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Evaluation of microorganisms associated with vended frozen fish in Ado Ekiti locality

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Abstract

Fishes are world widely consumed by all categories of works of life because of their richness in protein, readily available and affordable by all. 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 the environment of culture and their proliferation due to inadequate storage facilities. The aim of this study was focused on the isolation and identification of microorganisms from four frozen fish species sold in the Ado Ekiti metropolis. Frozen fish samples of *Scomber scombrus* (Atlantic Mackerel), *Clupea harengus* (Atlantic herring), *Urophycis tenuis* (White hake or mud hake) and *Trachurus trachurus* (Atlantic horse mackerel) from two markets in Ado Ekiti were microbiologically analyzed for possible microbial contamination. On the fish samples, the total heterotrophic count (THC) was 3.5×10^4 – 5.6×10^4 CFU/g, total coliform count (TCC) was 2.4×10^4 – 5.1×10^4 CFU/g, total *Salmonella*/*Shigella* count (TSSC) was 1.3×10^4 – 3.5×10^4 CFU/g, total *Vibrio* count (TVC) was 1.1×10^4 – 2.3×10^4 CFU/g and total fungal count (TFC) was 1.3×10^3 – 2.3×10^3 Spore/g were analyzed by cultural methods. There were variations in microbial loads among the fish species in the surveyed markets. The microorganisms identified with their percentage occurrence were *Bacillus cereus* (11.54%), *Streptococcus faecium* (13.46%), *Alcaligenes faecalis* (5.77%), *Salmonella enterica* serovar Typhi (5.77%), *Micrococcus luteus* (9.62%), *Vibrio cholerae* (7.69%), *Aerococcus viridans* (3.85%), *Pseudomonas aeruginosa* (7.69%), *Xanthomonas fragariae* (7.69%), *Staphylococcus aureus* (11.54%), *Clostridium butyricum* (7.68%), *Escherichia coli* (7.69%), *Aspergillus fumigatus* (11.11%), *Aspergillus flavus* (24.44%), *Aspergillus clavatus* (8.89%), *Aspergillus fishcheri* (6.69%), *Aspergillus terreus* (8.89%), *Mucor mucedo* (17.78%), *Penicillium digitatum* (13.33%) and *Aspergillus parasiticus* (8.89%). The results emphasized the microbial contamination of the fishes. The results obtained could serve as an awareness to consumers that microbial infection is possible from frozen fishes and as data for future reference in epidemiology or outbreak of disease from eating frozen fish.

1. Introduction

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 (Hastein *et al*., 2006). 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 resulted from its easy digestibility, readily available and high nutritional value. Fishes are found in different waters. Some are found in freshwater while some are found in saltwater (sea and oceans). According to the Center for Food Safety and Applied Nutrition in Washington (2001), most fish-related foodborne illness are traced to *Salmonella*, *Staphylococcus* spp., *Escherichia* spp., *Vibrio* spp., and *Clostridium* spp. It was further reported by these workers that harmful microorganisms could also enter the seafood processing chain because of inadequate process control, poor standards of hygiene and sanitation in processing plants and post-production contamination during incorrect handling or storage. Fish has been accepted as a good source of protein and other elements necessary for maintaining a healthy body but they deteriorate rapidly especially when storage facilities are lacking (Adebayo-Tayo *et al*., 2012).

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Kvenberg (1991) classified the bacterial pathogens associated with fish into two: the non-indigenous bacteria pathogens and the indigenous bacteria pathogens. The non-indigenous pathogens contaminates fish or fish's habitat in one way or the other and the pathogens include *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* species, *Shigella* species, *Escherichia coli*, etc. The indigenous bacteria pathogens are those naturally living in the fish's habitat such as *Vibrio* and *Aeromonas* species. Some bacteria that grow on fish, like *Pseudomonas* species, *Moraxella* species, *Alcaligenes* species, *Flavobacterium* species, etc. can survive freezing temperature and will resume growth when thawed (Frazier and Westhoff, 1988). 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). FAO (1989) report showed 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 fishnet along with the bottom sediments of water for a long time could result in exposing the fish to high bacterial contamination.

In view of the various ways fishes could be contaminated with microorganisms, the present research was therefore aimed at identifying the microorganisms associated with frozen fish sold in some markets in Ado Ekiti of Nigeria.

