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Microbiological Analysis of Three of Smoked Fish Obtained from the Ondo State, Nigeria

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Abstract The consumption of smoked fish usually obtained from the open shelf in most communities of the developing countries has raised some health related concerns. This research investigated the microbiological quality of three commercially important smoked fish, *Clarias gariepinus* (African mud catfish), *Sardinella eba* (herring) and *Oreochromis niloticus* (tilapia) obtained from fish mongers in three popular fish markets, Akure, Okitipupa and Akungba-Akoko in Ondo State, south west Nigeria. The total plate, coliform, *Salmonella-shigella*, *Staphylococcus aureus* and fungal counts of the heads, muscles and the heads of the fish samples were determined using standard methods. The ranges of the total plate, coliform, *Salmonella-shigella*, and *Staphylococcus aureus* were $1.3X10^4$ cfu/ml - $2.8X10^5$ cfu/ml, $3X10^3$ cfu/ml - $1.9X10^5$ cfu/ml, $1.2X10^4$ cfu/ml - $5.4X10^4$ cfu/ml and $1.4X10^4$ cfu/ml - $2.8X10^5$ cfu/ml respectively. The samples obtained from Akure market had the highest counts in most samples followed by those obtained from Okitipupa market while samples obtained from Akungba-Akoko market had the lowest counts. It is recommended that in order to prevent the spread of organisms that are of public health importance, fish should be processed, stored and distributed under safe hygienic conditions and good sanitary practices.

Keywords Smoking, Fish, Microorganisms, Pathogens, Health

1. Introduction

Fish constitutes an important source of protein intake of many people, particularly in the developing countries. In Nigeria, where about 41% of the total animal protein intake is obtained from fishery products, the total fish consumption rate has risen to 2.66 million metric tons annually [24]. [23] reported that fish is a major source of animal protein and an essential food item in the diet of Nigerians because it is relatively cheaper than meat. Fish contains most of the important essential amino acids, particularly, lysine, methionine and tryptophan that are lacking in plant proteins. It is also an important source of vitamins and minerals which are important for good living [2]. Apart from its food value, fish has been reported to possess medicinal values, such as, in the amelioration of asthma, arthritis, coronary heart diseases, goitre and cancer [15].

However, fish is a perishable food material that deteriorates soon after harvest at high ambient temperature [1], therefore, needs immediate preservation. The methods by which fish could be preserved include, freezing, salting, sun-drying, oven-drying, fermentation and smoking [10, 14]. In Nigeria, fish either obtained from a cultured pond or from the wild is sold to consumers as fresh, frozen, smoked or sun-dried. Smoked fish is highly desirable because of its enhanced flavour and texture in fish in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations [27]. [5] observed that smoking demonstrated a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content. [29] reported that smoke-drying had been used for centuries in preserving fish, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked. In industrialized countries, however, fish smoking is done for enhancement of flavour and texture, often producing value added products whose preservation is achieved by other means.

In many developing communities, smoked fish are usually hawked without taking cognizance of the microbial contamination from the environment. In Nigeria, smoked fish products could be contaminated with microorganisms from the processing units and the market centers before reaching the consumers because many processors and hawkers usually display them openly in a manner that could be potential sources of microbial contamination. The aim of this study, therefore, was to isolate the possible microorganisms associated with smoked fish with a view to

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assessing the level of their public health implications.

2. Materials and Methods

2.1. Sample Collection

The samples of dried smoked fish used for this study were obtained from fish mongers at popular fish markets in Akungba-Akoko, Akure and Okitipupa in Ondo State, Nigeria. The species of the smoked fishes sampled were catfish (*Clarias gariepinus*), herring (*Sardinella eba*) and tilapia (*Oreochromis niloticus*). The fish were purchased in batches and were brought to the laboratory of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko for microbiological analysis.

2.2. Preparation of Samples and Enumeration of Microorganisms

The fish samples were surface sterilized separately in 3.5% sodium hypochlorite solution (w/v) with constant agitation for 7 minutes, rinsed thoroughly with sterile distilled water until the traces of hypochlorite were removed and were then dried in an oven at 45°C for 24 hours. The heads, muscles and the tails of the fish samples were pulverized separately using a blender (maker). Five milliliters were taken from each sample into a sterile bottle containing 450 ml of sterile peptone physiological saline to form a stock culture. The sample bottles were placed on a rotator shaker at 120 RPM for 1 hour. 10-fold dilutions were subsequently prepared with peptone physiological saline. Aerobic mesophilic bacteria were enumerated on plate count agar (PCA, Oxoid) at 37°C for 24 hours and reported as total viable count (TVC). Lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe Agar (Merck) and incubated anaerobically at 30°C for 48 hours. Presumptive LAB was confirmed by oxidase and catalase tests, and confirmed counts were reported as lactic acid bacteria (LAB). Enterobacteriaceae were enumerated on Violet Red Bile Glucose at 37°C for 24 hours while Staphylococci were counted on mannitol salt agar (Oxoid) at 30°C for 48 hours). Yeasts and moulds were enumerated on Oxy-tetracycline glucose yeast extract agar (OGYEA, Oxoid CM0545) 25°C for 72 hours.

