Evaluation of Microbial Contamination of vended Frozen Fish in Ado Ekiti Locality

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Abstract

The basic nutrient of protein in fish that is so important in man’s diet also attracted microorganisms for their growth and multiplication. Meanwhile, the association of microorganisms in fishes depend on environment of culture and their proliferations due to inadequate storage facilities. This study examined microbial contamination of some frozen fishes (Atlantic Mackerel (Scomber scombrus), White hake (Urophysicus tenuis), Atlantic herring (Clupea harengus) and Trachurus trachurus (Atlantic horse mackerel) vended in Ado Ekiti metropolis. On the fish samples, total heterotrophic count (THC) of between $3.5 \times 10^3 – 5.6 \times 10^4$ colony forming unit per gram (Cfu/g), Coliform count in total (CCT) of $2.4 \times 10^3 – 5.1 \times 10^4$ Cfu/g, Salmonella/Shigella counts in total (SSCT) of $1.3 \times 10^3 – 3.5 \times 10^4$ Cfu/g, total Vibrio count (TVC) of $1.1 \times 10^3 – 2.3 \times 10^4$ Cfu/g and total fungal count (TFC) of $1.3 \times 10^3 – 2.3 \times 10^4$ Spore/g were analyzed by cultural methods. The microorganisms identified with their percent-age occurrence are Bacillus cereus (11.54%), Streptococcus faecium (13.46%), Alcaligenes faecalis (5.77%), Salmonella tph (5.77%), Micrococcus luteus (9.62%), Vibrio cholera (3.85%), Aerococcus viridans (3.85%), Pseudomonas aeruginosa (7.69%), Xanthomonas fragariae (7.69%), Staphylococous aureus (11.54%), Clostridium butyricum (7.69%), Escherichia coli (7.69%), Aspergillus fumigatus (11.11%), A. flavus (24.44%), A. niger (8.89%), A. fischeri (6.69%), A. terreus (8.89%), Mucor muscoides (17.78%), Penicillium digitatum (13.33%) and A. parasiticus (8.89%). The results obtained could serve as an awareness to consumers that microbial infection is possible from frozen fishes and also could serve as data for future reference in epidemiology or outbreak of disease from eating frozen fish.

Keywords: Ado Ekiti; Frozen Fish; Microbiological; Quality; Vended.

Introduction

Fish and their products are vital protein sources and have been reported that aquaculture made it possible for most of the fish that are consumed by humans [1]. Fish consumption by man has been for ages and till date served as important constituent in foods for number of some countries in the world. Fish are mainly consumed because of their high nutrient and the fact that when eaten digest easily. Fish inhabit different waters such as fresh water, salt water and is able produce toxins in fish when temperature is lowered to thawed [5]. Clostridium botulinum can in freezing temperature -2°C [5]. Fish are contaminated before freezing as result of poor maintenance of healthy body [3]. Pathogenic bacteria in fish are of two categories; those that are indigenous in fish and those not resident in fish. Bacteria pathogens such as Salmonella, Clostridium, Staphylococcus species, Escherichia coli and Listeria monocytogenes are examples of the non-indigenous and they contaminate fish habitats. The indigenous bacteria such as Aeromonas and Vibrio species are the naturally occurring pathogens found in the environment where fish inhabit [4]. Bacteria pathogens like Vibrio, Alcaligenes Pseudomonas, Flavobacterium and Moraxalla species can grow in fish, survive freezing temperature and are able to resume spoilage of fish when thawed [5]. Clostridium botulinum can in freezing temperature and is able produce toxins in fish when temperature is lowered to 3°C [5]. Fish are contaminated before freezing as result of poor...
design of plants and the methods of fish catching [6]. Due to the many ways fish can be contaminated, this study was aimed to identify the microorganisms associated with frozen fish vended in some markets in Ado Ekiti of Nigeria.

**Materials and Methods**

**Collection of Samples**

Four each of different fish species namely Atlantic Mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), White hake (*Urophycis tenuis*) and Atlantic horse mackerel (*Trachurus trachurus*). The fish samples were randomly purchased at different period of morning and evening from retailers in two different markets within Ado-Ekiti Metropolis. The purchased fish samples were wrapped in sterile polythene bag and contained in a cooler stocked within Ado-Ekiti Metropolis. The purchased fish samples were serially diluted to 10-10.

