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Research Article

Investigation on the microbial profile of frozen foods: Fish and Meat

S. Oranusi*, T.U. Obioha and B.T. Adekeye

Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria

*Corresponding author e-mail: orasoluche@gmail.com

Abstract

Freezing preserves food by stopping the growth and multiplication of microbes or by halting the foods own enzyme activity that would otherwise cause the food to rot. There have been reports of illness involving frozen foods due to contamination post-harvest. This study seeks to investigate the microbial profile of frozen fish and meat. Fifteen samples each of *Trachurus trachurus*, *Scomber scombrus*, *Urophycis tenuis*, *Clupea harengus* and poultry meat-chicken, turkey and gizzard was purchased from different cold rooms and retail stores for microbiological analysis. Coliforms were detected in all the meat samples and with counts ranging from 7.0×10^3 - 5.0×10^6 . Gizzard and chicken had significantly high ($P < 0.05$) coliform count. The head and intestinal regions of the fish have significantly high ($P < 0.05$) TAPC compared to the skin. The skin and the head region have significantly high ($P < 0.05$) coliform and fungi counts compared to the intestine. The microbial isolates from the fish and meat samples include *S. aureus*, *E. coli* and spp of Bacillus, Klebsiella, Salmonella, Flavobacterium, Listeria and Pseudomonas. Fungi isolates include spp of Aspergillus, Penicillium and Alternaria. Trichodina and Isopod spp of parasites were detected in some fish samples. Although freezing retard pathogens multiplication, post harvest contaminants can multiply during thawing to a level that can have a major impact on the quality of the final consumer product. It is advanced that frozen foods must be properly cooked before consumption and effective hazard analysis and critical control point implemented.

Keywords: Freezing , frozen fish and meat; Coliforms, TAPC, *S. aureus*, *E. coli*, *Aspergillus*, *Penicillium*.

Introduction

Freezing preserves food for extended periods by stopping the growth and multiplication of microorganisms that cause both food spoilage and foodborne illness and by halting the foods own enzyme activity that would otherwise cause the food to rot.

Most pathogens don't multiply at freezer temperature and many of them perish because their enzymes don't work properly to maintain normal cell activity. 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 preserved 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). Almost any kind of food can be frozen; some foods require special treatment before they can be frozen safely.

If defrosted correctly, frozen foods are generally as safe as their original condition; conversely freezing unwholesome food will not make it wholesome.

In vegetables, some enzymes have been reported to remain active at freezing temperature and continue to ripen and spoil them unless stopped with mild quick cooking (blanching), this process can cause some of the vitamin C (15 to 20%) to be lost. In spite of these losses, vegetables and fruits frozen soon after harvesting are often higher in nutrients than their "fresh" counterparts because fresh fruits and green vegetables can lose as much as 15% of their vitamin C content daily when kept at room temperature (Fellows, 2000).

Most types of meat, fish and fruits do not need to be blanched before freezing because their enzymes do not affect them in the same way when frozen. Freezing has very little effect on the nutrient content of foods. There are almost no vitamins and mineral losses from frozen meats, fish and poultry because protein, vitamins A and D and minerals are not affected by freezing. During the defrosting process, there is a loss of liquid containing water-soluble vitamins and mineral salts, which will be lost in the cooking process if this liquid is not recovered (Fellows, 2000, USDA Food Safety Information, 2013).

There are some disadvantages associated with frozen storage (Kropf and Bowers, 1992, Lundman, 2011) including freezer burn, product dehydration, rancidity, drip loss and product bleaching which can have an overall effect on the quality of the frozen foods. There have been reports of illness involving frozen foods, ice cream and strawberries, and contaminated ice (Archer, 2004). Freezing to 0 °F (-17.8 °C) or below inactivates any microbes (bacteria, yeasts, and molds) present in food. Once thawed, however, these microbes can again become active, multiplying under the right conditions to levels that can lead to foodborne illness. Since they will then grow at about the same rate as microorganisms on fresh food, thawed items must be handled as any perishable food (USDA Food Safety Information, 2013). The manner and condition in which frozen food is stored and the

