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ESTIMATION OF BACTERIA AND FUNGI IN SMOKED CATFISH (*Clarias gariepinus*) AVAILABLE IN OTA MARKETS

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ABSTRACT

This study was carried out to investigate the microbial quality of smoked Clarias gariepinus purchased from four different markets, namely Iyana-Iyesi (M1), Oju-Ore (M2), Oja-Ota (M3) and Sango-Ota (M4) in Ota metropolis. The samples from three retailers from the four markets were used for analyses for two weeks, making a total of 48 samples for the experiment. The microbiological procedure was carried out using Nutrient agar for bacteria load and Potato Dextrose agar for fungal load of the smoked catfish. The mean total plate count for bacteria ranged between 4.82 x 10^4 CFU/g for week 1 in Oju-Ore market and 4. 92 x 10^4 CFU/g for week 2 and Iyana-Iyesi market while fungi ranged from 4.85 x 10^4 for week 1 in Sango-Ota market and 4.92 x 10^4 CFU/g for week 2 in Oja-Ota and Sango-Ota markets. Vibrio cholerae, Staphylococcus aureus, Escherichia coli, Shigella spp and Salmonella typhi were the bacteria isolated from the smoked catfish. The presence of these organisms confirms microbial contamination either from poor smoking of the fish, poor personal hygiene of the processors or sellers, poor environmental conditions as well as packaging and storage of the fish. The adoption of good processing practice and the use of controlled temperature in processing and preserving of the smoked catfish are highly recommended.

Keywords: *Clarias gariepinus*, smoked, markets, microbes

INTRODUCTION

Fish is a very vital source of high quality protein and constitutes an important part of man's diet; it is one of the most important animal foods available in the tropics (Eyo, 2001). Catfish, (*Clarias gariepinus*) accounts for about 80% of aquaculture production in Nigeria (FDF, 2003). However, fish is a perishable food material that deteriorates soon after harvest at high ambient temperature (Aberoumand, 2010); therefore, needs immediate preservation.

Fish products are among the most perishable commodities worldwide mainly due to microbial spoilage. About one-third of the world's food production is lost annually as a result of microbial spoilage (Omojowo *et al*., 2010). Microorganisms inhabit nearly every niche of the earth and our food is no exception (Pelczar *et al*, 1993). Also, microorganisms in food may also be responsible for food poisoning and food-borne infections (Ogwan'g *et al*., 2005). Immediately fish dies, it remains in first class quality only for a short while (Eyo, 1997; 2001; Abolagba and Melle, 2008). Thus, the quality of fish as well as its potential keeping time deteriorates rapidly leading to food loss with regards to acceptable quality.

Furthermore, a number of processing techniques are in operation in Nigeria. These include chilling, freezing, salting, canning, drying and smoking. However, smoking is the most popular method of fish processing (Eyo, 2000) who reported that: smoking involves heat application to remove water and inhibit bacterial and enzymatic action on fish. Traditional methods are being used for the preservation of fish especially in rural areas (Chakrabarti and Varma, 1999). It imparts aroma, taste and colour on processed fish (Olley *et al.*, 1988). Meanwhile, smoking of fish from smouldering wood for its preservation dates back to civilization (Olokor, 2007). It is also noted that apart from giving the product a desirable taste and odour, smoking provides a longer shelf life through its anti-bacterial and oxidative effect, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting

as antagonist to spoilage agents (Eyo, 2001; Sengor *et al*., 2004). However, the effect of curing by smoking with respect to quality and shelf life of the product depends on the preparation of the raw material, the type of smoking, relative humidity, velocity, temperature, density, and composition of the smoke, and the time of smoking (Doe, 1998). To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products (Omojowo *et al*., 2010).

Microbial activity is responsible for spoilage of most fresh and of several lightly preserved sea foods (Lund *et al*., 2000). Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* spp., and *Clostridium botulinum* (Heintz and Johnson, 1998). Also, Abolagba *et. al*. (2011) and Abolagba and Uwagbai (2011) stated that bacteria such as *Staphylococcus aureus, Proteus, Bacillus, Micrococcus,* were the most common microorganism associated with smoked fish. Customers mostly assess the quality of fish by considering the appearance, smell and palatability of the fish when cooked hence it is necessary to produce good quality and safe smoked fish free from harmful microbial load (Abidemi-Iromini *et al*., 2011).

