Determination of Heavy Metals and Microbiological Contamination of Frozen Mackerel (*Scomber scombrus*) Sold in Eke-Awka Market, Awka, Anambra State, Nigeria

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KEYWORDS

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ABSTRACT

The determination of heavy metals and microbiological contamination of frozen mackerel fish (Scomber scombrus) sold in Eke-Awka market were carried out on five (5) randomly obtained samples with the codes; FOV (Frozen mackerel from vendor 1), FVT (Frozen mackerel from vendor 2), FTV (Frozen mackerel from vendor 3), FVF (Frozen mackerel from vendor 4) and FFV (Frozen mackerel from vendor 5). For the heavy metals analyses, concentrations of lead in the samples ranged from 0.00 to 4.00 x 10^{-3} mg/g. Apart from sample FFV which gave 0.00, all other samples showed lead contamination higher than Codex maximum limit of 0.3 x 10^{-3} mg/g. Mercury levels ranged from 0.10 to 0.40 x 10^{-3} mg/g, which were below the permissible guideline level of 0.5 x 10^{-3} mg/g as set by Codex for fish. This suggests that all the fish samples were well below permissible levels for mercury contamination. Chromium found in samples did not exceed the recommended daily intake. Arsenic and Cadmium were not detected in any of the samples. For microbiological analysis, the total heterotrophic bacterial count ranged from 1.0 x10^2 to 3.8 x 10^2 Cfu/g, total coliform count ranged from 1.4x10^2 to 2.5x 10^2 Cfu/g, total Salmonella count ranged from 1.0 x 10^2 to 1.2 x 10^2 Cfu/g, total Vibrio count ranged from 1.0 x 10^2 to 1.5 x 10^2 Cfu/g, and total fungal count ranged from 1.0 x 10^2 to 1.5x 10^2 Cfu/g. The study showed that the frozen mackerel fish samples were heavily contaminated with microorganisms, which may be attributed to their habitat, storage conditions and poor sanitary practices employed by the vendors.

INTRODUCTION

Over the years, there has been a slow but sure decline in the populations of fish species. This has been attributed to overfishing and environmental pollution (Sikoki, 2013). These pollutants include polyaromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), pesticides, metals, and recently, plastic wastes (Farrington and Takada 2014). Accordingly, the contamination of fish with pathogens and trace elements has become a major public health concern that requires constant monitoring. The food borne pathogens load in a fish product can be due to harvest environment or habitat, sanitary conditions, and practices associated with equipment and persons in the process environment (FDA, 2011). Fish can host varieties of pathogens on or inside its body as they are at the top of the food chain (Dahunsi *et al.*, 2012). Fishes are very susceptible to bacterial contamination due to their soft body organs. Use of fish as a bio-indicator of bacterial pollution can provide cumulative effect of different pollutant in the ecosystem (Santos *et al.*, 2011). Heavy metals accumulation in fish tissues depend on the concentration of the metal in the aquatic ecosystem and period of exposure (Sobhanardakani *et al*., 2011). Anthropogenic sources of heavy metals in aquatic ecosystems in Nigeria include effluents from the petroleum industry and agricultural discharge. In some cases, the concentrations of metals were found to exceed the maximum permitted levels, implying potential health risks to aquatic organisms and human consumers. Minerals (heavy metals) such as mercury, lead and cadmium are toxic even in trace amounts. However, essential minerals can also produce toxic effects at high concentrations. Industrial and municipal discharges, agricultural practices, and storm water runoff can put harmful substances into the water. Fishes absorb contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dioxins, and chlorinated pesticides from water, sediments and the food they eat (Salwa *et al.*, 2016). Consumption of frozen mackerel fish is widespread in Nigeria as a cheaper and supposedly healthier alternative to meat protein. Considering the nutritional benefits and the attendant risks associated with fish consumption, it has become important that the microbial and heavy metal contamination of frozen mackerel fish be assessed in order to ensure that they meet the requirements of food regulations, commercial specifications (Watermann, 2002), and also the safety level for consumers.

MATERIALS AND METHODS

Source of Materials

Five samples of frozen mackerel fish were randomly purchased from Eke-Awka market, Anambra state, Nigeria. The samples were transported to the laboratory in a cooler and preserved with ice-block under complete aseptic conditions, where they were processed and analyzed for heavy metals and microbiological contamination.

Experimental Design

Five samples of frozen fish (mackerel) were drawn randomly from various vendors using a completely randomized design (CRD). Table 1 shows the five samples and codes used.