way in which it is thawed can have a major impact on the quality of the final consumer product. Poor defrosting (thawing without refrigeration) encourage proliferation of microbial contaminants (Frazier and Westhoff, 1988, National Research Council, 2003) coupled with unhygienic handling and processing (inadequate cooking), has led to outbreaks of food borne illness (Baffone *et al.*, 2000, Farmer *et al.*, 2003; Su and Liu, 2007). The aim of this work is to investigate the microbial profile of frozen fish and meat sold in Ota Ogun state Nigeria, with a view to educating the public on proper handling and processing of frozen foods to prevent food borne diseases.

Materials and Methods

Sample collection

One hundred and five samples made up of fifteen each of Kote'- *Trachurus trachurus*, Titus – *Scomber scombrus*, Stock – *Urophycis tenuis*, Sawa – *Clupea harengus* and Poultry meat- chicken, turkey and gizzard were purchased from different sales outlets (cold rooms and retail stores) in Lagos and Ogun states of Nigeria. Triplicate samples were purchased from the different outlets and placed in sterile polyethylene containers. Samples were transported in cold pack to the laboratory for analysis within one hour of collection.

Microbiological Analysis of Samples

Twenty five gram (25g) samples of meat and samples obtained from the different parts of the fish (head region to include the gills, intestinal region and the outer skin surface) were used for analysis. Samples were respectively blended in sterile warring blender and diluted 10^{-1} to 10^{-7} in sterile peptone water (Oxoid). Aliquot 0.2ml volumes of each sample homogenate was spread plated in triplicate onto Nutrient agar, Eosin Methylene blue (EMB) agar and (SDA) Sabouraud dextrose agar (all from Biomark Laboratories, India), for Total aerobic plate count (TAPC), coliform count and fungal count respectively. Mannitol salt agar (Biolab, Hungary) was inoculated for isolation of *S. aureus* while Salmonella Shigella agar (Oxoid) was

inoculated after pre-enrichment in Selenite-F broth (Oxoid) for the isolation of salmonellae. A gram of each sample was inoculated into lactose broth in a capped test tube with inverted Durham tubes for coliform test. All cultures were incubated at 37°C for 24 to 48h except however, SDA for fungi isolation which was incubated at 28±3°C for 3 to 5 days and a plate of EMB incubated at 44°C and 24-48h for faecal coliform organisms. Culture plates were examined at the end of incubation period for colony counts and cultural characteristics of formed colonies was recorded for identification.

Confirmation of coliform organisms

Tubes of lactose broth with 1g sample showing gas production and /or colour change of dye after incubation were recorded as presumptive coliform test positive, similarly colonies on EMB inoculated into lactose broth in test tube with inverted Durham tubes and incubated for 24-48h at 37°C and 44°C and with gas production and/ or colour change of dye constituted a positive presumptive test (Speck, 1976; Oranusi *et al.*, 2004). Positive presumptive test cultures were treated for confirmatory test, typical colonies on EMB appearing brown and mucoid characteristic of *E. aerogenes* or black with greenish metallic sheen characteristic of *E. coli* and that is Gram negative non-spore bearing, confirmed the presence of coliform organisms. Isolates were stored on agar slants at 4°C refrigerator temperature for further characterization.

Enumeration and Identification of Microbial Isolates

Colony counts were made with colony counter (Stuart Scientific, UK), counts was expressed as colony forming units per gram (cfug⁻¹) sample. Characteristic discrete colonies on the different media were isolated, and purified by repeated sub-culturing on Nutrient agar. Pure cultures were stored on agar slants in the fridge at 4°C for further characterization. Identification of bacteria isolates was based on morphological characteristics, microscopy, biochemical tests (Speck, 1976) and using Biomerieux® Sa API biochemical test kits. Fungal isolates were identified based on their

morphological characteristics and microscopy with reference to standard identification keys and atlas (Hanlin and Ulloa, 1988; Tsuneo, 2010).

