Microbial load of some imported frozen fish species in Lagos, Nigeria

*[1]Taiwo, I. O., [2]Olopade, O. A. and [3]Bamidele, N. A.

Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, PMB 2240, Abeokuta. Nigeria
Department of Fisheries, University of Port Harcourt, Port Harcourt
Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture
*Corresponding E-mail: (iomtI@yahoo.com)

Abstract

Freezing preserves food by stopping the growth and multiplication of microbes or by halting enzymatic activities in foods that would otherwise cause spoilage. This study was conducted to identify and profile the microbes present in frozen fish. Five frozen fish species, Trachurus trachurus, Scombers combrus, Larimichthys crocea, Gadus chalcogrammus and Oreochromis niloticus, were purchased from Ijora–Olopa frozen fish sales outlet in Lagos State and transported in ice-flakes to the laboratory for microbiological analysis of the skin, muscles and gills. S. scombrus and G. chalcogrammus had the highest (5.30 x 10^2 cfu/g) and lowest microbial loads (1.85 x 10^2 cfu/g) respectively. The result however showed that L. crocea and G. Chalcogrammus were noticed to be significantly different ($P<0.05$) for coliform count on both the skin and the gills. These values were much higher than the recommended public health and safety standard values that ranged from 5.0 × 10^5 and 1.0 × 10^6 $CFUg^{-1}$, approved by Nigerian National Agency for Drug Administration and Control (NAFDAC). Hence, it is necessary that frozen foods should be properly cooked before consumption and effective hazard analysis and critical control points implemented. The predominant bacteria species isolated were Bacillus subtilis, Pseudomonas sp., Staphylococcus aureus, E. Coli and Salmonella sp while the predominant fungal species isolated were Apergillus flavus, A. fumigatus, A. niger and Yeast.

Keywords: Frozen fish, gills, muscles, skin, bacteria, fungi,

Introduction

Nigeria is one of the largest importers of fish and fishery products in Africa (Akande, 2000), this probably was due to the need to meet the short fall in domestic demand for fish. Fish is one of the cheapest sources of animal protein in Africa (The World Fish Center, 2009). It is one of the main food components of humans for many centuries and still constitutes an important part of the diet of many countries. The advantage of fish as a food is due its easy digestibility and high nutritional value. Since 70% of the earth's surface is covered by water, there are plenty sources to harvest fish from. Fishes are found in different waters. Some are found in fresh water while some are found in salt water (sea and oceans). However, the type of microorganism found associated with a particular fish depends on the water it was caught (Claucas and Ward, 1996).

Kvenberg (1991) and Rodricks (1991) classified the bacteria pathogens associated with fish into two: the non-indigenous bacteria pathogen and the indigenous bacteria pathogens. The non-indigenous pathogen contaminate fish or fish's habitat in one way or the other and the pathogens include *Clostridium botulinum, Listeria monocytogenes, Staphylococcus aureus, Salmonella* species, *Shigella s*pecies, *Escherichia coli*, etc. The indigenous bacteria pathogens are those naturally living in the fish's habitat. They are the *Vibrio*

species, *Aeromonas* species etc. Claucas and Ward (1996) also listed some organisms like *S. aureus, Salmonella, Vibrio parahaemolyticus, Monocytogenes, Shigella, Aeromonas, Yersenia* and *Pseudomonas* that could cause food-borne diseases when present in ready-to-eat sea foods.

Fishes have high water content and freeze between temperatures of 0 and 3°C with an average of about 2°C (Desrosier, 1978). Freezing kills some bacteria, but the ones not killed will grow once the fish thaws. Some bacteria such as *Pseudomonas* species, *Moraxella* species, *Alcaligenes* species, *Flavobacterium* species survive freezing temperature and will resume growth when thawed. At a temperature of 3°C or above, species of *Clostridium botulinum* can survive freezing and may grow and produce toxins (Frazier and Westhoff, 1988). The fish could be contaminated after being caught or during transportation to retail markets. After contamination and replication of microorganisms, decay occurs and the consumption becomes dangerous (Mol and Tosun, 2011; Alparslan *et al*., 2014). *Aspergillus flavus* is a fungus that was first recognized to cause aflatoxicosis in domestic animals and is the most important aflatoxin-producing species in food on a global basis.

