltophils*	-	-	-
6	*Proteus mirabilis*	+	-	+
7	*Crocinitomicaceae bacterium*	-	-	+
8	*Micrococcus luteus*	+	+	+
9	*Comamonas thiooxydans*	-	-	-
10	*Klebsiella aerogenes*	+	+	+
11	*Delftia tsuruhatensis*	-	-	-
12	*Salmonella enteric*	+	+	+
13	*Staphylococcus saprophiticus*			

Table 7: presents bacteria isolated from smoked panla fish purchased from different markets *Staphylococcus aureus*, *Klebsiella aerogenes* and *Micrococcus luteus* were isolate in all the samples bought from different markets while other isolates where absent in some samples and present in some.

Table 7: The bacteria species isolated from smoked panla fish bought from different markets

Samples	Bacterial isolates	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	*Staphylococcus aureus*	+	+	+
2	*Bacillus sp*	-	+	-
3	*Kelbsella pnemoniea*	-	-	-
4	*Enterobacter sp*	-	+	+
5	*Stenotrophomona smaltophils*	-	-	-
6	*Proteus mirabilis*	+	-	+
7	*Crocinitomicaceae bacterium*	-	-	-
8	*Micrococcus luteus*	+	+	+
9	*Comamonas thiooxydans*	-	-	-
10	*Klebsiella aerogenes*	+	+	+
11	*Delftia tsuruhatensis*	-	-	-
12	*Salmonella enteric*	-	+	+
13	*Staphylococcus saprophiticus*	+	+	+

Table 8 presents bacteria species isolated from catfish bought from different markets on the result *Staphylococcus aureus*, *Bacillius* sp. *Kelbisella pneumonia*, *Enterobacter* sp. *Proteus mirabilis*, *Micrococcus luteus*, were found in all the sample, while *Crocinitomicacae bacterium* and *Delftia tsuruhatensis* were not isolated. Other bacteria are present or absent in other samples.

Table 8: Enumeration of bacterial isolates from smoked catfish bought from different markets

Samples	Bacterial isolates	Eke Awka	Nkwo Mgbakwu	Amaenyi Awka
1	*Staphylococcus aureus*	+	+	+

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2	*Bacillus sp*	+	+	+
3	*Kelbsella pnemoniea*	+	+	+
4	*Enterobacter sp*	+	+	+
5	*Stenotrophomonas maltophils*	+	-	-
6	*Proteus mirabilis*	+	+	+
7	*Crocinitomicaceae bacterium*	-	-	-
8	*Micrococcus luteus*	+	+	+
9	*Comamonas thiooxydans*	-	-	+
10	*Klebsiella aerogenes*	+	+	+
11	*Delftiatsuruhatensis*	-	-	-
12	*Salmonella enterica.*	-	-	+
13	*Staphylococcus saprophiticus*	-	+	-

Neither bacteria nor fungi were isolated in both smoked fish and oven dried mackerel and sardine fishes. $0.8x10^2$ bacterial load was isolated in panla smoked fish and $0.2x10^2$ was isolated from oven dried panla fish no fungi was isolated.

Table 9: Total viable count of laboratory smoked and oven dried fish smoked packaged in polyethylene packaging materials and stored at room temperature

Days	Samples	Bacterial count of Smoked fish	Fungi	Bacterial count Oven dried	Fungal count
1day	Mackerel	-	-	-	-
	Sardine	-	-	-	-
	Panla	$0.8x10^2$		$0.2x10^2$	
	Catfish	$0.5x10^3$	-	-	-
	Mackerel	-	-	-	-
3^{rd} day	Sardine	-	-	-	-
	Panla	$1.0x10^2$	$2.2x10^3$	$0.9 x10^2$	-
	Catfish	$1.6x10^2$	$1.4x10^2$	-	-
7^{th} day	Mackerel	$1.3x10^2$	$2.0x10^3$	$0.6x10^2$	-
	Sardine	$1.7x10^2$	$0.9x10^2$	-	$1.7x10^2$
	Panla	$3.1x10^2$	$4.2x10^2$	$0.3x10^2$	$3.5x10^2$
	Catfish	$3.8x10^2$	$5.1x10^2$	$1.1x10^2$	$3.0x10^2$
14^{th} day	Mackerel	$3.7x10^2$	$7.2x10^2$	$2.6x 10^2$	$4.2x10^2$
	Sardine	$3.4x10^2$	$6.0x10^2$	$3.3x10^2$	$3.6x10^2$
	Panla	$3.9x10^2$	$4.9x10^2$	$2.5x10^2$	$4.1x10^2$
	Catfish	$5.2x10^2$	$8.5x10^2$	$3.3x10^2$	$3.2x10^2$
21^{st} day	Mackerel	$2.2.x10^6$	$7.8x10^6$	$1.2.x10^6$	$6.0x10^6$
	Sardine	$3.7x10^6$	$5.7x10^6$	$7.4x10^2$	$4.5x10^2$
	Panla	$7.1x10^6$	$8.2x10^6$	$5.3x10^6$	$6.6x10^6$
	Catfish	$3.5x10^6$	$6.9x10^6$	$4.1x10^6$	$5.0x10^6$
30^{th} day	Mackerel	$9.4x10^6$	$8.9x10^6$	$7.3x10^6$	$7.3x10^6$
	Sardine	$7.5x10^6$	$4.8x10^6$	$6.2x10^6$	$5.1x10^6$
	Panla	$9.0x10^6$	$7.4x10^6$	$6.6x10^6$	$6.6x10^6$
	Catfish	$5.1x10^6$	$7.5x10^6$	$9.3x10^6$	$5.6x10^6$