Identification of parasites

Two different methods were adopted for the identification of parasites in the fish and meat samples. The methods of Wafa (2010) modified, and following the description of Oranusi *et al.* (2012), fifty (50) grams of each sample was blended in sterile warring blender and homogenized in physiological saline. Sample homogenates was sieved to remove debris and then centrifuged at 2000rpm for 20 mins. The supernatant was discarded and the sediments carefully mixed. A drop was placed on a grease free slide, mixed with a drop of tincture of iodine and was examined for parasites under the microscope using x10 and x40 objective lenses. The digestion method as described by Rahdar and Salehi (2011) was also used. Fifty grams of samples were blended in sterile warring blender and digested overnight in 1.5% HCL and 0.5% pepsin at 28°C. Digested samples were sieved and centrifuged at 1500rpm for 5mins. The supernatant was discarded and the sediment mixed and then stained by Giemsa for microscopy. Parasites identification was based on the descriptions of Ghazi and Asmat, 2001; Heckmann, 2003).

Statistical analysis

The data obtained were presented as mean and standard deviation. The significance of differences among microbial load means was determined by the analysis of variance using SPSS 20.0 software for windows

Results

The mean microbial load values for TAPC, Coliform and Fungi count from the various frozen meat types are presented in Table 1. Coliforms were found in all the meat types and significantly high ($P < 0.05$) in gizzard and poultry-chicken compared to poultry-turkey. Similarly, the TAPC was significantly high ($P < 0.05$) in gizzard and poultry-chicken compared to poultry-turkey. There was no

significant difference at ($P < 0.05$) in the levels of fungi load in all three meat samples. Table 2 summarized the mean \log_{10} distribution of microbial populations in the different parts of the fish samples analysed. The head region including the gills and the intestinal region has significantly high ($P < 0.05$) TAPC compared to the skin while the head region and the skin has higher Coliform and fungi counts significant at ($P < 0.05$) compared to the intestine. The table also reveals that *Scomber scombrus* tend to be relatively less contaminated than the other fishes while *Urophycis tenuis* was more contaminated. Coliform was not detected in the intestinal region of the fishes except in *Urophycis tenuis*. The microbial isolates from the samples is presented in Table 3. *S. aureus*, *E. coli* and spp of Bacillus, Klebsiella, Salmonella, Listeria, and Pseudomonas are the most predominant bacterial isolates while Aspergillus, Penicillium, Alternaria and Actinomycetes are the predominant fungal isolates. Only two spp of parasite *Trichodina* and *Isopod* was detected in the fish samples, the meat samples yielded no parasite.

Discussion

Coliforms are indicator organisms signifying contamination of a product by fecal matter. The presence of coliform in all the meat samples could be explained to mean possible contamination from animal's own intestinal content via poor handling of evisceration process or from other carcasses due to cross contamination from the slaughter slabs /abattoir. It could also be attributed to contamination via possible use of polluted water either for washing meat before packaging, or for ice production in chilling (Zambuchini *et al.*, 2008). The personnel that handled the meat from slaughter, through packaging, transportation to sales could be the source of coliform to the meat product. The possibility of contamination of meat via utensils/equipment cannot be ruled out (Reilly, 2006; Zambuchini *et al.*, 2008). Contamination of meat and meat products via processing, personnel, utensils and environment have been reported by several authors (Wagner, 2013, Facts: Cross Contamination, 2013, Raw meat: cross contamination, 2013).

The significantly high coliform count ($P < 0.05$) on the skin and head region compared to the intestinal region in all the fish samples could be explained that contamination is from the water for ice production used in chilling, the personnel and from the utensils and equipment rather than from the feed consumed and water environments from which the fish was harvested. The composition of the intestinal flora of fish has been reported to be related to the level of contamination of water and food in the environment. The near absence of coliform in the intestinal region of the fish samples is in agreement to the fact that coliform is not a normal flora in the intestinal tract of fish (Geldreich and Clarke, 1966 ; Mandal *et al.*, 2009).

