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BACTERIOLOGICAL ASSESSMENT OF SOME FROZEN FISHES SOLD AT ATOR MARKET IN IKOT EKPENE METROPOLIS

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ABSTRACT

This research was carried out to assess the microbiological quality of frozen fishes sold at Ator market in Ikot Ekpene, Akwa Ibom State. Thirty fish samples from three species of fish were collected from different retailers at the market. Samples were prepared from the intestine the gills, and the skin. The microbial load of the different samples was determined using appropriate media. Total viable bacterial counts ranged between 3.02×10^4 to 52.0×10^4 cfu/g, total coliforms ranged between 9.32×10^4 to 47.4×10^4 cfu/g, total vibrio count ranged from 0.52×10^4 to 7.85×10^4 cfu/g while the total *Staphylococcal* counts ranged from 2.08×10^4 to 7.75×10^4 cfu/g. It was observed from the study that the skin and the intestines harboured more bacteria growth than the gills. The microbial load obtained in this study exceeds the international acceptable limit of ≤ 1.0×10^2 cfu/g for frozen products thus indicating the poor sanitary and poor hygienic conditions of the markets where these fishes are sold or countries where they were imported, from.
It is recommended that proper hygienic condition should be maintained during fish production, handling, storage, retailing and processing. Also, fish should be subjected to adequate cooking before consumption.

KEYWORDS: frozen fishes, coliforms, bacteria, microbiological quality.

INTRODUCTION

Fish and fish products are not only nutritionally important but also important in global trade as foreign exchange earner for a number of countries in the world (Yagoub and Ahmed, 2003). It is one of the most important source of animal protein available in the tropics and has been widely accepted as good source of protein and other elements for maintenance of a health body (Andrew, 2001; Al-jufalli and Opera, 2006). According to Claucas and Ward, (1996), it constitutes the cheapest source of animal protein in Africa.
The Food and Agriculture Organization (1994) asserted that fish contributes about 60% of the world's supply of protein and 60% of the developing world derives more than 30% of their annual protein from fish. However, in Nigeria, fish constitutes 40% of the animal protein intake (Olatunde, 1998). It is also a good source of vitamins, minerals such as fluorine and iodine which are necessary for the development of strong teeth and the prevention of goitre in man (Andrew, 2007). Fish is regarded as healthier than meat due to its high content of long chain polysaturated fatty acids which are associated with improving health and preventing diseases of old age (Kabaherd *et al*., 2009). Because of its high nutritive value, sea fishes are a major vehicle for pathogenic bacteria. Fishes can be contaminated by both aquatic environment and post-harvesting condition (Al-Sheraa, 2018).

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, cadaverine, organic acids, ketones, and sulfur compounds (Dalgaard *et al*., 2006; Doyle, 2007; Ezemba *et al.,* 2017).
Most families are used to consumption of frozen fish; especially mackerel (*Scomber japonicus*) because they are more commonly available in most Nigerian markets (Oyewole and Amosu, 2012). Majority of the frozen fishes are sold in the open markets. They are either imported frozen or caught in Nigerian waters and frozen on board (Oramadike *et al*., 2010). Marketing of fish in Nigeria is mostly carried out by local fishmongers at ambient temperature (Okoro *et al*., 2010); a condition that favours contamination and proliferation of microorganisms. Damp, unhygienic market environment and packaging/storage facilities further facilitate contamination of fish by microorganisms (Mahmuda *et al*., 2010). According to Arannilewa *et al*., (2005), protein decreased with increasing duration of frozen storage with fresh samples not frozen having higher protein content. Thus, product dehydration, rancidity, drip loss and product bleaching have an overall effect on the quality of frozen food (Kropf and Bowers, 1992). In spite of some disadvantages associated with frozen storage freezing is accepted as an effective way of preserving fish (Arannilewa *et al*., 2005).