5. DISCUSSIONS

The biochemical test and molecular characterization carried out on isolates from these research work showed the presence of *Staphylococcus aureus*, *Bacillus*, *Kelbsiella pneumonia*, *Enterobacter* spp., *Proteus mirabilis*, *Stenotropnomonas maltopilis*, *Crocinitomicaceae* bacterium, *Micrococcus* spp, *Comamonas thiooxydans*, *Klebsiella aerogenes*, *Delfia tsuruhatensis*, and *Salmonella* spp. As presented on Table 3 the isolation of *Staphlococcus aureus*, *Bacillus*, *Kelbsiella pneumonia*, *Enterobacter* spp., *Proteus mirabilis*, *Micrococcus* spp, *Klebsiella aerogenes* and *Salmonella* spp. From the samples is in agreement with (Felix *et al*., 2015) that also isolated some of the organisms from same species used in their research work. Amos, (2007) stated that isolation of pathogenic and spoilage organisms raises public health concerns about safety in consuming smoked fish product from the markets and causes a high rate of spoilage leading to shorter shelf and storage life of the product.

Isolation of *D.tsuruhatensis* might be as a result of the environmental factors since it can biodegrade organic pollutants, such as phenolic compounds and chlorobenzene (Jimenez *et al*., 2012, Ye *et al*., 2019). Although *D. tsuruhatensis* has been rarely

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associated with human infections, the Microorganism is a possible causative pathogen for life threatens infection in immunocompromised patients and patients with port related infections (Preiswerk *et al*., 2011; Tabak *et al*., 2013, Ranc*etal*., 2018). They have also been isolated from several other species that so far have been recovered from environmental samples.

Isolation of *Comamonas thiooxidan* from the samples purchased from Amaenyi market Awka might be as the result of the environmental factors, or from the water that was used to wash the fish or exposure and from touching from numerous customer during pricing. *Comamonas thiooxidan* might be capable of oxidizing thiosulfate under a mixotrophic growth condition was isolated from sulfur spring. *Comamonadace* are environmental bacteria from water and soil habitats (Narayan *et al*., 2010).

Isolation of *Stenotrophomonas maltophilia* from the sample might be from the water body in which it was harvested or from water used for washing of the fish, it has not be reported that *Stenotrophomonas maltophilia* has been isolated from fish and fish samples till the emergency of this research work. *S. maltophilia* is an aerobic gram-negative bacillus that is found in various aquatic environments, blood stream infection, Pneumonia, urinary tract infection, wound infection, cystic fibrosis, respiratory infection, burn wound infection (Gilligan, 2003). The presence of *Proteus mirabilis* may be due to dirty environment and deposition of dust particles on the smoked fish, which is in agreement with (Umeaku, *et al*., 2018). 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 is prone to contamination with microorganisms such as bacteria and fungi (Bukola*etal*.,2008). Thisindicates that spoilage of fish starts right from the aquatic ecosystem. Handling fishes are also prone to microbial attack especially in artisanal fishery due to unhygienic methods of reducing temperature.

The total viable counts for both bacteria and fungi of commercially purchased samples as presented in Tables 5-9 revealed that some exceeded the range of specified microbiological limits recommended for fish and fishery products while some are below the range of specified microbiological limits recommended for fish and fish products by international commission on microbiological specification for foods (ICMSF,1986). The protocol of ICMSF recommends a maximum bacterial count of $5x10^5$Cfu/g for good quality product and maximum count of 10^7 for marginally acceptable quality products (center for food and safety, 2014). During smoking period, smoking kilns used and overloading of the fishes on the smoking trays might lead to improper smoking and processing which can support bacterial and fungal growth. The environment in which smoked fishes are been displayed in the markets are not always hygienic, they are been displayed on contaminated tables, near refuse dump, dirty gutter. The method of selling also contributes seriously in contamination of the smoked fish in which the smoked fishes are being touched with unwashed hands of the prospective customers. This encourages bacteria and fungi attack and subsequent production of toxins. This is in agreement with report of Bukola *et al*., (2008) and Akande and Tobor (1992).

6. CONCLUSION

The study established that smoked fish sold in Awka markets and environs are heavily contaminated with both bacteria and fungi. There is need for public enlightenment of fish mongers on application of proper hygiene during fish processing and during selling, to limit contamination of fish products.

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