The presence of high TAPC and coliform in the meat and fish samples (except for fish intestinal region with low coliform), could be a reflection of poor initial meat and fish quality pre-freezing. Freezing only retard the growth and proliferation of contaminating organisms, it seldom destroy/kill the organism (Gill, 2002). Thawing of the samples prior to analysis could equally have encouraged proliferation of the contaminant (Frazier and Westhoff, 1988, National Research Council, 2003, Zahid *et al.*, 2010, USDA Food Safety Information, 2013).

The presence of coliform calls for concern because the presence of bacteria in this group indicates the possibility, that disease organisms may also be present in the in the meat and fish samples (Silliker and Gabis, 1976; Rompre' *et al.* 2002; Environmental fact sheet, 2010).

Though the raw meat and fish can become well processed before consumption, Cross contamination of other products eaten raw or with minimal processing can be very challenging.

S. aureus is a normal flora in man but not in fish and poultry, its presence in the samples could be attributed to contamination from the personnel and environment (Clucas and Ward, 1996, Wagner, 2013). The presence of Listeria and Salmonella spp in the frozen meat and fish calls for care in storage and processing to prevent cross contamination of

Table 1. Mean microbial count log₁₀ cfug-1 of frozen meat samples

| Sample | TAPC | Coliform Count | Fungi Count |
|------------------|-----------|----------------|-------------|
| Poultry- Chicken | 8.67±2.09 | 5.10±0.91 | 3.36±0.72 |
| Poultry- Turkey | 7.69±0.19 | 3.84±0.51 | 3.36±0.61 |
| Gizzard | 9.46±1.32 | 6.70±0.31 | 3.34±0.24 |

Table 2. Mean microbial count log₁₀ cfug-1 of frozen fish samples

| Microbial count | Sampling sites | | |
|---|----------------|-------------------|-----------|
| | Head region | Intestinal region | Skin |
| <i>Trachurus trachurus</i> (Kote´) | | | |
| TAPC | 11.07±1.21 | 9.46±0.29 | 4.59±0.39 |
| Coliform count | 5.17±0.65 | - | 5.69±0.85 |
| Fungal count | 4.15±0.46 | 1.49±0.01 | 3.66±0.42 |
| <i>Scomber scombrus</i> (Titus or Scombia) | | | |
| TAPC | 7.11±1.31 | 10.67±1.23 | 4.63±0.43 |
| Coliform count | 3.74±0.42 | - | 4.30±0.11 |
| Fungal count | 2.87±0.32 | 1.36±0.02 | 3.63±0.34 |
| <i>Urophycis tenuis</i> (Stock fish) | | | |
| TAPC | 8.93±0.54 | 11.82±2.11 | 6.79±0.32 |
| Coliform count | 5.92±0.46 | 2.43±0.72 | 4.45±0.42 |
| Fungal count | 5.08±0.18 | 3.36±1.33 | 3.56±0.52 |
| <i>Clupea harengus</i> (Sawa or Shawa) | | | |
| TAPC | 11.97±1.32 | 9.92±0.84 | 4.58±0.91 |
| Coliform count | 2.65±0.03 | 0.90±0.00 | 3.18±0.62 |
| Fungal count | 4.96±0.78 | 2.85±0.12 | 3.58±0.98 |