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The microbiological contamination concern has been on high loads of unspecified spoilage bacteria like *Salmonella* sp., *Vibrio* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Vazquez-sanchez *et al*, 2012; Zarei *et al*., 2012; Falaise *et al*., 2016). Initial micro-flora on fish is directly related to the surrounding aquatic environment while the bacterial flora in the gastrointestinal tract corresponds to the condition of the fish (Liston, 1980). It has been well known that fish can harbor human pathogenic bacteria particularly the coliform group (Adedeji *et al.,* 2012).

Microbiological quality is of importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. Microbial hazards causing infections and poor health are closely related to food safety concerns with animal proteins derived from marketed food - fish, fishery products, meat and meat products. This creates a burning question for all consumers with a high risk commodity with regard to pathogenic bacteria contaminations alarming to food safety challenge (Nilla *et al*., 2012).

Most of the frozen fishes sold in Ikot Ekpene Metropolis, Akwa Ibom State are exposed to dust and contaminated which encourages the growth of microorganisms due to their ubiquity and thus enhance transmission and prevalence of pathogens. Moreover, poor personal hygiene such as neglecting to wash hands after handling contaminated materials encourages contamination of frozen fish with pathogenic organisms. The aim of this study is to assess the microbiological quality of different frozen fishes sold in Ator market, Ikot Ekpene Metropolis Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

Study area and Sample Collection

A total of thirty (30) frozen fish samples were purchased from Ator Market, Ikot Ekpene Local Government Area of Akwa Ibom State. Ten samples each including Titus (*Scromber scrombus*), Shyna (*Trachurtus trachurus*) and Sardines (*Sardinella aurita*) were collected aseptically and placed in a food grade sterile container. The fish samples were immediately transported in a cooler containing ice blocks to the Department of Microbiology Laboratory, Ritman University within an hour for analysis.

Sterilization of Glasswares

The glass wares (pipettes, test-tubes, beakers, measuring cylinders, conical flasks etc.) were sterilized in a hot air oven at 160^0C for 1 hour. Pipettes were dried and kept in a canister before sterilization. Test-tubes were wrapped in aluminium foil; scalpels were wrapped in aluminium foil while the beakers, measuring cylinders and conical flasks were plugged with cotton wool before sterilization.

Preparation of Media

Media were prepared by weighing appropriate amount of the powder and dissolved in 1 litre of distilled water in a conical flask as stated by the manufacturer. The media were then homogenized by boiling before sterilizing in the autoclave at 121^oC for 15 minutes. The sterile media were allowed to cool to about 45^oC before being poured into sterile petri-dishes and allowed to set (Cheesbrough, 2006).

Nutrient agar

Nutrient agar (NA) was prepared according to the manufacturer's instruction. A 28g weight of the powder was weighed and dispensed into 1litre of distilled water. It was allowed to soak for 10 minutes, stirred to mix and sterilized by autoclaving at 121°C for 15minutes. The sterilized media was allowed to cool to 45°C. The sterile media was poured into sterile Petri dishes and allowed to solidify.

MacConkey agar

Macconkey agar (MCA) was prepared according to the manufacturer's instruction. An 49.53g weight of the powder was weighed and dispensed into 1litre of distilled water. It was allowed to soak for 10 minutes, stirred to mix and sterilized by autoclaving at 121°C for 15minutes. The sterilized media was allowed to cool to 47°C. The sterile media was poured into sterile Petri dishes and allowed to solidify.

Thiosulfate citrate bile salts sucrose agar

Thiosulfate citrate bile salts sucrose (TCBS) agar was prepared according to the manufacturer's instruction. An 88.1g weight of the powder was weighed and dispensed into 1litre of distilled water. It was allowed to soak for 10minutes, stirred to mix and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media was allowed to cool to 47°C. The sterile media was poured into sterile Petri dishes and allowed to solidify.