In hot smoking process, the fish is properly cooked with the temperature reaching 120˚C while the centre of the fish flesh may reach 60˚C. Hot smoking is the tradition method of fish smoking in the tropics. Fish is smoked until cooked in order to obtain a product with extended shelf life, since alternative preservation method such as refrigeration are absent in remote fishing villages where most fish processing takes place. The primary aim of hot smoking is to preserve the product, flavour and colour arising as a result of preservation function.

This study is aimed at understanding the safety or harm of smoked catfish products after smoking process. It is to identify microorganisms that contaminate the fish. The findings of the study should be used to enlighten the fish traders and consumers of the inherent harm and design steps necessary in lengthening shelf life of smoked catfish products.

MATERIALS AND METHODS

Study site

The smoked *Clarias gariepinus* samples were purchased from four different markets in Ota [Iyana-Iyesi (M1), Oju-Ore (M2), Oja-Ota (M3) and Sango-Ota (M4)] and Microbiological analyses were carried out at the Microbiology laboratory of the Department of Biological Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

Sampling size, collection and processing

The samples were purchased from the market and kept inside a polyethene bag to be carried to the laboratory for further analysis. A total of 6 hot-smoked catfish retailers were patronised per market, *Clarias gariepinus* was purchased at random from 3 retailers per market for every trial (R1, R2, R3). The samples from each 3 retailers were used for analyses for two weeks, making a total of 48 samples for the experiment. All samples obtained were ground with the use of an electric blender and fine fish samples were obtained.

Preparation of media

Nutrient Agar, Potato Dextrose Agar, Brilliant Green Agar and Mannitol Salt Agar were aseptically weighed, distilled water was transferred into the conical flask and the media were sterilized inside the autoclave for 15 minutes at 121˚C, allowed to cool and poured into the plates respectively. Thiosulphate Citrate Bile Salts Sucrose Agar and Salmonella-Shigella Agar were weighed, distilled water transferred and the media homogenized and made sterile with the use of magnetic stirrer and hotplate to boiling point for 10mins under frequent agitation and allowed to cool to about 45˚C before it was poured into the inoculated plate.

Cultivation and enumeration of bacteria

Fine grinded smoked catfish sample (1g) was aseptically weighed and was transferred into a MacCartney bottle containing (9 mLs) of sterile distilled water, and shaken thoroughly to make 10^{-1} dilution. Serial dilutions were carried out using sterile syringe that delivered the required volume 1 mL accurately to make decimal dilutions of 10^{-2} to 10^{-10}. Before every 1 mL dilution transfer,

the diluents were shaken well for proper mixing of the sample with the diluent. Using pour plate method, 0.1 mL of inoculum was transferred into sterile labelled Petridishes. About 20 mLs of sterilized molten Nutrient Agar, Potato Dextrose Agar, Brilliant Green Agar, Mannitol Salt Agar, Thiosulphate Citrate Bile Salts Sucrose Agar, cooled to about 45˚C, was poured into the inoculated Petridishes and allowed to gel. Some plates were also prepared as control to check on the sterility of the diluents. The plates were then incubated at 37˚C for 24 hours.

Purification of isolates

Nutrient Agar (20 mLs) was transferred to sterile Petridishes and allowed to solidify after which they were dried in a hot air oven at 30˚C; this was done to get rid of moisture on the cover of the plates and on the agar itself. Suspected colonies of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp e.t.c. were purified by streaking on Nutrient agar plates and were subjected to Gram staining and other Biochemical tests. Other microbiological activities carried out were gram staining reaction and other biochemical tests such as that of the Citrate, Kligler Iron agar, Sulphide Indole and Sugar Utilization.

RESULT

In this study, the results presented showed the microbial load from samples of smoked catfish obtained from three retailers in M1; (Iyana-Iyesi market), three retailers in M2; (Oju-Ore market), three retailers in M3; (Oja-market), three retailers in M4; (Sango-Ota market). Microbial analysis of the fish samples was carried out for 2 weeks.

