Microbial Quality Of Frozen Fish Sold In Uyo Metropolis

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ABSTRACT: Microbial quality of frozen fishes: Shinna (*Auxis thazard*), Bonga (*Ethmalosa fimbriata*) and Mackerel (*Scomber scombrus*) obtained from three different markets were carried out using standard methods. The total heterotrophic bacterial count ranged from 3.0 x10 ^{5} to 5.0 x 10 ^{5} cfu/g, 3.0x 10 ^{5} to 4.8 x10^5 cfu/g and 3.0 x10^5 to 6.3 x10^5 cfu/g for Shinna, Bonga and Mackerel. The Total coliform count ranged from 2.0x10 ^{5} to 4.0x 10 ^{5} cfu/g, 2.8x10 ^{5} to 3.9x10^5 cfu/g and 2.0 x10 ^{5} to 6.0x10^5 cfu/g for the three fishes respectively. The Samonella count ranged from 2.0 x 10^5 to 1.5 x10 5 cfu/g for Shinna and Bonga only. The total Vibrio count ranged from 1.0 x 10^5 to 1.5 x 10 ^{5} cfu/g for Shinna and Bonga only. Total fungi count ranged from 1.3 x 10 ^{5} to 3.0x 10 ^{5} cfu/g, 2.0 x 10 ^{5} to 4.0 x 10 ^{5} cfu/g and 1.0 x 10^5 to 2.4 x 10 ^{5} cfu/g for the three fishes respectively. The Bacteria isolated from frozen fish samples were Staphylococcus *aureus, Esherichia coli, Vibrio sp, Salmonella sp, and Pseudomonas sp Micrococcus sp* while fungi isolated were *Aspergillus niger, Penicillium sp, Rhizophus stolonifer and Monilia sp.* The bacterial isolates that occurred most frequently in the three different types of frozen fish samples includes *Staphylococcus aureus* (20.0%), *Escherichia coli* (20.0%), and *Pseudomonas sp* (20.0%). Others were *Micrococcus sp* (15.0%) and *Vibrio sp* (10.0%). Among the fungal isolates, *Aspergillus niger* was the most predominant (35.0%), followed by *Penicillium* sp. (30.0%), *Rhizopus stolonifer* (20.0%) and *Monilia sp* (15.0%) occurred least. The study showed that the frozen fish samples were heavily contaminated which may be as a result of poor sanitary practices employed by the vendors. This is of public health concern as these organisms are known causes of food-borne diseases.

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1. INTRODUCTION

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as good source of protein and other elements for maintenance of health body (Andrew, 2001). The less developed Countries capture 50% of the world harvest and a large proportion of the catch are consumed internally. In many Asian countries over 50% of the protein intakes comes from fish while in Africa the proportion is 17.50% (Willians and Dennis, 1988). In Nigeria, fish constitute 40% of the animal protein intake (Olatunde, 1998). Fish and fish products constitute an important part in the international trade, currently worth more than US and 50 billons indicating increasingly consumer interest in commodity. Generally, fish are good sources of vitamins B12 and B6. It is also good source of fluorine and iodine which are needed development of strong teeth and the prevention of goiter in man (Andrew, 2001). However, availability of these vital nutrients depends to a large extent on the methods of storage (Ryder et al., 1993), such as salting roasting, drying and freezing. Iced fish of different types are of great demand by the Nigeria consumers as a relatively cheaper source of animal protein (Ryder et al., 1993).

Also, seafood derived from wild fish as well as farmed fish has always been an important source of protein in the human diet (Yagoub, 2009). On a global scale, fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture (Håstein et al., 2006; Yagoub, 2009). Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Yagoub, 2009). Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits (Yagoub, 2009). In addition, they can also function as carriers of several microbial and other health hazards (Yagoub, 2009). Therefore maintenance of quality is of utmost importance in production and trade of fishery products. Most of current quality control techniques are time consuming and cumbersome

(Yagoub, 2009). Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub, 2009).