Table 1: Experimental design

S/N	Sample code	Sample description
1	FOV	Frozen mackerel fish from the first vendor
2	FVT	Frozen mackerel fish from the second vendor
3	FTV	Frozen mackerel fish from the third vendor
4	FVF	Frozen mackerel fish from the fourth vendor
5	FFV	Frozen mackerel fish from the fifth vendor

Heavy Metals Analyses

The muscle tissues of the frozen fish samples were oven dried to constant weight at 105 °C, ground into a fine powder and placed in bottles labeled accordingly. Triplicate digestion was performed according to the procedure of Turkmen and Ciminli (2007). Each of the samples was analyzed for Mercury, Arsenic, Cadmium, Lead, and Chromium by atomic absorption spectrophotometer.

Microbiological Analysis

Ten gram (10 g) of each sample of the digested frozen fish samples was put in 9ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1 ml was inoculated separately on to MacConkey agar, Nutrient agar and Potato Dextrose agar plates and spread evenly using sterile bent glass rod. The inoculated MacConkey agar, Nutrient agar and Potato Dextrose agar plates were incubated at 30 $^{\circ}$C and 35 $^{\circ}$C for 24 and 48 hours respectively. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per gram, cfu/g (Cheesebrough, 2006). Each of the bacterial colonies on the agar plates was subcultured and the pure culture obtained. The bacterial isolates were identified by carrying out tests which include; Gram staining, motility test and biochemical tests such as; catalase, coagulase, oxidase, citrate utilization and indole tests. For the fungal isolates, the pure cultures obtained were identified using morphological characteristics, spore formation, the production of fruiting body and biochemical reactions.

Statistical Analysis of Data

The data was analyzed using Statistical Package for Social Sciences version 23. All data were represented as mean of three replicates. The mean, range and standard deviation of each parameter was determined.

RESULTS AND DISCUSSION

Result of Heavy Metals Analyses from the five fish samples.

Table 2 shows the results of heavy metals in (mg/g) analyzed from the five fish samples, FOV, FFV, FTV, FVT, and FVF. The results indicate that concentrations of lead in the tissues of the fish samples ranged from 1.00 to 4.00 x 10^{-3} mg/g for FOV, FTV, FVT, and FVF, while no lead accumulation was detected in FFV. Lead concentrations in FVT and FVF were not significantly different from each other but are significantly different from the rest of the samples. Codex alimentarius commission (1995a) established maximum limit for lead contamination in fish as 0.3 mg/kg (0.3 x 10^{-3} mg/g) body weight for humans. In the present study, lead concentrations were higher than codex maximum limits. This agrees with the findings of Oluyemi and Olabanji (2011) in analysis of some heavy metals in frozen *C. harengus* and *S. scombrus* from Ibadan and Ile-Ife markets, Nigeria. The fish samples had mercury levels ranging from 0.10 to 0.40 x 10^{-3} mg/g, where FOV, FVT and FFV had no significant difference ($p > 0.05$), but were significantly different ($p < 0.05$) from FTV and FVF, which had same value of 0.10 x 10^{-3}mg/g. Mercury levels in the samples were below the permissible guideline level of 0.5 mg/kg (0.5 x 10^{-3} mg/g) as set by Codex alimentarius commission (1995a) for fish. This suggests that all the fish samples were well below permissible levels for mercury contamination. The mercury load was similar to the result of Zodape *et al*. (2011) in their work: contamination of heavy metals in seafood marketed from Vile Parle and Dadar markets of suburban areas of Mumbai (west coast of) India.

Table 2: Heavy Metals Contamination of the Five Fish Sample

Sample Code	Lead ($x10^{-3}$ mg/g)	Mercury ($x10^{-3}$ mg/g)	Chromium ($x10^{-3}$ mg/g)	Arsenic ($x10^{-3}$ mg/g)	Cadmium ($x10^{-3}$ mg/g)
FOV	4.00^{a}±0.00	0.40^{a}±0.00	4.00^{a}±0.00	ND	ND
FVT	2.00^{b}±0.01	0.30^{a}±0.00	2.00^{b}±0.00	ND	ND
FTV	1.00^{c}±0.00	0.10^{b}±0.00	0.40^{c}±0.00	ND	ND
FVF	2.00^{b}±0.00	0.10^{b}±0.00	0.40^{c}±0.00	ND	ND
FFV	ND	0.40^{a}±0.00	0.20^{c}±0.00	ND	ND

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$). FOV = Frozen mackerel obtained from the first vendor, FVT = frozen mackerel obtained from the second vendor, FTV = frozen mackerel obtained from the third vendor, FVF = frozen mackerel obtained from the fourth vendor, and FFV = frozen mackerel obtained from the fifth vendor. ND = not detected**.**

The Chromium load of the samples ranged from 0.20 to 4.00 x 10^{-3} mg/g. There was no significant difference ($p > 0.05$) among FTV, FVF and FFV, but significant difference exists between them and the rest of the samples. Estimates of the daily intake ranges from 0.025 to 0.2 mg/day (Codex, 1995b). Thus Chromium found in this study may not exceed the recommended daily intake. Arsenic and Cadmium were not detected in any of the samples as they all gave the value of 0.00 x 10^{-3} mg/g for both heavy metals.