**Sample Preparation**

The method of Obi and Krakowiaka [7] was used where 10 g each of head, middle and tail of test fish were cut ground with 10 ml of sterile water in sterile mortar and pestle. From the mixture, counts from other fish samples was between 1.3×10^4 - 3.5×10^5 CFU/g. However, no Vibrio count was recorded from *U. tenuis* purchased from Oba market. Counts from other fish samples was between 1.1×10^4 - 1.7×10^5 CFU/g. However, no Vibrio count was recorded from *S. scombrus* purchased from Oba market and least count of 1.3×10^4 CFU/g in *C. harengus* and *T. trachurus* purchased from Erinfun market respectively. Total Vibrio count was more with load of 2.3×10^4 CFU/g in *T. trachurus* purchased from Oba market. Counts from other fish samples was between 1.3×10^4 - 3.5×10^4 CFU/g. Fungal counts was recorded from *S. scombrus* and *U. tenuis* purchased from Oba market. Fungal counts was recorded in all the studied fish samples, with counts that ranged from 1.3×10^3 -2.3×10^4 Spore/g. *S. scombrus* purchased from Oba market had the highest fungal load of 2.3×10^4 Spore/g, followed by 2.1×10^3 Spore/g recorded from *T. trachurus* purchased from Erinfun and least count of 1.3×10^3 Spore/g from *U. tenuis* purchased from Erinfun market.

**Results and Discussion**

Total heterotrophic counts from the fish samples was more in *T. trachurus* with count of 5.6×10^4 CFU/g in the sample purchased from Oba market. This was followed by *S. scombrus* purchased from Erinfun market with load of 5.4×10^4 CFU/g of count of 3.5×10^4 CFU/g as the least count from *C. harengus* purchased from Oba market. Coliform bacteria were present in all the fish samples and the counts recorded was in the range of 2.4×10^3 to 5.1×10^4 CFU/g. *C. harengus* purchased from Erinfun market was the most coliform bacteria populated sample with load of 5.1×10^4 CFU/g. Following this was *S. scombrus* purchased from Erinfun market with load of 4.3×10^4 CFU/g and least count of 2.4×10^4 CFU/g from *U. tenuis* also purchased from Erinfun market. Count ranging from between 1.3×10^4 - 3.5×10^4 CFU/g was recorded for total Salmonella/Shigella species. The count was highest in *U. tenuis* with load of 3.5×10^4 CFU/g in the sample purchased from Oba market. This was followed by a count of 2.8×10^4 CFU/g from *S. scombrus* purchased from Oba market and least count of 1.3×10^4 CFU/g in *C. harengus* and *T. trachurus* purchased from Oba and Erinfun markets respectively. Total Vibrio count was more with load of 2.3×10^4 CFU/g in *T. trachurus* purchased from Oba market. Counts from other fish samples was between 1.1×10^4 - 1.7×10^5 CFU/g. However, no Vibrio count was recorded from *S. scombrus* and *U. tenuis* purchased from Oba market. Fungal counts was recorded in all the studied fish samples, with counts that ranged from 1.3×10^3 -2.3×10^4 Spore/g. *S. scombrus* purchased from Oba market had the highest fungal load of 2.3×10^4 Spore/g, followed by 2.1×10^3 Spore/g recorded from *T. trachurus* purchased from Erinfun and least count of 1.3×10^3 Spore/g from *U. tenuis* purchased from Erinfun market. Fungal counts was recorded in all the studied fish samples, with counts that ranged from 1.3×10^3 -2.3×10^4 Spore/g. *S. scombrus* purchased from Oba market had the highest fungal load of 2.3×10^4 Spore/g, followed by 2.1×10^3 Spore/g recorded from *T. trachurus* purchased from Erinfun and least count of 1.3×10^3 Spore/g from *U. tenuis* purchased from Erinfun market.