According to the Center for Food Safety and Applied Nutrition in Washington (2001), most fish related food borne illness are traced to *Salmonella*, *Staphylococcus spp.*, *Escherichia spp*., *Vibrio parahemolyticus, Clostridium perfringens, Clostridium botulinum E,* and *Enteroviruses* (Yagoub, 2009). Microorganisms are found mostly on the skin, gills, operculum and intestines of live and newly caught fish. The microbial loads vary enormously in the different parts of the fishes and reported the normal range of 10^{-2}- 10^{7} on skin surfaces. Fish contamination can also be linked to raw material, personnel, processing tools such as forklifts through leakage, opening in building and pests. Some pathogens may even become established in the processing plants from niches where they can survive for a long period of time. The quality of our fish is of major concern to the food processors, consumers and public health authorities and provisions of safe, wholesome and acceptable fish and its product as food to consumers and control of microorganisms is essential to meet these objectives. The potential of seafood to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Younes and Bartram, 2001; Wright et al., 2004). Water-related diseases continue to be one of the major health problems globally (Adebayo-Tayo et al., 2011a,b). Okonko et al. (2008, 2009) reported that both bacteria and fungi are common flora of frozen fish and fish related products during packaging.

The problem associated with identification of pathogens from seafoods demand development of accurate and rapid identification methods (Young-Jun et al., 2000). This study therefore, examined the microbiological quality in fishes sold in retail markets for consumption.

2. MATERIALS AND METHODS

2.1 Collection of samples

Frozen samples of frozen fish Mackerel, Bonga and Shinna were purchased from a retailer at Uyo, Itam and Appanadem markets at different period. The three different samples were placed in different sterile polythene bag and conveyed to the laboratory for microbiological analysis. The samples: *Scomber sombrous* (mackerel), *Ethmalosa fimbriata* (Bonga) and *Auxis thazard* (shinna) were aseptically removed from the polythene bag and were placed on a sterile trays and with the aid of sterile trays and with the aid of a sterile knife, cuts were made from the edible parts of the fishes and homogenized and about 10g taken for microbiological analysis.

2.2. Skin:

Sample from different locations of 150 raw fish of the skin was taken by rubbing the sterilized cotton swab over the skin and then inoculating into the nutrient broth.

2.3. Gills:

The sterilized cotton swab was wiped against the gill filaments by lifting the operculum with the help of a pair forceps. The sample was inoculated in the nutrient broth as well as swabbed on nutrient agar. A part of the gill filament removed aseptically was also placed in a separate (nutrient broth, MacConkey broth and Selinite F broth tubes) in order to isolate all the bacteria present on the gill filaments which might have escaped contact with the swab. The examined gills were taken from 150 raw fish.

2.4. Intestine and muscles:

This was done by cutting a part of intestine and muscle after sterilizing with red hot scalped and inoculation in the media (nutrient broth, MacConkey broth and Selinite F broth tubes). The samples included 150 intestines and 150 muscles from 150 raw fish.

2.5. Enumeration, Isolation and Identification of Bacterial and Fungi Isolates

Preparation of the media, Isolation and identification of the bacteria were done according to Cheesbourgh (1984). Sterilization of the media was done by autoclaving at 121°C for 15 min. Pour plate method was employed for the determination of microbial load of samples using different solid media. Multiple tube fermentation procedure (also known as the Most probable number procedure); a quantitative analysis of food and water samples was employed to give a statistical estimate of the number of bacteria that would give the observed result. It was used in the enumeration of coliforms especially feacal coliforms. Ten fold serial dilutions of the samples was made and 10^{-5} dilution of the samples from different location were plated out on Nutrient agar medium for total heterotrophic bacteria counts, MacConkey agar was used for total Coliform counts, Salmonella/Shigella agar for total Salmonella/Shigella counts, Mannitol Salt Agar for Staphylococcus counts, Thiosulphate citrate bile salt sucrose agar for total Vibrio counts and Sabouraud dextrose agar for total fungal counts using the pour plate technique. All samples were incubated at 37°C for 24 - 48 h. Counting was done according to Plate

count method, media used for Total bacterial count and *Pseudomonas* count (nutrient agar and Muller and Hinton), coliform count (MacConkey agar) *E. coli* count (EMB agar). Sabourand dextrose agar and potato dextrose agar (PDA, Difco) were used for the total fungal counts and incubated at 28 ± 1^{O}C for 5 days under 12 h photoperiod. After incubation, observed colonies were counted and then isolated. The bacterial isolates were further examined for their ability to ferment sugar, carbohydrate production of indole from tryptophan, citrate utilization, catalase production and oxidase test. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008). The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification (Samson and Varga, 2007).