FAO (1989) observed that fishes become contaminated at sea prior to freezing due to difficulty in designing the plant that would be able to operate satisfactorily at all time in adverse weather conditions at sea. The report further indicated that the method of catching fish contributes to the bacterial load of frozen fish and observed that trawling of fish net along the bottom sediments of water for a long time could result in exposing the fish to high bacterial contamination.

Fish quality is influenced by many factors as the source, cooling methods, processing, packaging and storage conditions. The quality and freshness of fish are rapidly deteriorated through microbial and biochemical mechanisms (Al–Jasser and Al–Jasass, 2014).Freezing preserves food for extended periods by stopping the growth and multiplication of microorganisms that cause both food spoilage and food borne illness and by halting the foods own enzyme activity that would otherwise cause the food to rot. Most pathogens do not multiply at freezer temperature. Also, pathogens need water to grow and freezing turns the available water into solid ice crystals. Freezing is a common practice in the meat, fish and other animal protein based industry, because it preserves the quality for an extended time and offers several advantages such as insignificant alterations in the product dimensions, and minimum deterioration in products colour, flavour and texture (Obuz and Dikeman, 2003; USDA Food Safety Information, 2013).

Frozen fish constitutes 45% of fish consumed in Nigeria (Tobor, 1984) and with the ever growing population and the need to store and transport the food (fish) from one place to another, food preservation becomes very necessary in order to increase its shelf life and maintain its nutritional value, texture and flavour. This study was therefore undertaken to assess the microbial load of five imported frozen fish species by identifying and isolating the pathogenic bacteria and fungal load.

Materials and methods

Culture media

Different media used for culturing were prepared and used, which are Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar and Potato Dextrose agar (PDA) for

fungi growth.

Preparation of samples and enumeration of microorganisms

The fish samples were surface sterilized separately in 3.5% sodium hypochlorite solution (w/v) with constant agitation for 7 minutes, rinsed thoroughly with sterile distilled water until the traces of hypochlorite were removed and blotted dry. The skins, muscles and the gills of the fish samples were pulverized separately using a blender. 5g were taken from each sample into sterile bottles containing 450 ml of sterile peptone physiological saline to form stock cultures. The sample bottles were placed on a rotator shaker at 120 RPM for 1 hour. 10-fold dilutions were subsequently prepared with peptone physiological saline. Aerobic mesophilic bacteria were enumerated on plate count agar at 37°C for 24 hours and reported as total viable count (TVC). Coliform were enumerated on MacConkey agar (MA) and incubated at 30°C for 48 hours. Presumptive *E. coli* were enumerated on MacConkey agar (MA) and incubated at 44°C for 48 hours. Moulds were enumerated on Potato dextrose agar (PDA) and incubated at 25°C for 72 hours (Felix *et al*., 2007).

Estimation of bacteria load

The bacteria load was estimated using the method described by Felix *et al*.(2007) for microbial count and involves the preparation of serial dilution and the prepared plates were inoculated in duplicate with 0.1 ml of the (10^{-2} to 10^{-5}) dilutions using pour plate method these were incubated at 37^0C for 24hr.

Calculation of bacteria counts

The method described by Collins *et al.* (1989) for estimating bacteria counts was used to enumerate the total viable counts of the isolates. Countable plates showing 1 to 32 colonies were selected and counted. The mean colony count on the pour plate of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram ($CFUg^{-1}$). The mean colony forming unit per gram ($CFUg^{-1}$) denoted by (x) was calculated as $\Sigma f\chi/\Sigma f$, where Σfx is the sum of the products of number of colonies and the colony forming unit per gram; while Σf is the summation of the number of colonies.

Identification of bacteria

Inocula were aseptically transferred from each slide into plates of respective media using a streak plate technique. The isolates were purified by repeated streaking on their respective media. Bacterial plates were incubated at 37°C for 24 hours while fungal plates at 25°C for 72hours. A 24hrs old culture was prepared from each plate for identification purposes.

