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MICROBIOLOGICAL ASSESSMENT OF THREE FROZEN MARINE COMMERCIAL FISHES

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ABSTRACT

The microbial status of Clupea harengus, Scomber scombrus and Micromesistius poutassou fish species sold in Owo market, Ondo state, Nigeria were determined at 0, 10 and 24 hr of exposure at open market temperature. Nine bacteria and seven fungi species were isolated and identified. The eight bacteria species identified were Micrococuss luteus, Salmonella typhimurium, Proteusmirabilis, Bacillus cereus, Leuconostoc lactis, Eschericha coli, Salmonella sonnel, Enterobacter aerogenes and Staphylococcus aureus. Proteus mirabilis and Bacillus cereus were present in all the three fish species. Clupea harengus had the highest bacteria count while Micromesistius poutassou had the lowest with respect to Micrococuss luteus. The fungal species identified were Rhizopus oryzae, Rhizopus stolonifer, Aspergillus flavus, Aspergillus fumigates, Candida krusei, Fusarium oxysporium and Schizosaccharomyces pombe. Aspergillus flavus and Candida krusei were isolated from all the three species. Micromesistius poutassou had the highest count of fungi while Scomber scombrus had the least. Bacteria load of the fish increases as the open market exposure duration increased. The highest bacterial (Micromesistius potassou) load ($4.0x10^5$)was recorded at 24 hours of open market exposure in while the lowest ($1.9x10^4$) was recorded at 10 hours. Fungal load of $7.6x10^3$ was recorded in Clupea harengus after 24 hrof exposure while there was no fungi growth at 0 hr. Fish contamination would be reduced with personal hygiene of fish handlers, availability of good storage and transportation facilities.

Keywords: microbial load, bacterial, fungal

INTRODUCTION

Fish and fish products constitute more than 60% of the total protein intake in Africa (Adekoya and Miller, 2004). Fish is considered a balanced food because it contains all classes of nutrient suitable for preventing malnutrition especially in Africa (Ogundiran et al., 2014; Olokor et al., 2007). Silva et al. (2008) reported that fish and fish product have high economic values. It is also a preferable source for animal protein with balanced amino acids, vitamins and essential nutrients. The inclusion of fish in the diets of groups of people such as pregnant and nursing mothers, infants and pre-school children is because of the polyunsaturated fatty acids essential for development of brain and nervous system found in fish (Steffens and Wirth, 1997). However, the increasing demand for fish and fish products all over the world is greatly challenged by microbial infection of fish and contamination of fish products especially (Pyatkin and Krivoshein, 1986). Fish contamination occurs at the point of sale in the open markets where preservation is lowest

Fish is highly perishable because of microorganisms on the skin, gastrointestinal tract and gills (Adedeji and Adetunji, 2004). According to Eyabi-Eyabi (1998), the limited shelf life of a dead fish is 16-36 hours under good conditions. The immune system of the fish fights against the microbes when the fish is alive, but after death, the immune system breaks down which allow multiplication of spoilage microbes. Spoilage in fish also increases due to contaminated surface of harvesting material, a conducive condition for the growth of spoilage organisms.

According to Olokor et al. (2007), freezing is the one of easiest and least time-consuming method of food preservation. It allows for retention of their natural colour, flavour, taste, texture and nutritional value in foods better than any other method. The freezing process is of beneficial effects because of low temperatures at which microorganism cannot grow, chemical reactions are reduced and cellular metabolic reactions are delayed (Delgado and Sun, 2000). During display of frozen raw fish for sale in the open market,

thawing occurs causing increase in temperature that is conducive for the growth of spoilage organisms.

Fish undergoing spoilage shows signs ranging from slime formation, discolouration, texture change, off-odours, off-flavours and gas production (Adedeji, 2012). Therefore, there is a need to assess the microbial load of frozen fish to ascertain the quality of fish sold in open market.

MATERIALS AND METHODS

Nine samples each of three fish species: common mackerel *(Scomber scombrus),* Atlantic hearings *(Clupea harengus)* and blue whiting *(Micromesistius poutassou* were obtained from three different cold rooms at Owo town, Ondo state, August 2016. The samples were collected early in the morning in a sterile polythene bag containing ice cubes and transported to the Laboratory of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo state for microbial analyses. Morphometric readings of the fish samples were taken immediately the samples got to the laboratory. Morphometric readings were weight, total length, head length, body depth, caudal penduncle length and depth, eye diameter and standard length.

For the microbial analysis of the samples of *Scomber scombrus, Clupea harengus* and *Micromesistius poutassou*, the method as described by Slaby et al. (1981) was adopted. Morphological characteristics of the various bacteria isolates were noted in the agar plates after gram staining reactions and series of biochemical procedures. The procedure was done in triplicates. The values obtained were analysed using one-way analysis of variance (ANOVA) using statistical package for social science (SPSS v.20.0). T-test was used to test for significance of means.