**Table 1. Average microbial counts from fish samples in the surveyed markets.**

| Fish samples | Market  | THC (CFU/g) | TCC (CFU/g) | TSSC (CFU/g) | TVC (CFU/g) | TFC (Spore/g) |
|--------------|---------|-------------|-------------|-------------|-------------|---------------|
| *C. harengus* | Oba     | 5.5×10^4   | 2.6×10^4   | 1.3×10^4   | 1.1×10^4   | 1.6×10^5      |
|              | Erinfun | 4.2×10^4   | 5.1×10^4   | 3.4×10^4   | 1.3×10^4   | 1.4×10^5      |
| *S. scombrus* | Oba     | 4.6×10^4   | 3.6×10^4   | 2.8×10^4   | -           | 2.3×10^5      |
|              | Erinfun | 5.4×10^4   | 4.3×10^4   | 1.7×10^4   | 1.5×10^4   | 2.0×10^5      |
| *T. trachurus* | Oba     | 5.6×10^4   | 2.6×10^4   | 2.5×10^4   | 2.3×10^4   | 1.7×10^5      |
|              | Erinfun | 4.3×10^4   | 3.7×10^4   | 1.3×10^4   | 1.4×10^4   | 2.1×10^5      |
| *U. tenuis*  | Oba     | 4.6×10^4   | 3.6×10^4   | 3.5×10^4   | -           | 1.6×10^5      |
|              | Erinfun | 5.2×10^4   | 2.4×10^4   | 1.4×10^4   | 1.7×10^4   | 1.3×10^5      |
region were Streptococcus faecium, Salmonella typhi and Micrococcus luteus, while Streptococcus faecium and Vibrio cholerae were isolated from the tail region. Aerococcus viridans, Streptococcus faecium and Pseudomonas aeruginosa were respectively isolated from the head and middle regions of Scomber scombrus while Xanthomonas fragariae and Streptococcus faecium were isolated from the tail region. From the head of Trachurus trachurus, Xanthomonas fragariae and Staphylococcus aureus were isolated, from the middle region were Clostridium butyricum and Escherichia coli, while Streptococcus faecium and Aerococcus viridans were found at the tail region. From the head of Urophycis tenuis, Micrococcus luteus and Staphylococcus aureus were isolated, Vibri cholerae, Escherichia coli and Alkaligenes faecalis were isolated from the middle region, while Xanthomonas fragariae and Streptococcus faecium were isolated from the tail region.

The most frequently occurred bacteria specie was M. luteus with 16.3%. This was followed by S. aureus with 11.1%, while the least occurred bacteria was V. cholerae with 3.4% (Figure 1).

Spoilage of fish is dependent in quality based on the differences in the environment where they inhabit and species type. There were variations in microbiological quality among the studied fish in respect to microbial population in the surveyed markets. Total heterotrophic counts from the fish ranged between $3.5 \times 10^4$ - $5.6 \times 10^4$ denoting that all the isolated fish were microbiologically certified as been of good quality hence the counts was within the permissible level of international standard. ICMSE [11] accepted total count limit for coliform in frozen fish is $<100$MPN/g. The total coliform counts from the fish samples as recorded in this study was between $2.4 \times 10^4$ - $5.1 \times 10^4$. The encounter of coliform in the sampled fish is an indication of faecal contamination by either animals or humans of the water they were caught, though could also result from human handling and environment of storage. Salmonella and Vibrio bacteria species were encountered in majority of the evaluated fishes. By the standard of International Association of Microbiology Society, these species of bacteria should not the encountered in frozen fish. From this perspective, we cannot ascertain good quality of the fish samples despite the counts of other isolated pathogens that were within standard level. However, species of Salmonella and Vibrio have been reported in frozen fish by Adebayo-Tayo et al. [3], Popovic et al. [12], Sanjee and Karim [13]. Aspergillus flavus, the most dominant fungus among the isolated fungi have been reported in fish and salt water and have been identified as diseases causing fungi in animal and human as reported by Saleem et al. [14], Oransu and Olarewaju, [15], Oranusi et al. [16].

