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Keywords : chrysichthys nigrodigitatus, smoked fish, microbial load, health.

I. Introduction

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis, (Abolagba and Mella, 2008). Fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo *et al.,* 2008).

Fish is soft and easily damaged; therefore rough handling and bruising results in contamination of fish flesh. Fish will become unfit for human consumption within about one day of capture, unless it is subjected to some form of processing or preservation. Even after the fish has been processed, particularly if traditional methods have been used, the fish is still subject to many forms of loss and spoilage (Shewan, 2000). The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species; hence, the indigenous microbial populations of fish can vary significantly (Shewan, 2000). Fish, because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and invasion on the fish are prevented by the body's natural defence system during life but after death the defence system breaks down and the bacteria multiply and invade the flesh. And also immediately fish dies, it remains in first class quality only for a short while (Clucas and Ward, 1996). However, spoilage soon sets in which is occasioned by an increase in the ambient temperature that triggers favourable conditions for microorganisms to strive.

According to (Aberoumand, 2010), *Escherichia coli* is a classic example of enteric bacteria causing gastroenteritis. *E coli* including other coliforms and bacteria as *Staphylococcus spp* and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Scientists have shown that the contamination of food of fish origin with pathogenic *E. coli* probably occur during handling of fish and during the production process (Jimoh *et al.,* 2009). The microorganisms associated with smoked fish pose a great threat to the populace as the transfer of the microorganisms attack the immune

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system of the consumer, usually man, thereby, giving room for the invasion of disease.

II. Materials and Methods

a) Sample Collection

Three samples each of smoked fish (*Chrysichthys nigrodigitatus*) were purchased from major market (Lafenwa and Makun-omi) of fish processors from Oyan lake and Ogun waterside in Ogun state, Nigeria. A total of six (6) samples of smoked *Chrysichthys nigrodigitatus* were purchased and collected with a sterile aluminium foil (3 smoked *Chrysichthys nigrodigitatus* from each location) and samples were transported to the Veterinary Microbiology Laboratory, Federal University of Agriculture, Abeokuta in a well covered ice cold cooler for analysis and these samples were labelled; OL1, OL2, OL3, OW1, OW2, OW3. A sterile scalpel was used to cut a large portion of the skin and flesh of the fish in a sterile container and analysed for bacteria and fungi.

b) Microbiological Analysis

The samples were analyzed for Total Plate Count (TPC), Total Coliform Count (TCC), occurrence of bacteria and fungi and identification of bacteria and fungi.

c) Sample Preparation

One gram of the fish sample for microbiological evaluation was weighed into 9ml of sterile water in the bijou bottle. This was done for samples gotten from each location and was taken as the original stock sample of each market location. One millimeter of the original stocks solution was transferred into 9ml of sterile distilled water and mixed thoroughly to give 10^{-2} dilution of the original sample and this was done for each market sample and the bottles were labelled: S1(10^{-1}), S1(10^{-2}), S2(10^{-1}), S2(10^{-2}).....S6(10^{-1}), S6(10^{-2}). The serial dilution was carried out using a sterilized micropipette from tube one to the last tube. The NA, PDA and MA were prepared according to manufacturer instruction and sterilized using autoclave for 15 minutes at 121^0 C. It was then removed and allowed to cool before it was poured into plates. The plates were then allowed to set, after which one millimetre of the serial diluted sample of 10^{-8} dilution was inoculated on the surface of the well dried NA, PDA and MA and gently swirled to completely spread. The inoculated NA and MA plates were incubated at 37°C for period of 18-24 hours while the PDA was incubated at room temperature for 5 days. Each bacteria isolates obtained was counted and estimated according to method of Miles and Mistra (Baker and Beach, 2000) while the fungi colonies were also counted and estimated.

d) Estimation of bacteria total count according to Hedges (2002)

To 90ml of sterile water was homogenized 10g of the fish sample to make 1/10 dilution, while 9ml of sterile water was put in 8 sterile test tubes and serial dilution of the homogenized samples was made to the 8th tube. One ml was discarded from the 8th tube and 1ml of the dilution was spread on well dried Nutrient agar. It was allowed to dry and incubated at 37^0C for 18 – 24 hours. Bacteria colonies seen were counted and estimated accordingly.

e) Estimation of fungi total count according to Hedges (2002)