RESULT AND DISCUSSION

Bacteria isolated from the three fish species were *Micrococcus luteus, Proteus mirabilis, Bacillus cereus, Leuconostoc latis, Escherichia coli, Staphylococcus aureus, Salmonella sonnel, Salmonella typhimurium, Enterobacter aerogenes.* Fungi isolated from fish samples are *Rhizopus oryzae, Aspergillus fumigates, Candida krusei, Rhizopus stolonifer, Fusarium oxysporium, Schizosaccharomyces pombe, Aspergillus flavus.*

At 0 hour, *Scomber scombrushad* the least bacteria load ($4.0x10^4$ cfu/ml) while *Micromesistius poutassou* had highest ($5.9x10^4$cfu/ml). Bacteria count of *Scomber scombrus* was the least at 10 hours ($8.7x10^4$ cfu/ml) and that of *Micromesistius poutassouwas* the highest ($1.5x10^5$ cfu/ml). The highest bacteria count ($5.9x10^5$ cfu/ml) at 24 hours was recorded with *Scomber scombrus* while the lowest was $5.0x10^5$ cfu/ml for *Micromesistius poutassou. Micromesistius poutassou* had the highest fungi count ($1.7x10^4$ sfu/ml) at 0 hour with the lowest in *Clupea harengus* ($2.3x10^3$ sfu/ml).

Bacteria isolated from *Scomber scombrus* were *Micrococcus luteus, Bacillus cereus, Escherichia coli, Salmonella typhimrium, Leuconostoc lactisand Enterobacter aerogenes.* Bacteria isolates reported for *Micromesistius poutassouwere Micrococcus luteus, Proteus mirabilis, Escherichia coliand Enterobacter aerogenes. Clupea harengus contained Micrococcus luteus, Leuconostoc lactis, Proteus mirabilis, Salmonella sonnel,Bacillus cereusand Salmonella typhimrium* (Table 2).

Fungal species isolated from *Scomber scombrus* were *Aspergillus fumigates, Aspergillus flavus, Candida krusei and Fusarium oxysporium.Candida krusei, Rhizopus stolonifer, Aspergillus fumigates*, and *Schizosaccharomyces pombe* were fungi genus isolated from *Micromesistius poutassou*. From *Clupea harengus*, fungi genus isolated are *Rhizopus oryzae, Candida krusei, Rhizopus stolonifer, Aspergillus flavus,* and *Fusarium oxysporium* (Table 3).

The result indicates different microbes associated with frozen commercial marine fishes such as *Scomber scombrus, Micromesistius poutassou,* and *Clupea harengus.* The presence of some pathogenic bacteria isolates such as *Escherichia coli* reveals the pollution of the aquatic habitats with faecal matter either from sewage disposal or from human activities. Most of the organisms isolated causes food poisoning such as *shigellosis, salmonellosis* caused by *Shigella* and *Salmonella spp* respectively.

Table 1: Analysis of Microbial Loads of *Scomber scombrus, Micromesistius poutassou,* and *Clupea harengus* after 0, 10 and 24 hoursof open market exposure

Time	Microbial Loads	*Scomber scombrus*	*Micromesistius poutassou*	*Clupea harengus*
0 Hour	Bacteria (cfu/ml)	$4.0x10^4$	$5.9x10^4$	$4.6x10^4$
	Fungi (sfu/ml)	$6.8x10^3$	$1.7x10^4$	$2.3x10^3$
	p-value	0.35	0.36	0.36
	t-test	1.20	1.19	1.19
10 Hours	Bacteria (cfu/ml)	$8.7x10^4$	$1.5x10^5$	$1.4x10^5$
	Fungi (sfu/ml)	$7.1x10^3$	$1.2x10^4$	$8.6x10^3$
	P value	0.36	0.36	0.36
	t-test	1.19	1.18	1.18
24 Hours	Bacteria (cfu/ml)	$5.9x10^5$	$5.0x10^5$	$5.3x10^5$
	Fungi (sfu/ml)	$9.0x10^3$	$2.3x10^4$	$1.6x10^4$
	P-value	0.36	0.36	0.36
	t-test	1.17	1.17	1.17

Table 2: Analysis of bacteria species isolated from *Scomber scombrus, Micromesistius poutassou,* and *Clupea harengus* after 0, 10 and 24 hours of open market exposure

	Fish species								
	Scomber scombrus			*Micromesistius poutassou*			*Clupea harengus*		
Bacteria	0 hour	10 hours	24 hours	0 hour	10 hours	24 hours	0 hour	10 hour	24 hours
Bacillus cereus	$5.2x10^4$	-	$2.3x10^4$	-	-	-	-	-	$2.1x10^5$
Enterobacter aerogenes	-	-	$1.2x10^5$	-	$1.9x10^4$	$1.9x10^4$	-	-	-
Escherichia coli	-	-	$3.2x10^5$	$2.1x10^4$	-	-	-	-	-
Leuconostoc lactis	-	$6.7x10^3$	-	-	-	-	$4.2x10^4$	$1.0x10^4$	$1.3x10^5$
Micrococcus luteus	$3.4x10^4$	-	$1.2x10^5$	-	$1.4x10^4$	$4.0x10^5$	-	-	-
Proteus mirabilis	-	$1.3x10^4$	-	$5.4x10^4$	-	-	$1.0x10^5$	-	-
Salmonella sonnel	-	-	-	-	$4.0x10^3$	-	-	-	-
Salmonella typhimurium	-	$2.0x10^4$	-	-	-	-	-	$3.5x10^4$	$1.8x10^5$
Staphylococcus aureus	-	-	-	$8.0x10^4$	-	-	-	-	-