Microbiological Analysis

The results of the total heterotrophic count of bacteria on the nutrient agar are shown in Table 3. The highest number of bacterial count was obtained from FOV which was 3.8 x 10^2 Cfu/g, while the least count of 1.0 x 10^2 Cfu/g was FFV. Total *Coliform* count ranged from 1.4 x 10^2 Cfu/g to 2.5 x 10^2 Cfu/g, which was similar to the result obtained by Adebayo-Tayo *et al*. (2012) in Microbial quality of fresh fish sold in Uyo Metropolis.

Table 3: Microbiological Analysis

Sample Code	THC ($x10^2$Cfu/g)	TCC ($x10^2$Cfu/g)	TSC ($x10^2$Cfu/g)	TVC ($x10^2$Cfu/g)	TFC ($x10^2$Cfu/g)
FOV	3.8^{a} ±0.1	2.3^{b}±0.1	NG	1.5^{a}±0.1	1.0^{a}±0.5
FVT	3.5^{b} ±0.1	2.0^{c}±0.1	1.2^{a}±0.1	NG	1.5^{a}±0.1
FTV	2.6^{c} ±0.1	2.5^{a}±0.1	NG	NG	1.4^{a}±0.1
FVF	2.0^{d} ±0.1	2.2^{b}±0.1	1.3^{a}±0.1	1.0^{b}±0.1	NG
FFV	1.0^{e} ±0.1	1.4^{d}±0.1	1.0^{b}±0.1	1.2^{b}±0.1	NG

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$). Where FOV = Frozen mackerel from the first vendor, FVT = frozen mackerel from the second vendor, FTV = frozen mackerel from the third vendor, FVF = frozen mackerel from the fourth vendor, FFV = frozen mackerel from the fifth vendor. THC = total heterotrophic count, TCC = total *Coliform* Count, TSC= total *Salmonella* Count, TVC = total *Vibrio* count, TFC = total Fungal count and NG = no growth observed.

The total heterotrophic count ranged from 1.0 x 10^2 Cfu/g - 3.8 x 10^2 Cfu/g, total *Salmonella* Count ranged from 1.0 x 10^2 Cfu/g - 1.3 x 10^2 Cfu/g, total *Vibrio* count ranged from 1.0 x 10^2 Cfu/g - 1.5 x 10^2 Cfu/g and total Fungal count also ranged from 1.0 x 10^2 Cfu/g - 1.5 x 10^2 Cfu/g. The highest *Vibrio* count obtained was from FOV (1.5 x 10^2 Cfu/g) while the least count obtained was from FVF (1.0 x 10^2 Cfu/g), but there was no count for FVT and FTV. Total Fungi count ranged from 1.0 x 10^2 Cfu/g - 1.4 x 10^2 Cfu/g, where FVT was the highest and FOV the lowest. From the obtained results, it can be deduced that all the samples harbored heterotrophic bacteria and *Coliform* while samples FOV and FTV were free from growth of *Salmonella*. Also, samples FVT and FTV were free from *Vibrio* growth and no fungal growth was detected for samples FVF and FFV. Arannilewa *et al*. (2006) in "effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (*Sarotherodun galiaenus*)" found that the total *coliform* count range in fish was between 3.0 x 10^3 - 7.5 x 10^6 with increasing values, as the duration of storage increases.

Morphological Identification of the Bacterial Isolates

Table 4 shows the result of morphological identification of bacterial isolates from the frozen fish samples. Gram stain of the isolated *Escherichia coli* and *Salmonella spp* was negative and the motility was positive. They were also rod shaped. *Staphylococcus aureus* was positive for gram stain and negative for motility. It had a cocci shape which is characteristic of cocci bacteria. Faecal contamination of water supplies and contaminated food handlers has frequently been implicated in outbreaks caused by *Escherichia coli* and *Salmonella spp*. These organisms have been implicated in the consumption of some acidic foods.