In week 1, smoked *Clarias gariepinus* sampled from Iyana-Iyesi market recorded the highest mean bacterial count of 4.90 $X10^4$ CFU/g and the least fungal count of 4.86 $X10^4$ CFU/g; Oju-Ore market samples recorded the highest fungal count of 4.90 $X10^4$ CFU/g and the least bacterial count of 4.82 $X10^4$ CFU/g; Oja market samples the highest fungal count of 4.90 $X10^4$ CFU/g and the least bacterial count of 4.87 $X10^4$ CFU/g while Sango-Ota market samples recorded the highest fungal count of 4.85 log cfu/g and the least bacteria count of 4.77 $X10^4$ CFU/g as shown in Figure 1. Similarly, Figure 2 showed the microbial-bacterial and fungal counts of smoked *Clarias gariepinus* sampled from the four markets. The fish sampled in Iyana-Iyesi market recorded the highest mean bacteria count of 4.92 $X10^4$ CFU/g and the least fungal count of 4.88 $X10^4$ CFU/g; Oju-Ore market samples recorded the highest fungal count of 4.92 $X10^4$ CFU/g and the least bacteria count of 4.90 $X10^4$ CFU/g; Oja market samples recorded the highest fungal count of 4.92 $X10^4$ CFU/g and the least bacteria count of 4.88 $X10^4$ CFU/g while Sango-Ota market samples recorded the highest bacteria count of 4.87 $X10^4$ CFU/g and the least fungal count of 4.82 $X10^4$ CFU/g.

The biochemical characterization of bacteria isolates from the smoked Catfish sampled from the markets: Iyana-Iyesi, Oju-Ore, Oja and Sango-Ota for week 1 was determined with the occurrence of the following organisms: *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella* spp and *Salmonella typhi* as shown in Table 1. Likewise, Table 2 presented the biochemical characterization of bacteria isolates from the smoked Catfish sampled from the four markets, which was determined with the occurrence of the following organisms: *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi.*

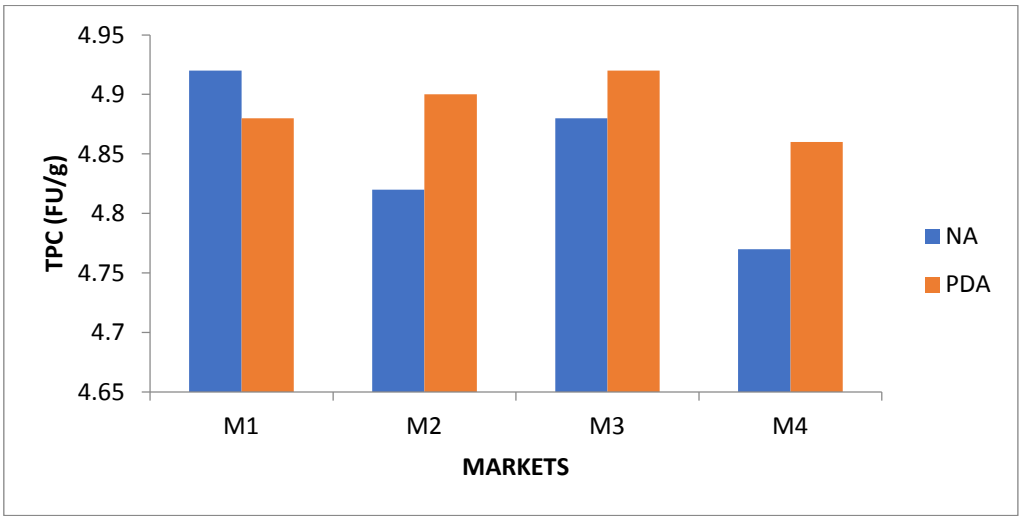

Figure 1: Bacteria and Fungi count of smoked catfish samples from the markets in week 1