Key: -= Not present

Table 3: Analysis of fungal species isolated from *Scomber scombrus, Micromesistius poutassou,* and *Clupea harengus* after 0, 10 and 24 hoursof open market exposure

	Fish species								
	Scomber scombrus			*Micromesistius poutassou*			*Clupea harengus*		
Fungi	0 hour	10 hours	24 hours	0 hour	10 hours	24 hours	0 hour	10 hour	24 hours
Aspergillus flavus	-	$5.4x10^3$	-	-	-	-	-	$2.3x10^3$	-
Aspergillus fumigates	$7.1x10^3$	-	-	-	$5.6x10^3$	$1.1x10^4$	$2.1x10^3$	-	$6.4x10^3$
Candida krusei	-	$1.3x10^3$	$2.6x10^3$	$5.4x10^3$	-	-	$4.8x10^3$	-	$2.6x10^3$
Fusarium oxysporium	-	-	$6.3x10^3$	-	-	-	-	-	$7.6x10^3$
Rhizopus oryzae	-	-	-	-	-	-	-	-	-
Rhizopus stolonifer	-	-	-	$7.2x10^3$	$6.8x10^3$	-	$1.6x10^3$	-	-
Schizosaccharomyces pombe	-	-	-	-	$4.6x10^3$	$1.1x10^4$	-	-	-

Key: -= Not present

The fungal contamination of fish could be attributed to improper sanitation during catching, handling, manufacturing, storage, transportation and marketing of fish. These findings weresupported by Hassan*et al* (2012) which reported that presence of some pathogenic bacteria isolates such as *Escherichia coli* reveals the pollution of the aquatic habitats with faecal matter either from sewage disposal or from human activities.

Table 4: Percentage prevalence of Bacteria and Fungi in *Scomber scombrus, Micromesistius poutassou,* and *Clupea harengus* after 0, 10 and 24 hours

Bacteria	0 Hour	10 Hour	24 Hour
Bacillus cereus	11.11	0	22.22
Enterobacter aerogenes	0	11.11	22.22
Escherichia coli	11.11	11.11	11.11
Leuconostoc lactis	11.11	11.11	11.11
Micrococcus luteus	22.22	11.11	22.22
Proteus mirabilis	11.11	0	22.22
Salmonella sonnel	0	11.11	11.11
Salmonella typhimurium	0	11.11	11.11
Staphylococcus aureus	11.11	0	0
Fungi			
Aspergillus flavus	0	28.57	0
Aspergillus fumigates	14.29	14.29	14.29
Candida krusei	28.57	14.29	28.57
Fusarium oxysporium	0	0	28.57
Rhizopus oryzae	14.29	0	14.29
Rhizopus stolonifer	28.57	14.29	0
Schizosaccharomyces pombe	0	14.29	14.29

The findings similarly revealed that after 24 hours of exposure in open market, bacteria loads were greater than the fungi loads on the three fish species. The results of this study shows microbiological contamination of frozen fishes to be *Staphylococcus aureus* and *Escherichia coli* are common pathogenic bacteria found associated with frozen fish. Type and number of microorganism 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 existence of microorganisms and contamination by handlers and market sellers (Thatcher and Clark, 1973). Isolation of *Staphylococcus spp* and *Escherichia spp* in the frozen fish samples supports the report of Okonta and Ekelemu (2005) who reported that both bacteria species are the predominant spoilage agent in frozen fish. Fish of good quality should have bacterial count

less than 10^5 per gram as recommended by Food and Agricultural Organisation (Emikpe *et al.,*2011).

The presence of *Aspergillus spp* reported in this study, might lead to the production of toxins like aflatoxin, territrems, cyclopiazonic acid produced by this fungal isolates. This could become the possible source of Aspergillosis transmission among consumers (Doyle, 2007). *Fusarium spp, Aspergillus spp* and *Rhizopus spp* isolated in the three fish species have been incriminated in the cases of pulmonary and urinary tract infections, meningitis, arthritis, osteomyelitis, dermatitis, endocarditis and eye infection (Mossel and Shennan, 2006). The result of this study revealed that frozen fish when not properly cooked could be a source of food borne bacteria and fungal pathogens.

CONCLUSION

The determination of bacteria and fungi contamination of frozen fishes carried out in the study was necessary in safeguarding public health. This is because of all the pathogens isolated are of food and public health implication; hence, hazardous and injurious to human health if consumed. Therefore, there is a need for all hygienic measures to be adhere to during harvesting, handling and processing.

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