To 90ml of sterile water was homogenized 10g of the fish sample to make 1/10 dilution, while 9ml of sterile water was put in 5 sterile test tubes and serial dilution of the homogenized samples was made to the 5th tube. 1ml was discarded from the 5th tube and 1ml of the dilution was spread on well dried Sabouraud Dextrose agar. It was allowed to dry and incubated at room temperature for 5 days. Fungi colonies seen were counted and estimated accordingly.

f) Bacteria identification according to Monical (1996)

Homogenized fish sample was cultured on Blood agar and MacConkey agar using sterile wire loop and incubated at 37^0C for 18 to 24 hours in aerated condition. Colonies seen were purify on nutrient agar and their colonial morphology such as shape, colour, consistency, edges, elevation, pigmentation and hemolysis were individually examined.

g) Biochemical Test

Each bacteria colony was identified by their Gram stain and biochemically characterized by their sugar fermentation test, coagulase, catalase, indole, citrate, oxidase, H_2S production and urea.

III. Results

Table 1 shows the average bacteria count obtained from the skin of smoked *Chrysichthys nigrodigitatus* from Makun-omi market (Ogun waterside) was 3.1×10^6 while the mean count for intestine was 4.9 $\times10^6$ and the gill recorded average microbial count of 4.2×10^6cfu/g. The average bacteria count obtained from the skin, intestine and gill of smoked *Chrysichthys nigrodigitatus* from Lafenwa market (Oyan lake) were 13.8×10^6, 10.7×10^6 and 6.8×10^6cfu/g respectively. The result showed that bacteria load obtained from the smoked fish (*Chrysichthys nigrodigitatus*) in Oyan lake were higher than the recommended value but fell within range in Ogun waterside. Table 2 shows the average fungi count obtained from smoked *Chrysichthys nigrodigitatus* while Table 3 and 4 revealed the results of percentage of occurrence of bacteria and fungi in smoked *Chrysichthys nigrodigitatus.* Table 5 and 6 shows the cultural and morphological characteristics of bacteria and fungi isolates while Table 7 revealed the biochemical test used in characterization of the bacteria isolates from smoked fish (*Chrysichthys nigrodigitatus).*

Table 1 : The average bacterial count in the fish samples

Location and fish specie	Body parts	Average Bacterial Count (X 10^6CFU/g)
OW *(Chrysichthys nigrodigitatus)*	Skin	3.1
	Intestine	4.9
	Gill	4.2
OR *(Chrysichthys nigrodigitatus)*	Skin	13.8
	Intestine	10.7
	Gill	6.8

KEY: CFU/g= colony forming unit per gram.

Table 2 : Average fungi count in the fish samples

Location and fish specie	Body parts	Average FungiCount (X 10^6CFU/g)
OW *(Chrysichthys nigrodigitatus)*	Skin	0.0
	Intestine	0.2
	Gill	0.0
OR *(Chrysichthys nigrodigitatus)*	Skin	0.3
	Intestine	0.3
	Gill	0.0

KEY: CFU/g= colony forming unit per gram.

Table 3 : Percentage occurrence of bacteria in the fish samples (*Chrysichthys nigrodigitatus*)

Locations	Isolate	Number	Percentage (%)
Ogun waterside	*Bacillus spp*	2	10.0
	Staphylococcus saprophyticus	1	5.0
	Micrococcus spp	2	10.0
	Escherichia coli	2	10.0
	Staphylococcus aureus	1	5.0
Oyan lake	*Bacillus spp*	2	10.0
	Staphylococcus saprophyticus	2	10.0
	Micrococcus spp	2	10.0
	Escherichia coli	3	15.0
	Staphylococcus aureus	3	15.0

Table 4 : Percentage occurrence of fungi in the fish samples (*Chrysichthys nigrodigitatus*)

Locations	Isolate	Number	Percentage (%)
Ogun waterside	*Fusarium spp*	1	14.3
	Mucor spp	1	14.3
	Aspergillus fumigatus	0	0.0
Oyan lake	*Fusarium spp*	2	28.6
	Mucor spp	2	28.6
	Aspergillus fumigatus	1	14.3

Table 5 : Cultural and morphological characteristics of bacteria in the fish samples (*Chrysichthys nigrodigitatus*)

Suspected isolates	Morphology	Microscopic
Bacillus spp	Creamy white, raised with rough edges	Gram positive bacilli
Staphylococcus saprophiticus	Creamy, slightly raised with smooth edges	Gram positive cocci in cluster
Micrococcus spp	Creamy deep yellow, slightly raised with smooth edges	Gram positive cocci
Eschericia coli	Whitish, raised with rough edges	Gram negative bacilli
Staphylococcus aureus	Golden yellow, slightly raised with smooth edges	Gram positive cocci in cluister