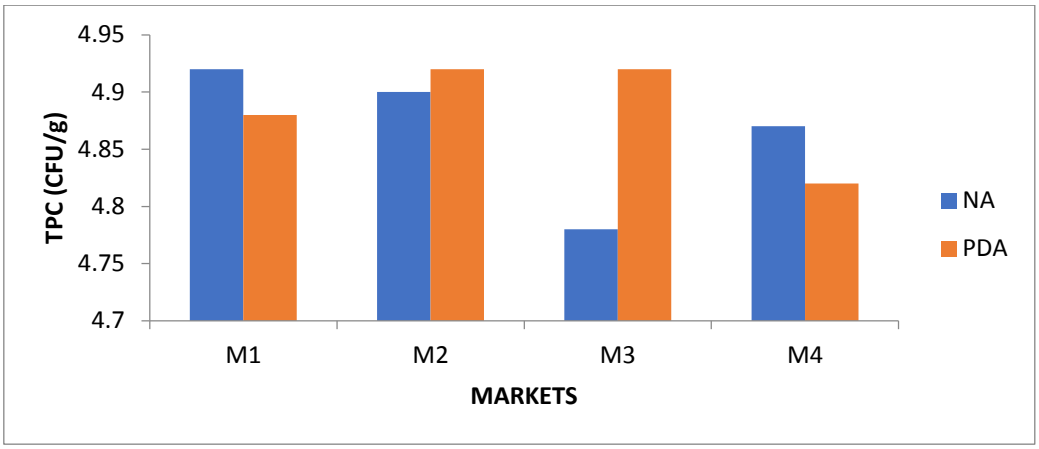

Figure 2: Bacteria and Fungi count of smoked catfish samples from the markets in week 2

Table 1: Identification of Bacterial Isolates from Different Markets in Week 1

Sample	CC	GR	Shape	K (Slant)	A (Butt)	H_2S	G	IND	MOT	CIT	CAT	H_2S	OXI	GLU	Identification
							M1								
R1(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
R2(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R3(BGA)	White	-	Rod	+	+	+	-	-	+	+	+	-	-	+	*Salmonella typhi*
(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
							M2								
R1(EMB)	Metallic sheen	-	Rod	+	+	-	-	+	+	+	+	-	-	-	*Escherichia coli*
(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R2(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R3(SSA)	Colourless	-	Bacilli	+	-	-	-	+	-	-	+	-	-	+	*Shigella* spp
M3															
R1(BGA)	White	-	Rod	+	+	+	-	-	+	+	+	-	-	+	*Salmonella typhi*
(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
							M4								
R1(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
(BGA)	White	-	Rod	+	+	+	-	-	+	+	+	-	-	+	*Salmonella typhi*
R2(BGA)	White	-	Rod	+	+	+	-	-	+	+	+	-	-	+	*Salmonella typhi*

Key: CC-Colonial character, GR-Gram reaction, K-Lactose production, A-Glucose production, H_2S-Hydrogen sulphide, G-Gas, IND-Indole, MOT-Motility, CIT-Citrate, CAT-Catalase, OXI-Oxidase, GLU-Glucose, M-Market, R-Retailer.

Table 2: Identification of Bacterial Isolates from Different Markets in Week 2

Sample	CC	GR	Shape	K (Slant)	A (Butt)	H_2S	G	IND	MOT	CIT	CAT	H_2S	OXI	GLU	Identification
							M1								
R1(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R2(EMB)	Metallic sheen	-	Rod	+	+	-	-	+	+	+	+	-	-	-	*Escherichia coli*
(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R3(EMB)	Metallic sheen	-	Rod	+	+	-	-	+	+	+	+	-	-	-	*Escherichia coli*
(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
							M2								
R1(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
R2(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
(EMB)	Metallic sheen	-	Rod	+	+	-	-	+	+	+	+	-	-	-	*Escherichia coli*
(SSA)	Colourless with black center	-	Bacilli	+	-	-	-	+	-	-	+	-	-	+	*Salmonella* spp
							M3								
R2(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
R3(MSA)	Yellow	+	Cocci	+	-	+	-	-	-	+	+	-	-	+	*Staphy. aureus*
							M4								
R1(BGA)	White	-	Rod	+	+	+	-	-	+	+	+	-	-	+	*Salmonella typhi*
R2(TCBS)	Yellow	-	Rod	+	-	+	-	-	+	+	+	-	+	+	*Vibrio cholerae*
R3(SSA)	Colourless with black center	-	Bacilli	+	-	-	-	+	-	-	+	-	-	+	*Salmonella* spp

Key: CC-Colonial character, GR-Gram reaction, K-Lactose production, A-Glucose production, H_2S-Hydrogen sulphide, G-Gas, IND-Indole, MOT-Motility, CIT-Citrate, CAT-Catalase, OXI-Oxidase, GLU-Glucose, M-Market, R-Retailer.