3. RESULTS ANALYSIS

The results of the total heterotrophic count of bacteria on the nutrient agar are shown in Table 1. The highest number of bacteria count was obtained from mackerel which was 6.3 x 10^5 cfu/g in Uyo, while the least count of 3.0 x 10^5 cfu/g was from Shinna, Bonga and Mackerel from Anpkanadem markets respectively. Also, Table 1 showed the result obtained for the total coliform count ranged from 2.0 x 10^5 to 4.0 x 10^5 cfu/g, 2.5 x 10^5 to 3.9 x 10 ^{5}cfu/g, 2.0 x10^5 to 6.0x 10^5 cfu/g for Shinna, Bonga and Mackerel from different markets respectively. Total Salmonella- shigella count was 2.0 x 10^5 cfu/g and 1.1 x10^5 cfu/g for Shinna and Bonga, no count for mackerel. The highest Vibrio count obtained was from Bonga 1.1 x 10^5 cfu/g from Uyo market while the least count obtained was from Shinna 1.0 x 10^5 cfu/g, from Uyo market with no count for mackerel. Total fungi count ranged from 1.3 x 10^5 to 2.6 x10^5 cfu/g, 2.0x10^5 to 4.0 x10^5 cfu/g and 1.0 x 10^5 to 2.4 x 10^5 cfu/g for Shinna, Bonga and Mackerel in the three different markets respectively.

Results of cultural and morphological characteristics showed that most isolates were gram negative rods. Two Gram positive cocci were isolated. The bacterial isolates from the three types of fish samples (Bonga, Mackerel and Shinna) included *Staphylococcus aureus, Escherichia coli, Salmonella sp, Vibrio sp, Pseudomonas sp* and *Micrococcus sp* are shown in Table 2. The bacterial isolates that occurred most frequently in the three different types of frozen fish samples includes *Staphylococcus aureus* (20.0%), *Escherichia coli* (20.0%) and *Pseudomonas sp* (20.0%). Others were *Micrococcus sp* (15.0%) and *Vibrio sp* (10.0%).

Table 1: Total Bacterial Count of Frozen Fish Sold in Uyo Metropolis

FISH	MARKET	THC (Cfu/g)	TCC (Cfu/g)	TSSC (Cfu/g)	TVC (Cfu/g)	TFC (Cfu/g)
Shinna	Uyo Main	4.2x10^5	2.8x10^5	-	1.0x10^5	3.0x10^5
	Itam	5.0x10^5	4.0x10^5	2.0x10^5	-	1.3x10^5
	Akpanandem	3.0x10^5	2.0x10^5	-	-	2.6x10^5
Bonga	Uyo Main	4.8x10^5	2.8x10^5	1.1x10^5	1.5x10^5	4.0x10^5
	Itam	4.2x10^5	3.9x10^5	-	-	2.1x10^5
	Akpanandem	3.0x10^5	2.5x10^5	-	-	2.0x10^5
Mackerel	Uyo Main	6.3x10^5	6.0x10^5	-	-	2.9x10^5
	Itam	4.8x10^5	4.4x10^5	-	-	2.0x10^5
	Akpanandem	3.0x10^5	2.0x10^5	-	-	1.0x10^5

Keys: THC: Total Heterotrophic count; TCC: Total Coliform count; TSSC: Total Salmonella/Shigella Count; TVC: Total Vibrio Count; - : No count

Table 2: Frequency of Occurrences for Bacterial Isolated From Frozen Fish

Location/fish	Bacterial Isolates	No. (%)
AS,US,IB,UM,AB	*Staph. aureus*	5(25.0)
IM,UM,US,IB	*E. coli*	4(20.0)
IS,UB	*Salmonella sp*	2(10.0)
IB,UM	*Vibrio sp*	2(10.0)
US,UB,IM,AM	*Pseudomonas sp*	4(20.0)
UB, IS, AB	*Micrococcus sp*	3(15.0)
Total		**20(100.0)**

Keys: US= Uyo Shinna; UM=Uyo Mackerel; IS=Itam Shinna; AS= Akpandem Shinna; UB= Uyo Bonga; IM= Itam Mackerel; IB= Itam Bonga; AB= Akpandem Bonga

The fungal isolates include *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium sp*, and *Monilia sp* were shown in Table 3. *Aspergillus niger* was the most predominant among the fungi isolates with (35.0%), followed by *Penicillium sp* (30.0%), *Rhizopus stolonifer* (20.0%) and *Monilia sp* (15.0%) occurred least (Table 3).