2. Materials and methods

2.1 Collection of samples

Four different fish species namely *Scomber scombrus* (Atlantic Mackerel), *Clupea harengus* (Atlantic herring), *Urophycis tenuis* (White hake or mud hake) and *Trachurus trachurus* (Atlantic horse mackerel). Five each of the fish samples were randomly purchased at the different period from retailers in two different markets within Ado-Ekiti Metropolis. The purchased fish samples were wrapped in a sterile polythene bag and contained in a cooler stocked with ice cubes. The fish were taken to the laboratory where they were microbiologically analyzed under aseptic condition.

2.2 Sample preparation

Sample preparation was made using the method described by Obi and Krakowiaka (1983). Approximately 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples were macerated in a sterile mortar with 10 mL sterile water. From the macerated sample, 1 mL was serially diluted tenfold.

2.3 Inoculation, enumeration and identification of isolates

Serially diluted fish samples (1 mL) were obtained with sterile pipette and pour plated on culture media for enumeration and preliminary identification. Bacterial plates were incubated at 37°C for 24 hrs, while fungal plates of potato dextrose agar were incubated at room temperature (28±2°C) for 72 hrs. The samples were cultured on plate count agar for bacterial enumeration, *Salmonella*/*Shigella* agar for *Salmonella* and Shigella species, mannitol salt agar for *Staphylococcus aureus*, violet red bile glucose agar for *Enterobacteriaceae*, Thiosulfate Citrate bile salts sucrose agar for *Vibrio*, eosin methylene blue agar for *Escherichia coli* and nutrient agar for any other bacteria that might be present in the samples. Resultant colonies that were identifiable as discrete were carefully examined macroscopically for cultural characteristics before colony enumeration using the method of Sharmin *et al*. (2014). The colonies were then sub-cultured into fresh agar plates to obtain a pure culture of isolates.

The purified isolates were Gram-stained to observe for cell morphology and uniformity. Identification of isolates was carried out based on the methods described by Cheesbrough (2002) and Holt *et al*. (1994), where Gram stain, citrate, oxidase, methyl red (MR) – Voges Prauskauer (VP) tests, triple sugar iron (TSI), catalase, coagulase and indole production, were the biochemical tests performed to identify the bacteria to species level. Fungal isolates were sub-cultured on potato dextrose agar and identified based on their morphological and cultural characteristics as described by the method of Barnett *et al*. (2000).

3. Results and discussion

3.1 Microbial load of fish samples

Total heterotrophic counts from the fish samples were more in *T. trachurus* with the count of 5.6×10^4 CFU/g in the sample purchased from the Oba market. This was followed by *S. scombrus* purchased from Erinfun market with load of 5.4×10^4 CFU/g and least count of 3.5×10^4 CFU/g from *C. harengus* purchased from Oba market. Coliform bacteria were present in all the fish samples with counts that ranged from 2.4×10^4 to 5.1×10^4 CFU/g. *C. harengus* purchased from Erinfun market was the most coliform bacteria populated sample with a load of 5.1×10^4 CFU/g. Following this was *S. scombrus* purchased from Erinfun market with a 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 a load of 3.5×10^4 CFU/g in the sample purchased from the Oba market. This was followed by a count of 2.8×10^4 CFU/g from *S. scombrus* purchased from Oba market and the 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 a load of 2.3×10^4 CFU/g in *T. trachurus* purchased from Oba market. Counts from other fish samples were in the range of 1.1×10^4 - 1.7×10^4 CFU/g. However, no *Vibrio* count was recorded from *S. scombrus* and *U. tenuis* purchased from the Oba market. Fungal counts were recorded in all the studied fish samples, with counts that ranged from 1.3×10^3 - 2.3×10^3 Spore/g. *S. scombrus* purchased from Oba market had the highest fungal load of 2.3×10^3 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). Fish is highly nutritious as known for its compositions and this has made man to source for it as a healthy diet. Meanwhile, the nutrient contents of fish highly encourage the growth of microorganisms. These microorganisms need the nutrients for growth and multiplication and not intended to cause harm to man. But the consumption of pathogen contaminated fish does result in infection and probably disease manifestation. Also, contaminated fish with food spoilage microorganisms may set a foundation of loss to fish retailers if the microbiological quality is not evaluated to sound warning on adequate preservation. Studying frozen fish's quality microbiologically paves the way for the update and adoption of quality storage methods.