DISCUSSION

In this study, evaluation of microbial qualities of smoked catfish was carried out to determine the absence and presence of the target food borne pathogens such as *Salmonella, Staphylococcus aureus*, *Escherichia coli*. The total plate counts for both bacteria and fungi did not exceed the range of specified microbiological limits recommended for fish and fishery products by International Commission on Microbiological Specification for Foods (ICMSF, 1986). The protocol of ICMSF recommends a maximum bacterial count of 5×10^5 CFU/g for good quality product and a maximum count of 10^7 for marginally acceptable quality products (Nwachukwu and Madubuko, 2013).

The biochemical test carried out on isolates from these smoked catfish showed the presence of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella* spp and *Salmonella typhi* which according to Ramos, (1999) that isolation of pathogenic and spoilage organisms such as *E. coli*, *Staphylococcus aureus, Listeria monocytogenes*, *Aspergillus flavus* etc., raises public health concerns about safety in consuming smoked fish products from our markets and cause a high rate of spoilage leading to shorter shelf/storage life of the product. . Meanwhile, organisms that cause food-borne diseases have been reported to include *E.coli,Bacillus* species, *Clostridium botulinum*, molds, fungi and yeast (Osakue *et al*, 2016).

Tainting of fish with these organisms is attributed mainly to poor handling by processors and traders who expose smoked fish to unsanitary conditions. *E. coli* is often implicated in gastroenteritis associated with poor handling of food. Some of the diseases caused by these microbes are listeriosis manifesting as meningitis, abortion and pre-natal septicaemia affecting mostly immune-compromised individuals, pregnant women and infants. *E. coli* causes life threatening epidemic gastroenteritis in humans for example, travellers' diarrhoea (ETEC) also called "Delhi belly". *Bacillus cereus* produces toxins that cause a disease that is more an intoxication than a food borne infection. Also *S. aureus* is known to cause enterotoxigenicity due to the production of enterotoxin and also known to cause *Staphylococcus* food poisoning which is a major type of food intoxication (Nwachukwu and Madubuko, 2013).

Pathogenic bacteria associated with fish and fishery product can be categorised into three general groups: Bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*). Enteric bacteria (non- indigenous bacteria) that are present due to faecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and Bacterial contamination during processing, storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp.) (Lyhs, 2009). The presence of enteric bacteria in fish and fishery product is therefore seen as a sign of poor standards of process hygiene and sanitation (Dalsgaard, 1998).

Similarly, fish are transported in non-insulated open trucks, where both the fish and traders occupy the back of the open trucks. This post-harvest infection of smoked fish is in line with Dillon *et al* (1994), studying microbiology of smoked fish in Canada found out, that the level of micro-organisms in fish reduces with smoking but increases with storage period and during transportation. Also, fish from the processing villages do not reach the outlet markets in time, as most of the feeder roads to these areas are poor and are impassable during rainy season. This observation corroborates Poulter *et al.* (1988) findings in Zambia.

The presence of these organisms confirms microbial contamination either from poor smoking of the fish, poor personal hygiene of the processors or sellers, poor environmental conditions as well as packaging and storage of the fish. Also, the isolation of *Staphylococcus* in the smoked catfish sample may be attributed to post processing contamination and *Vibrio cholerae* may be as a result of poor sanitation practice or contamination from the water that the fish had been harvested.

There was no significant difference in variation in the bacterial counts of the catfish purchased from the market for 5 weeks they were all within the same range.

CONCLUSION

It is envisaged in this study that fish processors be advised to choose high quality fish products. This is because, people eat smoked fish due to the flavour and texture that the fish acquires on smoking and they deserve to eat products of high quality. Also it is noted that the contamination of the fish samples is as a result of post-processing handling of smoked catfish products which is not properly done. The smoked catfish is observed to be displayed on newspaper on a flat basket and opened for flies to perch on. The houseflies contaminate them with dirt from the surrounding environment. Some of these smoked fish products are prepared or processed poorly that if they do not reach the markets the same day, they get spoilt and cause loss to the fish processor.

Recommendation

Nigerians should be educated more on the post-processing handling of the smoked catfish products on how to ensure that they are well packed in well ventilated baskets and transported in proper sanitized trucks. The adoption of good processing practice and the use of controlled temperature in processing and preserving of the smoked catfish are highly recommended.

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