Table 3: Frequency of Occurrences for Fungal Isolated From Frozen Fish

Location/fish	Fungal Isolates	No. (%)
IS, AS, US, IB, UM, AB, IM	*Aspergillus niger*	7(35.0)
IS, AS, UB, UM, US, IB	*Penicillium sp*	6(30.0)
IM, UM, AS, UB	*Rhizopus stolonifer*	4(20.0)
IS, UM, UB	*Monilla sp*	3(15.0)
Total		**20(100.0)**

Keys: US= Uyo Shinna; UM= Uyo Mackerel; IS= Itam Shinna; AS= Akpandem Shinna; UB= Uyo Bonga; IM= Itam Mackerel; IB= Itam Bonga; AB= Akpandem Bonga

4. DISCUSSION

Fish is an important food commodity in the international trade but they deteriorate rapidly especially when storage facilities are lacking. It has been widely accepted as a good source of protein and other elements necessary for the maintenance of

healthy body. Frozen fish (Shinna, Bonga, and Mackerel) was examined for the presence of microorganisms ; the total heterotrophic bacterial count gave the following 3.0x 10^5 to 4.8 x 10^5 cfu/g, 3.0x 10^5 to 4.8 x 10^5 cfu/g and 3.0 x 10^5 to 6.3 x 10^5 cfu/g for Shinna, Bonga and mackerel of three different markets. These values are within the permissible range of ice fish product. The total coliform count of the three fishes were 2.0x 10^5 to 4.0 x 10^5 cfu/g, 2.5x 10^5 to 3.9 x 10^5 cfu/g and 2.0 x 10^5 to 6.0 x 10^5 cfu/g respectively. For Salmonella, the mean count is 1.1 x 10^5 to 2.0 x10^5 cfu/g for Shinna and Mackerel, with Bonga having no count. Arannilewa et al. (2006) also found that the total coliform count range in fish was between 3.0 x 10^3 - 7.5 x 10^6 with increasing values, as the duration of storage increases. Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g).

From the result of this study, it can be seen that frozen fish sold in the market has high contamination may be as a result of certain factors like temperature which favours some organisms and the character of fish handler, by not maintaining personal hygiene, contaminated water taken in by the fishes which may contain faecal matter in their ecosytem which resulted in the isolation of enteric organism like *Escherichia coli, Salmonella typhi*, *Salmonella paratyphi and Vibrio cholerae* as well as other microorganisms which causes contamination like that of *Aspergillus niger* which may produce toxic material, when fish is consumed by man. This is in agreement with earlier report by Itah et al. (1996) on bacteriological and chemical characteristics of rural water supplies in Calabar and that of Agbu et al. (1998) in Kastina in terms of high viable counts of coliform density in the water ecosystem. These may be one of the reasons of the discovery of *E. coli*, in the fishes of this study. It is important to note that samples containing high level of coliform density and high viable bacterial counts are ill-fit for human consumption.

Apart from the enteric – organisms, *Staphylococcus aureus* encountered in this study are known enteriotoxic producing agent and a microorganism which is poisonous. This is in agreement with the previous study by some authors in Nigeria and outside Nigeria (Okonko et al., 2008, 2009). Other bacteria isolated from iced fish include *Salmonella sp, Vibrio sp*, and *Micrococcus sp.* In a study by Yagoub (2009), *Enterobacteriaceae* were isolated from gills, skin, muscles and the intestine of 83 out of 150 (55%) randomly collected fishes, the most dominants isolates were *E. coli*, *Citrobacter spp*, *Enteriobacter spp* and *Klebsiella spp*. This also confirms the findings of Koutsoumanis and Nychas (2000); Gonzalez-Podriguez et al. (2001), Yagoub et al. (2004), Herrera et al. (2006) and Yagoub (2009) who isolated similar organisms from fish and fish products. Thampuran et al. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform were *Escherichia coli* is the most common contaminant and is often encountered in high numbers. The presence of *E. coli* as well as verotoxigenic *E. coli* O157:H7 in fish meal was also investigated by some other authors (Ayulo et al., 1994; Hwang et al., 2004; Thampuran et al., 2005; Ristori et al., 2007).

In this study, *Pseudomonas spp* was isolated from the fish samples collected from the three locations. The isolation of *Pseudomonas spp* from the fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. This is in accordance with what was previously reported by Koutsoumanis and Nychas (2000), Jeyasekaran et al. (2006) and Yagoub (2009) who identified *pseudomonads* as a good spoilage index.