Biochemical tests

The following biochemical tests were carried out to determine the presence of microorganisms in the samples: Catalase test, Coagulase test, Oxidase test, Indole test, Citrate utilization test. Sugar fermentation test. Other test carried out to determine the presence of bacteria were Motility test (positive result showed motile bacterium darting across the microscopic field); Urease test **(**Negative test retain the brown colour with no growth**).** Hydrogen sulphide production**test** was used in the identification of enterobacteria and also to differentiate between bacteria such as *Bacteriodes* and *Brucella* species. Methyl red test was used to differentiate enterobacteria. Voges-proskauer (VP) test was used to differentiate some enterobacteria.

Preparation of frozen samples for fungi enumeration

10g of each of the samples purchased was mashed and thoroughly homogenized in 100ml of sterile normal saline. To 90mls of sterile distilled water, 10ml of the

homogenized sample was added in a sterile beaker. Then, 9mls of sterile physiological normal saline was dispensed each into different 10 test-tube and 1ml of the fish homogenized sample was transferred using a pipette into first test tube to give a dilution factor of 10^{-1}, and serial dilution was made to the last tube while 1ml of the last tube was discarded. 1ml from 10^{-3} and 10^{-4} dilutions were plated out on Potato dextrose agar (PDA) using the pour plate method these were incubated at room temperature for 72h. This procedure was performed aseptically on a bench-work swabbed with methylated spirit. Also, a spirit lamp was lit to prevent contamination with micro-organisms.

Identification/characterization of fungi isolates

Fungi isolates were identified macroscopically viz: colour, texture and pigment; and microscopically viz by using lactophenol cotton blue. A drop of 95% ethanol was placed on a microscopic slide. Using a sterile inoculating needle, a small portion of fungal growth was gently removed from midway between the colony centre and the edge. It was ensured that both the aerial and substrate (vegetative) mycelium was included in the specimen. With two dissecting needles, the fungus was gently teased so that it is thinly spread out on the mounting medium. When most of the ethanol had evaporated, a drop of lactophenol cotton blue was added and covered with cover glass. The fungus was examined microscopically.

Results

Microbial load count of the skin, muscles and gills is shown in Table 1. The predominant bacteria species isolated were *Bacillus subtilis, Pseudomonas* sp*, Staphylococcus aureus, E. coli, Salmonella* sp while the predominant fungal species isolated were *Apergillus flavus, Aspergillus fumigatus, Aspergillus* niger and Yeast (Table 2).

The microbial load count on the skin of the fish samples, total viable count was highest in *L. crocea* withmean value of 9.60 × 10^5 CFU. Both *L. crocea* and *G. chalcogrammus* were significantly different (P<0.05) from *T. trachurus*. Total coliform count was highest in *S. scombrus* and lowest in *L. crocea* with 7.45 × 10^2 and 4.55 × 10^2cfu/g respectively. *L. crocea* and *S. scombrus* were significantly different (P<0.05). Total *E. coli* count on the skin was highest and lowest in *L. crocea* and *G. chalcogrammus* with mean values of 2.10 × 10^1 and 0.85 × 10^1cfu/g respectively. *L. crocea* and *G. chalcogrammus* were significantly different (P<0.05) from the other species. Total Mould count of the skin was highest in *O. niloticus* and lowest in *L. crocea* with mean values of 2.25 and 1.15 x 10^2cfu/g respectively.

Microbial load count found in the muscle, TVC in all fish species ranged between 5.90 and 3.65 × 10^5cfu/g, Total Coliform Count was highest in *S. scombrus* and lowest in *G. chalcogrammus* with mean values of 5.30 and 1.85 × 10^2 cfu/g respectively. Total *E. coli* count was highest in *L. crocea* and lowest in *G. chalcogrammus* with mean values of 1.65 and 0.25 × 10^1cfu/g respectively while Total Mould Count was highest in *L. crocea* and lowest in *G. chalcogrammus* with mean values of 0.95 and 0.30 × 10^2cfu/g respectively.