Mannitol salt agar

Mannitol Salt Agar (MSA) was prepared according to the manufacturer's instruction. A 111g weight of the powder was weighed and dispensed into 1litre of distilled water. It was allowed to soak for 10 minutes, stirred to mix and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media was allowed to cool to 47°C. The sterile media was poured into sterile Petri dishes and allowed to solidify.

Preparation of Samples for Microbiological Analysis

Bacterial counts of the external surfaces, intestines and gills were estimated as follows:

Skin surfaces: Sample from different locations of the skin of 30 raw fishes representing each of the fish types were taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of sterile distilled water. A ten (10) fold serial dilution of the bacterial suspension already inoculated in sterile distilled water was prepared in duplicates and viable aerobic bacterial counts were enumerated using 0.1ml inoculum in standard plate count agar as described by Slaby *et al.*, (1981)**,** and then incubated at 37^oC for 24 hrs.

Intestines and Gills: Ten grams of intestines and gills of the frozen fish were collected using a dissecting set. These were separately added to 90 ml of sterile distilled water and homogenized in a blender. A 1 ml weight of the homogenate was transferred to a test tube containing 9 ml peptone water to obtain a dilution of 10^{-1}. In a similar manner, 1 ml was transferred from this dilution to a test tube containing 9 ml diluents and the process was repeated until a dilution of 10^{-6} was obtained according to the method of Esther *et al*., (2010) and Slaby *et al.,* (1981).

Enumeration of bacteria

Total viable bacterial counts were determined using spread plate method with nutrient agar. Total coliform bacteria were enumerated with MacConkey agar. *Staphylococcus* spp were enumerated using Mannitol

salt agar and Thiosulphate Citrate Bile Salt Sucrose agar for pathogenic *Vibrio spp*. The plates were incubated at 37^{o}C for 24 hours. The observed colony growth were counted using Coulter™ colony counter according to plate count method. Only plates with colonies between 30 to 300 cfu/g were used in enumeration and were expressed as colony forming unit per gram (cfu/g) of the samples (Ashok, 2008; Esther *et al*., 2010).

RESULTS

The results of the assessment of the bacteriological quality of frozen fishes (*Scomber scombus Sardinella aurita* and *Trachurus trachurus*) sold at Ator market, Ikot Ekpene, Akwa Ibom State are as presented in figures 1, 2 and 3.

Results obtained from the total viable bacterial counts (TVBC) for all the fishes ranged from 3.02×10^4 to 52.0×10^4 cfu/g with the skin and gills of *Scomber scombrus* having the highest and lowest counts respectively. The skin and gills of *Scomber scombrus* also had the highest (47.4×10^4 cfu/g) and the lowest (9.32×10^4 cfu/g) total coliform counts (TCC) for all the three species of fishes examined. Total Vibrio count (TVC) was highest (2.85×10^4 cfu/g) in the skin of *Trachurus trachurus* and lowest (0.52×10^4 cfu/g) in the gills of *Scomber scombrus*. The intestine of *Scomber scombrus* and the gills of *Sardinella aurita* recorded the highest (7.75×10^4 cfu/g) and lowest (2.08×10^4 cfu/g) total staphylococcal counts (TSC) respectively.

Figures 1, 2 and 3 show a graphical representation of microbial counts (Log_{10} cfu/g) of *Scomber scombrus*, *Sardinella aurita* and *Trachurus trachurus* respectively.