The total fungal counts ranged from 1.3 x 10^5 to 3.0x 10^5 cfu/g, 2.0 x 10^5 to 4.0 x 10^5 cfu/g and 1.0 x 10^5 cfu/g to 2.4 x 10^5 cfu/g for the three fishes in the three market respectively. The highest count 4.0 x 10^5 cfu/g is Uyo in Bonga while the lowest count 1.0 x 10^5 cfu/g ifs from mackerel in Akpanadem market. The fungi isolates includes *Aspergillus niger, Penicillium sp, Rhizopus stolonifer and Monilia sp.* Heinitz et al. (2000) found that 10% of imported and 2.8% of domestic raw seafood was positive for *Salmonella. Enterococcus sp* and *Aeromonas sp*, fecal and total coliform, the presence of *Listeria sp* and *Salmonella spp* from the external surface of tilapias were shown by Morales et al. (2004). Håstein et al. (2006) outlined and discussed the hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood that may pose a risk to human health.

In this study, the presence of contaminating bacteria in sea foods could be attributed to cross-contamination from environment, source, and handling by the sellers (Bryan et al., 1981; Bryan, 1988). The microorganisms reported in this study are similar to what has been reportedly isolated in other studies in Nigeria (Okonko et al., 2008, 2009; Chukwuka et al., 2010; Akinmusire, 2011; Akintobi et al., 2011; Al-Hindi et al., 2011; Adebayo-Tayo et al., 2011a,b, 2012). It showed that the organism isolated from frozen suggest the high level of contamination of water body were these fishes are

caught, meaning that water body is not free from microorganisms. The isolates obtained from this study are similar in character to hose reported by previous work on frozen fish by Jay (1996) with the following isolates *Bacillus sp, Escherichia coli and Staphylococcus sp*.

The importance of *Vibrio spp* as a contaminant of raw or under cooked seafood has been well established (Gopal et al., 2005; Lucan et al., 2008). Species such as *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. fluvialis, V. furnissii, V. metschnikovii, V. hollisae* and *V. damsela* are human pathogens (Adeleye et al., 2010). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye et al., 2010). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi et al., 2004). The presence of other species of *Vibrio* (*Vibrio parahaemolyticus, Vibrio fluvialis,* and *Vibrio mimicus*) agrees favourably with previous studies by Gopal *et al*. (2005), Colakogu *et al.* (2006), Ali (2010) and Adebayo-Tayo et al. (2011a,b) in a similar study on shellfishes. Ristori et al. (2007) isolated *Aeromonas spp., Plesiomonas shigelloides, Vibrio cholerae* 01, *Vibrio parahaemolyticus, and Vibrio vulnificus* from different organs of fishes. Young-Jun et al. (2000) isolated Vibrio strains from imported frozen seafoods. It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (Hwang et al., 2004; Yagoub, 2009).

The microbial composition of fish depends upon the microbial counts of water in which they live. However, fresh and internal organs of freshly caught healthy fish from tropical and temperature water are normally sterile because the scale and slime covering the fish serve as biological barriers to the entry of microorganisms (Frazier and Weesthoft 1991; Jay 1996). Even though epidemiological evidence on outbreak of food borne disease is scarce, there are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipments/utensils and the raw food materials (Edema et al., 2008). Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food borne illness (Moyo and Baudi, 2004; Ajao and Atere, 2009). However, contamination of the fish may occur from food handlers and retailer who sell these items to the public for consumption (Adebayo-Tayo et al., 2011a,b).

5. CONCLUSION

The findings of this present study may be considered as additional knowledge to enhance proper controlling of the storage life of fish, and fish product quality in Nigeria. This study revealed that raw fish sold at different markets in Uyo metropolis in Akwa-Ibom State, South-Southern region of Nigeria could be a source of food-borne bacterial and fungal pathogens. It has also shown that samples of fresh fish and frozen fishes used in this study were grossly contaminated by pathogenic organisms such as *Staphylococcus aureus, Escherichia coli, Salmonella sp, Vibrio sp, Pseudomonas sp* and *Micrococcus sp,* Aspergillus *niger*, *Rhizopus stolonifer*, *Penicillium sp*, and *Monilia sp* and thus, constitute potential public health hazard due to the unhygienic nature of fish handlers which predisposes frozen fishes to contamination by pathogenic microorganisms. This call for public health concerns and improvements in handling and processing are needed to minimize the prevalence of the pathogens. The total viable counts strongly suggest the urgent need to improve the quality control and assurance systems. The results of this study also constitute an indicator of bacteriological contamination of a variety of fishes. However, fishes should be properly cooked before consumption and good quality control measures should be adopted in culturing, processing, harvesting and consumption of sea foods. To limit the microbial loads of frozen fish, we suggest the provision of the adequate storage facilities i.e. refrigerator by retailer so as to avoid the multiplication of microbes under atmospheric temperature in the market.

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