Table 4: Morphological Identification of the Bacterial Isolates

Isolates	**Gram stain**	**Motility**	**Shape**
Escherichia coli	-	+	Rod
Salmonella typhi	-	+	Rod
Staphylococcus aureus	+	-	Cocci

Key: Negative (-), Positive (+)

Biochemical Identification of the Bacterial Isolates

Table 5 shows the result of biochemical identification of bacterial isolates on the five fish samples. The result revealed the presence of *Escherichia coli, Salmonella spp* and *Staphylococcus aureus* in the fish samples when subjected to different biochemical tests. Catalase test was positive in the identification of all the bacteria. Coagulase test was negative for *Escherichia coli*, not tested for *Salmonella spp* and positive for *Staphylooccus aureus.* Coagulase test is used to specifically differentiate *Staphylococcus aureus* (positive) from Coagulase Negative Staphylococcus (CONS). Oxidase test was positive for *Escherichia coli* and *Salmonella spp* but negative for *Staphylococcus aureus.* Indole test was positive in the identification of *Escherichia coli* on the sample. Citrate test was negative in the identification of *Escherichia coli* and *Salmonella spp* but was positive for *Staphylococcus aureus.* The result revealed the presence of *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus* in the fish samples when subjected to different biochemical tests.

Table 5: Biochemical Identification of the Bacterial Isolates

Isolates	Catalase test	Coagulase test	Oxidase test	Citrate test	Indole test
E. coli	+	-	+	-	+
Salmonella spp	+	NT	+	-	-
S. aureus	+	+	-	+	-

Key: Negative (-), Positive (+), Not tested (NT).

Bacterial and Fungal Isolates from the Five Mackerel Samples

Table 6 shows that the major bacterial isolates from the samples evaluated after five days of incubation were mostly *Staphylococcus aureus, Escherichia coli, Salmonella spp, Vibrio spp, Pseudomonas spp* and *Micrococcus spp*. Major fungal isolates include: *Aspergillus niger, Penicillium spp* and *Rhizopus stolonifer.* Several studies have reported that the microbes that have been widely isolated from fish samples belong to the genera *Streptococci, Staphylococcus, Escherichia, Salmonella, Shigella, Enterobacter, Klebsiella, Serratia, Bacillus, Lactobacilli, Clostridium, Proteus, Pseudomonas, Corynebacterium, Micrococcus*, and *Aeromonas* (bacteria); *Saccharomyces, Aspergillus, Penicillin, Fusarium, Candida, Rhizopus, Geotrichum,* and *Mucor* (fungi). Results from table 6 shows that *Micrococcus spp, Pseudomonas spp, Vibrio spp, Penicillium spp* and *Aspergillus niger* appeared in all the 5 samples used in the study. Sample FOV had the highest percentage (23 %) of isolated microbes, followed by both FTV and FVF (20.5 %), and lastly by FVT and FFV (18 %). Presence of *E. coli* and *Salmonella spp* indicates possible contamination by faecal matter. Findings from this study showed that storage conditions, hygienic practices of frozen fish handlers and duration of storage may have affected the overall microbial load and contamination of the samples.

Table 6: Bacteria and Fungi isolated from frozen fish samples after 5 days of incubation.

Sample codes	*Aspergillus niger*	*Penicillium Spp*	*Rhizopus Stolonifer*	*Staphylococcus aureus*	*E. coli*	*Salmonella spp*	*Vibrio spp*	*Pseudomonas spp*	*Micrococcus spp*
FOV	+	+	+	+	+	+	+	+	+
FVT	+	+	+	-	+	-	+	+	+
FTV	+	+	+	+	-	+	+	+	+
FVF	+	+	+	+	+	-	+	+	+
FFV	+	+	-	+	+	-	+	+	+

FOV = Frozen mackerel from the first vendor, FVT = frozen mackerel from the second vendor, FTV = frozen mackerel from the third vendor, FVF = frozen mackerel from the fourth vendor, FFV = frozen mackerel from the fifth vendor.

CONCLUSION

This study revealed that frozen mackerel fish sold in Eke-Awka market, Anambra State, Nigeria could be a source of heavy metals, pathogenic bacterial and fungal contamination for humans. The mercury and chromium contamination appeared to be within safe limits, while that of lead was far beyond acceptable limits. There seems to be no danger from cadmium and arsenic contamination as

they were not detected. The frozen mackerel fish samples were grossly contaminated by pathogenic organisms such as: *Staphylocccocus aureus, Escherichia coli, Salmonella spp, Vibrio spp, Pseudomonas spp, Micrococcus spp, Aspergillus niger, Rhizopus stolonifer* and *Penicillum spp*., though below permissible limits, but in significant enough amounts to be of health concern. However, the absence of some of the microorganisms in some of the samples show that holding and storage conditions, period of storage and handling conditions may have contributed to the microbial contamination and load. This calls for public proper training and improvements in handling and storage conditions of frozen fish in order to minimize the frequency and extent of contamination.

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