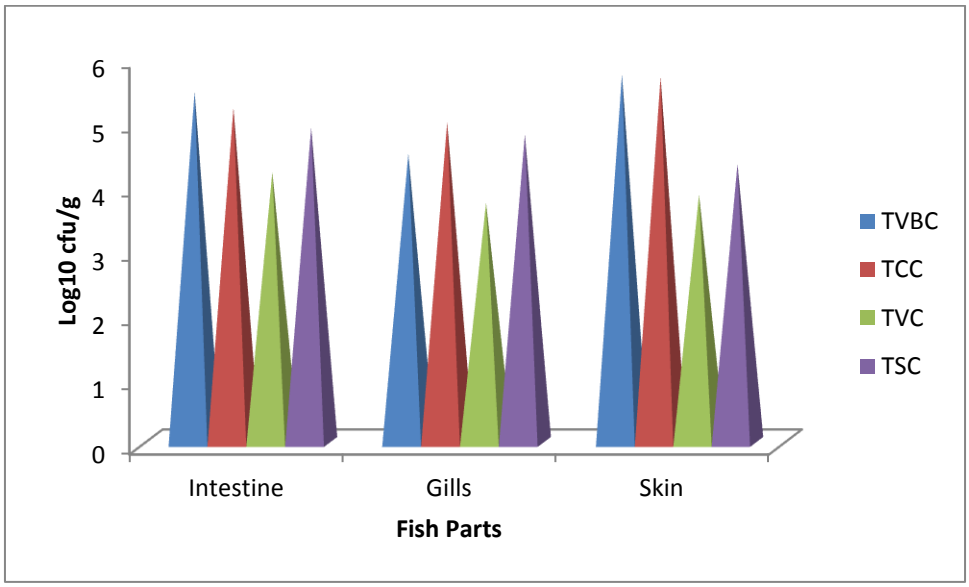

Fig. 1: Microbial counts from different parts of *Scomber scombrus*

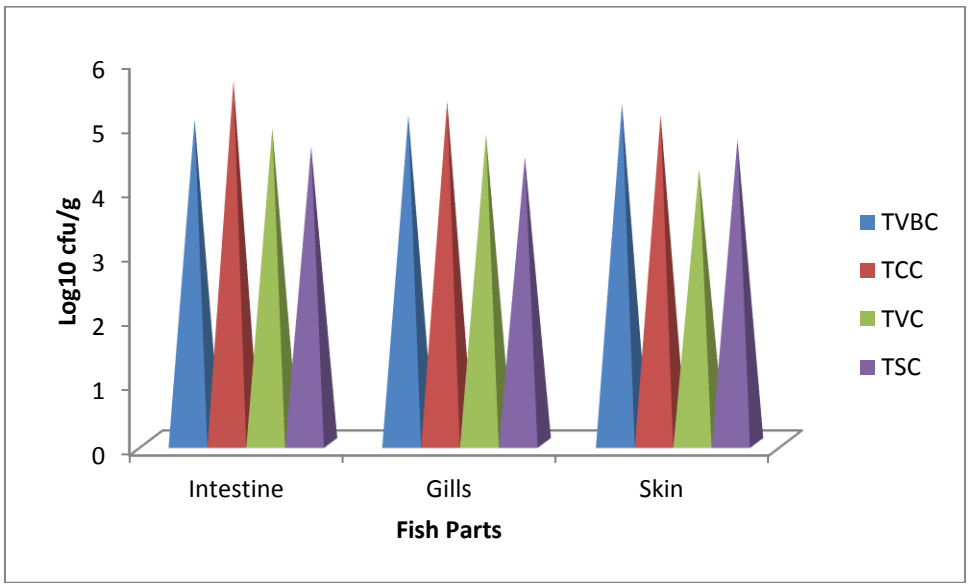

Fig. 2: Microbial counts from different parts of *Sardinella aurita*

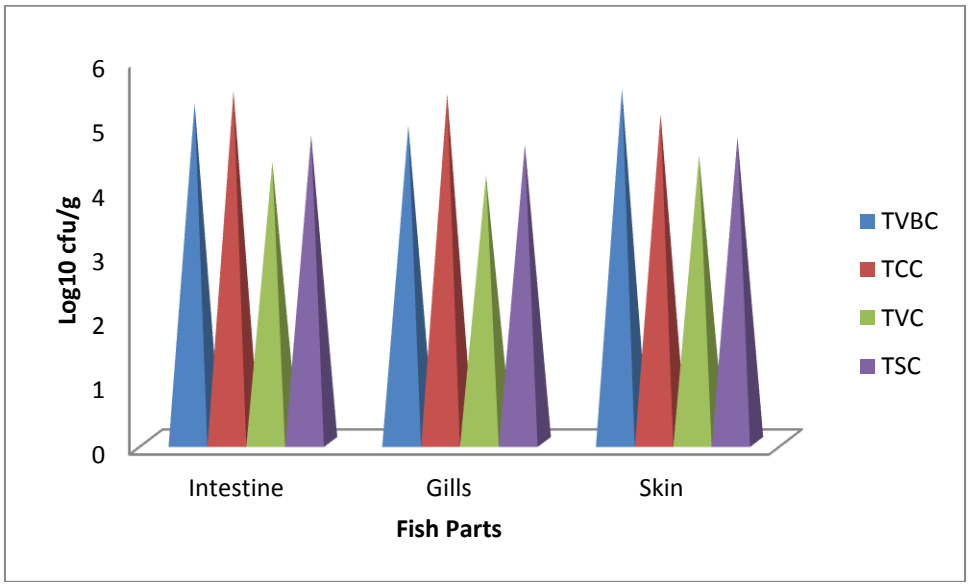

Fig. 3: Microbial counts from different parts of *Trachurus trachurus*

DISCUSSION

The spoilage of fish caused by different bacteria is not uncommon. It is thus essential to ensure the quality of fish as well as the consumer's safety. High microbial loads were found in all the species of fish examined. This study revealed that the microbial load of fish samples is largely dependent on the fish species, with the fatty fish most likely to support microbial growth than the low fat fish. This assertion agrees with the observation of Ezemba *et al.,* (2017) who opined that provided all conditions remain constant, low fat fish withstands microbial deterioration better than the fatty fish counterpart. Also other factors which influenced the microbial counts are the various parts of the fish samples, with the highest counts obtained from the skin though closely followed by the intestine for *Trachurus trachurus* and *Scomber scombrus*. Poor hygiene in handling and packaging might have been the reason for high microbial load on the skin Preservation in low quality ice, handling with contaminated hands and storage could also be responsible for higher loads of viable bacteria as experienced in this study. Fish are highly susceptible to contamination with different bacteria because of their high protein content (Shankar *et al.,* 2009).