3.2 Isolated microorganisms from fish samples

A total of twelve bacteria species were isolated from the frozen fish samples. Isolated from the head of *Clupea harengus* include *Bacillus cereus*, *Alcaligenes faecalis* and *Streptococcus faecium*. From the middle region were *S. faecium, Salmonella enterica* serovar Typhi and *Micrococcus luteus*, while *S. faecium* and *Vibrio cholerae* were isolated from the tail region. *Aerococcus viridans; S. faecium* and *Pseudomonas aeruginosa* were respectively isolated from the head and middle regions of *Scomber scombrus* while *Xanthomonas fragariae* and *S. faecium* were isolated from the tail region. From the head of *Trachurus trachurus*, *X. fragariae* and *Staphylococcus aureus* were isolated, from the middle region were *Clostridium butyricum* and *Escherichia coli,* while *S. faecium* and *A. viridians* were found at the tail region. From the head of *Urophycis tenuis, M. luteus and S. aureus* were isolated, *V. cholerae*, *E. coli* and *Alcaligenes faecalis* were isolated from the middle region, while *X. fragariae*\and *S. faecium* were isolated from the tail region.

The most frequently occurred bacteria species was *S. faecium* with 13.46%. This was followed by *B. cereus* and *S. aureus* with 11.54%, while the least occurred bacteria specie was *A. viridans* with 3.85% (Table 2). Fish are perishable and liable to extremely large variations in quality regarding 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 3.5×10^4 - 5.6×10^4 CFU/g denoting that all the evaluated fish were microbiologically certified as been of good quality hence the counts were within the permissible level of international standard. ICMSE (1986) accepted limit of the total coliform count for frozen fish is <100 MPN/g. The total coliform counts from the fish samples as recorded in this study was between 2.4×10^4 - 5.1×10^4 CFU/g. The encounter of coliform from the fish samples indicates animal or human faecal contamination 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 the majority of the evaluated fishes. By the standard of the International Association of Microbiology Society, these species of bacteria should not the encountered in frozen fish. From

Table 1. Microbial counts from fish samples in the surveyed markets

Fish	Market	THC (CFU/g)	TCC (CFU/g)	TSSC (CFU/g)	TVC (CFU/g)	TFC (Spore/g)
Clupea harengus	Oba	3.5×10^4	2.6×10^4	1.3×10^4	1.1×10^4	1.6×10^3
	Erinfun	4.2×10^4	5.1×10^4	3.4×10^4	1.3×10^4	1.4×10^3
Scomber scombrus	Oba	4.6×10^4	3.6×10^4	2.8×10^4	-	2.3×10^3
	Erinfun	5.4×10^4	4.3×10^4	1.7×10^4	1.5×10^4	2.0×10^3
Trachurus trachurus	Oba	5.6×10^4	2.6×10^4	2.5×10^4	2.3×10^4	1.7×10^3
	Erinfun	4.3×10^4	3.7×10^4	1.3×10^4	1.4×10^4	2.1×10^3
Urophycis tenuis	Oba	4.6×10^4	3.6×10^4	3.5×10^4	-	1.6×10^3
	Erinfun	5.2×10^4	2.4×10^4	1.4×10^4	1.7×10^4	1.3×10^3