Microbial load count on the gills, Total Viable Count in all fish species ranged between 11.40 and 5.05 × 10^5 with *L. crocea* and *G. chalcogrammus* with the highest and lowest counts respectively. Total Coliform Count was noticed to be highest in *S. scombrus* and lowest in *T. trachurus* with mean values of 9.00 and 3.10 × 10^2cfu/g

respectively, Total *E. coli* count was noticed to be highest in *S. scombrus* and lowest in *G. chalcogrammus* with mean values of 2.70 and 0.55 × 10^1cfu/g respectively. Total Mould Count was observed to be highest in *S. scombrus* and lowest in *G. chalcogrammus* with mean value of 2.30 and 1.10 × 10^2cfu/g respectively.

Table 2: Isolated microorganisms from fish species

Fish species	Bacterial species	Fungal species
Trachurus trachurus	*Bacillus subtilis, Pseudomonas* sp, *Staphylococcus aureus, E. coli, Salmonella* sp	*Apergillus flavus, A. fumigatus, A. niger,* Yeast
Oreochromis niloticus	*B, subtilis, Pseudomonas* sp, *S. aureus, E. coli, Salmonella* sp	*A. flavus, A. niger,* Yeast
Scomber scombrus	*B. subtilis, Pseudomonas* sp, *S. aureus, E. coli, Salmonella* sp	*A. flavus, A. fumigatus, A. niger,* Yeast
Larimichthys crocea	*B. subtilis, Pseudomonas* sp, *S. aureus, Salmonella* sp	*A. flavus, A. niger,* Yeast
Gadus chalcogrammus	*Pseudomonas* sp, *S. aureus,E. coli,*	*A. fumigatus, A. niger,* Yeast

Discussion

The mean viable count of the microorganisms on the skin, muscles and gills were higher than that recommended by the Nigeria Agency for Drug Administration and Control (NAFDAC). The standard recommended by NAFDAC for public health is between 5.0 × 10^5 and 1.0 × 10^6cfu g-1. According to Eze *et al*. (2011), the mean viable count although cannot be taken as an absolute figure. This is because the number and type of bacteria found on frozen fish is dependent on many factors, of which source of the fish is a major factor. This supported earlier report by Thatcher and Clark's (1973) that the kind and number of microorganisms found on frozen fish is dependent on the source of the fish, additional contamination introduced in the fishing boat, freezing temperature during storage, severity of freezing process with respect to lethality to microorganisms and contamination by handlers and market sellers.

The predominant bacteria species isolated are *Bacillus subtilis, Pseudomonas sp, Staphylococcus aureus, E. coli, Salmonella* sp. The microorganisms isolated from this studywere similar to the microorganisms reported by Olawale *et al*. (2005) and *Oranusi et al. (2014)* for both bacterial and fungal isolates which include *S. auerus*, *E. coli*, among other organisms. The presence of *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *Enterobacter* sp, *E. coli*, *Flavobacterium* sp., *Staphylococcus* sp. reported in this study are in agreement with the findings of Adesokan *et al*. (2005) who reported the presence of *Bacillus* sp. and *E. coli* among other organisms and also report by*Ehigiator et al. (2014) onthe presence of Pseudomonas sp., E. coli, S. aureus, Bacillus sp*. in the fish samples.

Coliforms are indicator organisms signifying contamination of a product by faecal matter. The presence of high bacteria and coliform in the fish samples could be a reflection of poor initial fish quality pre-