Although the preponderance of microbe also depends on the type of microbe at a particular fish part, for example, vibro spp was higher in the intestine of the fishes. This however contradicts the report of Kabaherda *et al.,* (2009) who reported highest counts from the internal organs while the least counts were obtained from the skin. The TVBC values were within the ranges in the findings of Emikpe *et al.,* (2011) and Ezemba *et al.,* (2017). Indicator organisms, *E. coli* and other total coliform bacteria were found in all the samples analyzed. The presence of coliform suggests external contamination of fish because coliforms are not normal intestinal flora in fish (Emikpe*et al.,* 2011). None of the fish samples met the international acceptable limit of ≤ 1.0×10^2 cfu/g for frozen products.

In conclusion, fishery products are important not only for its nutrition but as an item of trade and foreign exchange globally. Also worthy of note, as indicated in this research is that exposing fish for a long time without refrigeration is highly unsafe. This practice makes it prone to gross microbial deterioration. Fish is a food to man, they say and everything consumed by man should be kept clean.

From this study, the microbiological assessment of most commonly consumed fishes in this part of the world, has further revealed the low quality of our frozen fishes. This could be due to unsanitary and unhygienic conditions prevalent in the markets and the countries where some of the fishes are imported from.

High level of microbial loads were obtained from each of the frozen fish samples, it is therefore advised that eating fish in its raw form should be completely avoided.

It is recommended that fish should be subjected to adequate cooking and safe preparation before consumption by humans. Adequate hygiene practice from production, retail and processing should be upheld by fish vendors to avoid both the introduction of new microbial species and cross contamination.

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