Table 2. Frequency of occurrences for bacterial isolated from frozen fish

Fish	Market	Isolated Bacteria	No (%)
Clupea harengus	Oba/Erinfun	*B. cereus*	2
Scomber scombrus	Oba/Erinfun	*B. cereus*	1
Trachurus trachurus	Oba/Erinfun	*B. cereus*	1
Urophycis tenuis	Oba/Erinfun	*B. cereus*	2
			6(11.54)
Clupea harengus	Oba/Erinfun	*S. faecium*	3
Scomber scombrus	Oba/Erinfun	*S. faecium*	2
Trachurus trachurus	Oba/Erinfun	*S. faecium*	1
Urophycis tenuis	Oba/Erinfun	*S. faecium*	1
			7(13.46)
Clupea harengus	Oba/Erinfun	*A. faecalis*	1
Scomber scombrus	Oba/Erinfun	*A. faecalis*	0
Trachurus trachurus	Oba/Erinfun	*A. faecalis*	1
Urophycis tenuis	Oba/Erinfun	*A. faecalis*	1
			3(5.77)
Clupea harengus	Oba/Erinfun	*S. enterica ser. Typhi*	1
Scomber scombrus	Oba/Erinfun	*S. enterica ser. Typhi*	1
Trachurus trachurus	Oba/Erinfun	*S. enterica ser. Typhi*	0
Urophycis tenuis	Oba/Erinfun	*S. enterica ser. Typhi*	1
			3(5.77)
Clupea harengus	Oba/Erinfun	*M. luteus*	2
Scomber scombrus	Oba/Erinfun	*M. luteus*	1
Trachurus trachurus	Oba/Erinfun	*M. luteus*	1
Urophycis tenuis	Oba/Erinfun	*M. luteus*	1
			5(9.62)
Clupea harengus	Oba/Erinfun	*V. cholerae*	1
Scomber scombrus	Oba/Erinfun	*V. cholerae*	0
Trachurus trachurus	Oba/Erinfun	*V. cholerae*	2
Urophycis tenuis	Oba/Erinfun	*V. cholerae*	1
			4(7.69)
Clupea harengus	Oba/Erinfun	*A. viridans*	0
Scomber scombrus	Oba/Erinfun	*A. viridans*	1
Trachurus trachurus	Oba/Erinfun	*A. viridans*	1
Urophycis tenuis	Oba/Erinfun	*A. viridans*	0
			2(3.85)
Clupea harengus	Oba/Erinfun	*P. aeruginosa*	0
Scomber scombrus	Oba/Erinfun	*P. aeruginosa*	1
Trachurus trachurus	Oba/Erinfun	*P. aeruginosa*	1
Urophycis tenuis	Oba/Erinfun	*P. aeruginosa*	2
			4(7.69)
Clupea harengus	Oba/Erinfun	*X. fragariae*	1
Scomber scombrus	Oba/Erinfun	*X. fragariae*	1
Trachurus trachurus	Oba/Erinfun	*X. fragariae*	1
Urophycis tenuis	Oba/Erinfun	*X. fragariae*	1
			4(7.69)
Clupea harengus	Oba/Erinfun	*S. aureus*	2
Scomber scombrus	Oba/Erinfun	*S. aureus*	2
Trachurus trachurus	Oba/Erinfun	*S. aureus*	1
Urophycis tenuis	Oba/Erinfun	*S. aureus*	1
			6(11.54)
Clupea harengus	Oba/Erinfun	*C. butyricum*	1
Scomber scombrus	Oba/Erinfun	*C. butyricum*	1
Trachurus trachurus	Oba/Erinfun	*C. butyricum*	2
Urophycis tenuis	Oba/Erinfun	*C. butyricum*	0
			4(7.69)
Clupea harengus	Oba/Erinfun	*E. coli*	1
Scomber scombrus	Oba/Erinfun	*E. coli*	0
Trachurus trachurus	Oba/Erinfun	*E. coli*	1
Urophycis tenuis	Oba/Erinfun	*E. coli*	2
			4(7.69)
Total			52(100)

this perspective, we cannot ascertain the good quality of the fish samples despite the counts of other isolated pathogens that were within the standard level. However, species of *Salmonella* and *Vibrio* have been reported in frozen fish by Samaha (2017), Popovic *et al*. (2010), and Sanjee and Karim (2016). *Aspergillus flavus*, the most dominant fungus among the isolated fungi have been reported in fresh and saltwater and implicated as a pathogen in animal and human diseases as reported by Saleem *et al*. (2012), Oranusi and Olarewaju, (2013), and Oranusi *et al*. (2013).

The presence of *S. faecium* as the most dominant bacteria identified from the fish samples simplifies that the offshore water was contaminated with faecal of either man or animals. Though the infectious dose of this bacterium is unknown, the diseases it manifests such as urinary tract infection, wound infection, endocarditis and bacteraemia may be zoonotic and communicable. Meanwhile, this bacterium has been found to be multiple drug-resistant (Shimoda *et al*., 1984).

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 the environment (Wagner, 2013). Similarly, cross-contamination via utensil has been well documented by Roche *et al*. (2003), Harrington, (2010), and Berrang *et al*. (2013).

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, *A. flavus* and *Aspergillus clavatus* respectively. Isolated from *S. scombrus* are *A. flavus* from the head region, *Mucor mucedo*, *Aspergillus fischeri* and *Aspergillus terreus* from the middle region, while *Penicillium digitatum, Aspergillus terreus* and *M. mucedo* were from the tail region. From the head region of *T. trachurus*, *M. mucedo*, *A. fumigatus* and *A. flavus* were isolated. From the middle region were *A. fumigatus* and *A. clavatus*, while from the tail were *A. flavus, M. mucedo* and *Aspergillus parasiticus*. *Urophycis tenuis* was inhabited with *A. flavus* at the head region, *M. mucedo* and *Penicillium digitatum* from the middle region and *A. flavus* and *A. fumigatus* from the tail region.

The result obtained from the fish denotes varied microbial contamination. From the fungal contamination perspective, *Aspergillus* species were dominant in the fish samples from different regions. Meanwhile, *A. flavus* was the most frequently occurred among the isolated fungi with 24.44% occurrence, followed by *M. mucedo* (17.78%) and *A. fischeri* (6.67%) as the least frequently occurred (Table 3).