Table 1: Microbial load count of the Skin, Muscle and Gills

Species	TVC($\times 10^5$)			TCC($\times 10^2$)			TEC($\times 10^1$)			TMC($\times 10^2$)		
	Skin	**Muscle**	**Gills**	**Skin**	**Muscle**	**Gills**	**Skin**	**Muscle**	**Gills**	**Skin**	**Muscle**	**Gills**
Gadus chalcogrammus	7.20 ± 0.71^{b}	3.65 ± 0.21^{c}	5.05 ± 0.21^{c}	5.65 ± 0.78^{bc}	1.85 ± 0.07^{b}	4.60 ± 0.57^{c}	0.85 ± 0.21^{c}	0.25 ± 0.07^{d}	0.55 ± 0.21^{c}	1.70 ± 0.14^{ab}	0.30 ± 0.00^{a}	1.10 ± 0.14^{a}
Trachurus trachurus	8.85 ± 0.78^{ab}	4.85 ± 0.64^{abc}	6.75 ± 0.21^{b}	5.45 ± 0.35^{bc}	2.40 ± 0.28^{b}	3.10 ± 0.28^{d}	1.30 ± 0.14^{bc}	0.50 ± 0.14^{c}	0.90 ± 0.14^{bc}	1.85 ± 0.21^{ab}	0.55 ± 0.21^{a}	1.45 ± 0.49^{a}
Oreochromis niloticus	8.95 ± 0.07^{ab}	5.90 ± 0.28^{a}	6.30 ± 0.14^{b}	6.75 ± 0.35^{ab}	4.95 ± 0.21^{a}	6.15 ± 0.07^{b}	1.30 ± 0.14^{bc}	0.95 ± 0.07^{b}	1.15 ± 0.07^{b}	2.25 ± 0.07^{a}	0.65 ± 0.07^{a}	1.35 ± 0.21^{a}
Larimichthys crocea	9.60 ± 0.14^{a}	4.45 ± 0.21^{bc}	11.40 ± 0.28^{a}	4.55 ± 0.21^{c}	2.40 ± 0.28^{b}	5.30 ± 0.14^{c}	2.10 ± 0.14^{a}	1.65 ± 0.07^{a}	2.65 ± 0.21^{a}	1.15 ± 0.07^{b}	0.95 ± 0.21^{a}	1.85 ± 0.07^{a}
Scomber scombrus	8.35 ± 0.35^{ab}	5.15 ± 0.35^{ab}	11.10 ± 0.57^{a}	7.45 ± 0.35^{a}	5.30 ± 0.42^{a}	9.00 ± 0.28^{a}	1.75 ± 0.21^{ab}	0.75 ± 0.07^{b}	2.70 ± 0.14^{a}	1.40 ± 0.42^{b}	0.65 ± 0.35^{a}	2.30 ± 0.42^{a}

[abc]Means (± Standard deviation) in the same column having different superscript are significantly different ($P<0.05$)
(TVC = Total Viable Count; TCC = Total Coliform count; TEC = Total *E.coli* count; TMC = Total Mould count)

freezing. The total coliform count of T. trachurus was not significantly different from that recorded by Oranusi et al. (2014) while the total coliform count of S. scombrus on the skin was higher than that reported by Oranusi *et al.* (2014). Previous researches by Rompre´ *et al. (2002) and* Environmental Fact Sheet (2010) has indicated that high presence of coliform calls for concern because the presence of bacteria in this group indicated the possibility, of the presence of disease organisms in the fish samples.

The presence of *S. aureus*, *Salmonella* sp. and *Shigella* spp in fish samples also calls for concern because these are pathogenic organisms of public health concern and *their* significant levels in these frozen seafood products might be due to contamination of the processed frozen seafood products from the source as a result of handling by processors asimproper handling and hygiene might lead to the contamination of frozen foods and may consequently affect the health of the consumers (Dunn *et al*., 1995; Adebolu and Ifesan, 2001; Bankole *et al*., 2004; Afolabi, 2005; Omemu and Bankole, 2005; Okonko *et al*., 2008). The isolation and identification of these micro-organisms that are contaminants to frozen fish will help educate the public on the need to adopt various measures to prevent the multiplication of these micro-organisms. Freezing does not destroy these micro-organisms, it is especially important to make sure food is wholesome before freezing. The safety of frozen foods depends on the condition and handling of the food before being frozen, and the freezing temperature.

Conclusion

The fish muscles contained less TEC and TCC count than the skin and gills. It is therefore, advisable not to eat the gills and to remove the skin of fish or properly cook fish before consumption. Frozen fish contains micro-organisms above NAFDAC recommended standard and preserving fish through freezing could reduce microbial load but not completely eliminate them. The presence of high bacteria and coliform in the fish samples indicates that the fish samples were contaminated with faecal matter. This could have occurred before the fish were frozen which could be an indication poor and unhygienic handling of captured fish species.

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