Table 3. Microorganisms isolated from fish and meat samples

| Sample | | Microorganisms Isolated* |
|----------------|----------------------------|--|
| Local name | Major name | |
| Kote´ | <i>Trachurus trachurus</i> | <i>Staphylococcus aureus</i> , <i>Trichodina spp</i> , <i>Aspergillus spp</i> , <i>Streptococcus spp</i> , <i>Klebsiella spp</i> , <i>Flavobacterium</i> , <i>Bacillus spp</i> . |
| Titus/ Scombia | <i>Scomber scombrus</i> | <i>Pseudomonas spp</i> , <i>Klebsiella spp</i> , <i>Trichodina spp</i> , <i>Aspergillus spp</i> , <i>Fusarium spp</i> . |
| Stock fish | <i>Urophycis tenuis</i> | <i>Staphylococcus aureus</i> , <i>Bacillus spp</i> , <i>Salmonella spp</i> , <i>Aspergillus spp</i> , <i>Penicillium spp</i> , <i>Enterobacter spp</i> , <i>Flavobacterium</i> , <i>Isopod</i> |
| Sawa/Shawa | <i>Clupea harengus</i> | <i>Bacillus spp</i> , <i>Salmonella spp</i> , <i>Aspergillus spp</i> , <i>Actinomycetes</i> , <i>Alternaria spp</i> , <i>Saprolegnia spp</i> |
| Chicken | Poultry- Chicken | <i>Staphylococcus aureus</i> , <i>Bacillus spp</i> , <i>Klebsiella spp</i> , <i>Escherichia coli</i> , <i>Listeria spp</i> , <i>Salmonella spp</i> , <i>Aspergillus spp</i> , <i>Pseudomonas spp</i> . |
| Turkey | Poultry- Turkey | <i>Staphylococcus aureus</i> , <i>Serratia marcescens</i> , <i>Enterobacter spp</i> , <i>Micrococcus spp</i> , <i>Salmonella spp</i> , <i>Aspergillus spp</i> . |
| Gizzard | Gizzard | <i>Staphylococcus spp</i> , <i>Bacillus spp</i> , <i>Listeria spp</i> , <i>Escherichia coli</i> , <i>Klebsiella spp</i> , <i>Salmonella spp</i> , <i>Pseudomonas spp</i> , <i>Aspergillus spp</i> . |

* Microorganisms isolated in 40% and above of specific sample types

other products in the fridge/freezer or by use of same utensils. *Listeria* and *Salmonella* spp are known to be highly pathogenic with meat and meat products as a major source of infection for man. It has been reported that a drop of fluid containing *Listeria* is enough to cause contamination of products. Similarly cross contamination via utensil have been well documented (Roche *et al.* 2003, Gombas *et al.* 2003, Harrington, 2010, Wagner, 2013, Berrange *et al.*, 2013). Effective HACCP application and GMP in the handling of meat and fish products is imperative.

Fungi spp of *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Saprolegnia* are spore bearers and common environmental contaminants of food and food products. The isolation of these organisms in fish and meat is in tandem with the reports of (Refai *et al.*, 2010; Junaid *et al.*, 2010, Iqbal *et al.*, 2012, Iqbal and Mumtaz, 2013). These fungi are observed as pathogen or opportunistic pathogens in fresh and salt water fishes and have also been implicated in veterinary and human diseases (Saleem *et al.* 2012, Oranusi and Olarewaju, 2013a, Oranusi *et al.* 2013b). Fish do not have indigenous flora, the presence of these fungi in fish could be associated to contamination from the environment, the personnel, water and utensils (Geldreich and Clarke, 1966, Lund, 2000, Adams and Moss, 2000, Loir *et al.*, 2003).

Conclusion

Freezing preserve food for extended periods of time because it prevents the growth of micro-organisms that cause both food spoilage and foodborne illness. Freezing to a large extent doesn't destroy these micro-organisms, it is especially important to make sure food is wholesome before freezing. Once thawed, microbes that were present when the food was frozen, including bacteria and molds, can become active again. If conditions are right, these microbes can multiply and lead to foodborne illness. The safety of foods that have been frozen depends on the condition and handling of the food before and after being frozen, and the freezer temperature. It is advanced to freeze meats and poultry in meal-size quantities so that they can be defrosted quickly,

maintain hygienic practices with food – make sure hands, chopping boards and utensils are clean and avoid cross-contamination; all frozen foods must be properly cooked before consumption and effective HACCP application is necessary (sea food health facts, 2013).

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