Table 6 : Cultural and morphological characteristics of fungi in the fish samples (*Chrysichthys nigrodigitatus*)

Suspected isolates	Morphology	Microscopic
Fusarium spp	Whitish cotton aerial	Elongated ovoid curved microconidia
Mucor spp	Yellow-white fluffy strand with brown reverse side	Hyphae without rhizoids, dispersed, branched, large globose sporangiophore
Aspergillus fumigates	Creamy yellow filamentous colonies	Large/globose conidiphore, loose columna with biseriated hypha

Table 7 : Biochemical test of the bacteria isolated from the fish samples (*Chrysichthys nigrodigitatus*)

GRAM	MOTILITY	GLUCOSE	LACTOSE	MANNITOL	MALTOSE	INDOLE	METHYL RED	VOGUES PROKAMER	CITRATE	H_2S	SUCROSE	UREA	OXIDASE	COAGULASE	CATALASE	ORGANISM ISOLATED
-	+	+	+	+	+	+	+	-	-	-	N/A	-	--	N/A	+	*Escherichia coli*
+	+	+	+	+	+	-	-	+	-	-	+	-	-	N/A	+	*Bacillus spp*
+	-	+	+	+	+	N/A	+	-	N/A	N/A	+	N/A	+	-	+	*Micrococcus spp*
+	N/A	+	+	+	+	N/A	`-	+	N/A	N/A	+	-	-	+	+	*Staphylococcus aureus*
+	N/A	+	+	+	+	N/A	-	+	N/A	N/A	+	-	+	+	+	*Staphylococcus saprophyticus*

KEY:
- : NEGATIVE
+ : POSITIVE
N.A : NOT APPLICABLE

IV. Discussion

This study shows that pathogenic bacteria and fungi are present in smoked *Chrysichthys nigrodigitatus* in Lafenwa and Makun-omi markets in Ogun State. According to International Commission on Microbiological Specification for Food (ICMSF, 1986), the maximum recommended bacteria count for good quality product is 5.0×10^5 (5.7Log cfu/g). The bacteria load obtained from the smoked fish (*Chrysichthys nigrodigitatus*) in Oyan lake was higher than the recommended value lake but fell within range in Ogun waterside. Bacteria present in the fish samples include, *Bacillus spp, Staphylococcus saprophiticus, Micrococcus spp, Eschericia coli* and *Staphylococcus aureus.* The occurrence of *Staphylococcus aureus* and *Eschericia coli* in the smoked-dried fish samples were in accordance with Martin (1994) when he stated that these organisms were the commonest micro-organisms associated with smoked fish. The presence of *Staphylococcus aureus* in fish samples according to Okonko *et al.* (2008) might have been through contamination by handling.

The bacteria group of *Staphylococcus aureus* according to Herman *et al.* (2011) reported that it was one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting in a diseased

condition. This bacteria class may also cause superficial and systemic infections such as boils, impetigo and folliculitis while more serious and more common infections could be pneumonia, bacteremia and other infections of the bones and wounds. Also, *Eschericia coli* usually cause diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections. The fungi present in the fish samples are *Fusarium spp, Mucor spp* and *Aspergillus fumigatus.* It was observed in this study that the presence of fungi particularly aflatoxigenic molds in these fish specie is very significant as it was indicated by food safety standard that aflatoxigenic molds produce mycotoxins which have pathogenic effects on man; it destroys the liver and kidney resulting to death. The presence of the organisms could be as a result of handling during smoking and also cross contamination during storage, after smoking and handling during sales of smoked fish.

V. Conclusion

In conclusion, smoked fish (*Chrysichthys nigrodigitatus*) from Lafenwa market were heavily contaminated with many bacteria species including members of the genera *Escherichia* and *Staphylococcus* due to the greater unhygienic conditions of the environment than Makun-omi market. The public health concern of smoked *Chrysichthys nigrodigitatus* is therefore the poor handling and processing either by the processors, marketers or the consumers. This has greatly contributed to the contamination of these products by various pathogenic micro organisms which make their consumption hazardous to health.

VI. Recommendation

Environmental sanitation education and orientation should be organized for fish processors; this will enable them to reduce the unattractive environment that makes their operations smelly and repulsive. The relevant national and municipal authorities must ensure improve quality of smoked fish to safeguard public health and enhance food safety in the country.

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