The microorganisms identified in this study seemed to be common among other species of frozen fishes in reports of researchers elsewhere. Okonko *et al*. (2008), Chukwuka *et al*. (2010), Akinmusire, (2011), and Adebayo-Tayo *et al*. (2012) have isolated similar microorganisms from different frozen fishes in Nigeria. Whereas, Edris *et al*. (2017) has isolated them from Egypt and Murad *et al.* (2013) from Iran. Popovic *et al*. (2010), have isolated *Salmonella* sp, *E. coli*, *S. aureus* and *V. cholerae* from fresh and frozen seafoods in Croatia.

Thatcher and Clark (1973) earlier report 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, the severity of freezing process with respect to lethality to microorganisms and contamination by handlers and market sellers. For this, it, therefore, calls for great attention to handle fish in more hygienic ways to keep up to the microbiological standard of the use of fishing equipment which often serves as a route of contamination of frozen fish and storage environment. Brooks *et al*. (2004) however, concluded that one of the sources of infection is contaminated food. Foodstuffs safety are generally ensured by a preventive approach, which could be achieved by the implementation of the practice of good hygiene and application of the procedures based on hazard analysis and critical control point (HACCP) principles. Popovic *et al*. (2010), have therefore reported that microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. Some 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.

4. Conclusion

Both pathogenic and spoilage microbes were isolated from the frozen fish and it signals that microorganisms in frozen fish can serve as a 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 the pathogenic microorganisms isolated emphasized that frozen fish can serve as a possible vehicle for human infection. Every home in Nigeria consumes fish on daily basis in their diet. The consumed fish are either in inform of dried, boiled or fried and to date, there is no literature or data on disease outbreak as a result of consuming frozen or processed fishes.

Table 3. Occurrence frequency of the isolated fungi from fish samples

Fish	Market	Isolated Bacteria	No (%)
Clupea harengus	Oba/Erinfun	*A. fumigatus*	2
Scomber scombrus	Oba/Erinfun	*A. fumigatus*	0
Trachurus trachurus	Oba/Erinfun	*A. fumigatus*	2
Urophycis tenuis	Oba/Erinfun	*A. fumigatus*	1
			5(11.11)
Clupea harengus	Oba/Erinfun	*A. flavus*	5
Scomber scombrus	Oba/Erinfun	*A. flavus*	2
Trachurus trachurus	Oba/Erinfun	*A. flavus*	2
Urophycis tenuis	Oba/Erinfun	*A. flavus*	2
			11(24.44)
Clupea harengus	Oba/Erinfun	*A. clavatus*	3
Scomber scombrus	Oba/Erinfun	*A. clavatus*	0
Trachurus trachurus	Oba/Erinfun	*A. clavatus*	1
Urophycis tenuis	Oba/Erinfun	*A. clavatus*	0
			4(8.89)
Clupea harengus	Oba/Erinfun	*A. fischeri*	1
Scomber scombrus	Oba/Erinfun	*A. fischeri*	2
Trachurus trachurus	Oba/Erinfun	*A. fischeri*	0
Urophycis tenuis	Oba/Erinfun	*A. fischeri*	0
			3(6.67)
Clupea harengus	Oba/Erinfun	*A. terreus*	1
Scomber scombrus	Oba/Erinfun	*A. terreus*	2
Trachurus trachurus	Oba/Erinfun	*A. terreus*	0
Urophycis tenuis	Oba/Erinfun	*A. terreus*	1
			4(8.89)
Clupea harengus	Oba/Erinfun	*M. mucedo*	1
Scomber scombrus	Oba/Erinfun	*M. mucedo*	3
Trachurus trachurus	Oba/Erinfun	*M. mucedo*	3
Urophycis tenuis	Oba/Erinfun	*M. mucedo*	1
			8(17.78)
Clupea harengus	Oba/Erinfun	*P. digitatum*	1
Scomber scombrus	Oba/Erinfun	*P. digitatum*	2
Trachurus trachurus	Oba/Erinfun	*P. digitatum*	1
Urophycis tenuis	Oba/Erinfun	*P. digitatum*	2
			6(13.33)
Clupea harengus	Oba/Erinfun	*A. parasiticus*	1
Scomber scombrus	Oba/Erinfun	*A. parasiticus*	3
Trachurus trachurus	Oba/Erinfun	*A. parasiticus*	3
Urophycis tenuis	Oba/Erinfun	*A. parasiticus*	1
			4(8.89)
Total			45(100)

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 a useful reference in future.

Conflict of interest

The authors declare no conflict of interest.

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