The presence of Streptococcus faecium as the most dominant bacteria identified from the fish samples simplifies that the offshore water was contaminated with faecals of either man or animals. Though the infectious dose of this bacterium is unknown, the diseases it manifest such as urinary tract infection, wound infection, endocarditis and bacteremia may be zoonotic and communicable. Meanwhile, this bacterium has been found to be multiple drug resistance [17].

S. aureus been one of the pathogens isolated from the frozen fish, is a normal flora in man but not in fish, its presence in the samples could be attributed to contamination from personnel and environment [18]. Similarly, cross contamination via utensil have been well documented by Roche et al. [19], Harrington, [20], Berrange et al. [21].

Eight species of fungi were isolated from the frozen fish. From the head region of Clupea harengus, Aspergillus fumigatus and Aspergillus flavus were isolated, from the middle and tail regions, Aspergillus flavus and Aspergillus clavatus respectively. Isolated from Scomber scombrus are Aspergillus flavus from the head region, Mucor mucedo, Aspergillus fischeri and Aspergillus terreus from middle region, while Penicillium digitatum, Aspergillus terreus and Mucor mucedo were from the tail region. From the head region of Trachurus trachurus, Mucor mucedo, Aspergillus fumigatus and Aspergillus flavus were isolated. From the middle region were Aspergillus fumigatus and Aspergillus clavatus, while from the tail were Aspergillus flavus, Mucor mucedo and Aspergillus parassitus. Urophycis tenuis was inhabited with Aspergillus flavus at the head region, Mucor mucedo and Penicillium digitatum from the middle region and; Aspergillus flavus and Aspergillus fumigatus from the tail region.

The result obtained from the fish denotes varied microbial contamination. From fungal contamination perspective, Aspergillus species were dominant in the fish samples from different regions. Meanwhile, A. terreus was the most frequently occurred among the isolated fungi with 18.6% occurrence, followed by M. mucedo (15.2%) and A. fumigatus (8.1%) as the least frequently occurred (Fig 2).

The microorganisms identified in this study seemed to be common among other species of frozen fishes in reports of researchers elsewhere. Research studies by some authors in Nigeria such as Okonko et al. [22], Chukwuka et al. [23], Akintusire, [24], Adebayo-Tayo et al. [3] have isolated similar microorganisms from different frozen fishes in Nigeria. Edris et al. [25] from Egypt, Murad et al. [26] from Iran, Popovic et al. [12], have isolated Salmonella sp, E. coli, S. aureus and V. cholerae from fresh and frozen sea foods in Croatia.

It has been reported that microbial type and number in frozen fish is relatively on fish source, contamination from boat use in fishing, storage temperature, inconsistency in freezing process

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**Figure 1. Frequency of occurrences for bacteria isolated from frozen fish.**
and wrongful human handling [27]. For this, it therefore calls for great attention to handle fish in more hygienic ways to keep up to microbiological standard of the use of fishing equipment which often serve as a route of contamination of frozen fish and storage environment. Conclusion by Brooks et al. [28], was that contaminated food is one of infection sources in man. Foodstuffs safety are generally ensured by a preventive approach, which could be achieved by implementation of good hygiene practice and the use of the principles of hazard analysis and critical control point (HACCP). Popovic et al. [12], have therefore reported that microbiological criteria are useful in the procedure of validation and HACCP verification procedures; and other control measures relating to good hygiene practice. Most of the isolated microbes might not be directly associated with the fish but it is cleared that microorganisms localize frozen fish and it is a message that fish must be properly processed either by cooking, frying or drying for safe consumption.

Conclusions

Both pathogenic and spoilage microbes were isolated from the frozen fish and it signals that microorganisms in frozen fish can serve as health hazard to consumers if not properly processed before consumption and labour loss to sellers if adequate preservation of fish is not relatively considered as important factor. Though the microbial load recorded from the fishes is minimal and can be accepted but the pathogenic microorganisms isolated emphasized that frozen fish can serve as possible vehicle for human infection. Every home in Nigeria consume fish on daily bases in their diet. The consumed fish are either in informal of dried, boiled or fried and till date there is no literature or data on disease outbreak as result of consuming frozen or processed fishes. However, this research evaluated that infection with microbial pathogens could be possible from frozen fish and also parts of the results obtained could serve as useful reference in future.

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