2.2.1. Identification of Bacteria

Inocula were aseptically transferred from each slide into plates of respective media using a streak plate technique. The isolates were purified by repeated streaking on their respective media. Bacterial plates were incubated at 37°C for 24 hours while fungal plates at 25°C for 72 hours. A 24 hour old culture was prepared from each plate for identification purposes. Bacteria isolates were identified based on their cultural characteristics, Gram staining reaction and various identification tests. Isolates were identified according to [19]. Lactic acid bacteria were also identified by assaying in API 50 CHL galleries (BioMerieux).

2.2.2. Identification of Fungi

Mould isolates were cultured by three point inoculation on CYA and MEA at 25°C for 5 days. The young cultures of the isolates were stained with lactophenol-blue and identified to the genus level by colony and cell morphology and biochemical tests according to [7].

2.3. Determination of Water Content

The water contents in the smoked fish samples were determined through a standard laboratory procedure as described by [9].

3. Results and Discussion

The mean values of the total, coliform, *Salmonella-shigella* and *Staphylococcus* counts of the smoked fish samples are shown in Table 1. Among the tilapia species, the highest total count ($2.9x10^5$ cfu/g) was found in the samples obtained from Akure (AKR) market while the lowest count ($1.3x10^4$ cfu/g) was observed in the samples hawked in Akungba-Akoko (AKG). A similar trend was also observed in the coliform count of the tilapia sample with the highest value ($1.37x10^5$ cfu/g) and lowest ($1.2x10^4$ cfu/g) obtained in in the AKR and AKG markets, respectively. The highest *Salmonella-Shigella* ($5.2x10^4$ cfu/g) and *Staphylococcus* ($2.9x10^5$ cfu/g) counts were obtained in the tilapia samples displayed in Okitipupa (OKP) market in comparison to the lower counts of $3.0x10^4$ cfu/g (*Salmonella-Shigella*) and $1.4x10^4$ cfu/g (*Staphylococcus*) found in the AKR and AKG samples, respectively.

The catfish samples obtained from AKR market had the highest total viable count ($2.9x10^5$ cfu/g) in comparison to the lowest value ($2.0x10^4$ cfu/g) obtained in AKG. Coliforms, *Salmonella-Shigella* and *Staphylococcus* species were prominently found in the fish samples hawked across the markets. The various bacteria isolated from the samples are shown in Table 2. *Bacillus* species were prominently encountered on the catfish samples in all the locations. *Staphylococcus aureus, Escherichia coli, Salmonella* spp and *Proteus* spp also featured in the other fish species. A variety of fungi species were observed in the fish samples hawked across the study area (Table 3). *Aspergillus fumigatus, A. flavus, A. niger, Spiromyces minutes* and *Geotrichum albidum. Aspergillus fumigatus* were isolated in samples of tilapia (OKP), catfish (OKP) and herring (AKR) while *A. flavus* was found in tilapia (AKR), catfish (OKP) and herring (AKG). *A. niger, S. minutes* and *G. albidum* were isolated in herring (AKG and AKR) and catfish (OKP). The moisture contents in the studied smoked fish samples are shown in Table 4.

The microbial flora associated with fish could be from the environment in which the fish are harvested and not specific to a particular species [26, 28]. In this study, the fish samples which were smoked on charcoal/wood barbecue were either displayed on dirty floor/mats/trays/open containers or untidy

tables in the markets for sale. [12] reported that processed fish are easily contaminated with microorganisms in nature, through handling, during processing and if the post-processing handling is not properly done under hygienic conditions. The quality of smoked products is dependent on several factors, including, the quality of the fish at the time of smoking, the preparation of the raw material, the nature of wood and the type of the smoking procedure employed [16].

Table 1. Microbial Counts (cfu/g) in Dried Fish Samples

Location/Fish	Total count	Coliform	*Salmonella-Shigella*	*Staphylococcus*
Akungba-Akoko				
Herring	$5x10^4$	$3x10^3$	$2x10^4$	$9x10^3$
Tilapia	$1.3x10^4$	$1.2x10^4$	$3.2x10^4$	$1.4x10^4$
Catfish	$2x10^4$	$1x10^4$	$1.2x10^4$	$6x10^3$
Okitipupa				
Tilapia	$2.8x10^5$	$7.3x10^4$	$5.2x10^4$	$2.9x10^5$
Catfish	$2.94x10^5$	$1.8x10^5$	$4x10^3$	$2.84x10^5$
Akure				
Herring	$2.89x10^5$	$1.89x10^5$	$5.4x10^4$	$3.9x10^4$
Tilapia	$2.94x10^5$	$1.37x10^5$	$3x10^4$	$2.2x10^4$
Catfish	$2.99x10^5$	$1.90x10^5$	$4.1x10^4$	$4.6x10^4$

Table 2. Isolated bacteria on Fish Samples in the Study area

Fish/Location	*Staphylococcus aureus*	*Bacillus* spp	*Escherichia coli*	*Salmonella spp*	*Proteus spp*
Tilapia					
Akungba-Akoko	+				
Okitipupa			+		
Akure	+			+	
Catfish					
Akungba-Akoko		+			
Okitipupa	+	+			
Akure		+			+
Herring					
Akungba-Akoko	+				
Akure		+			

+: microbial presence

Table 3. Implicated fungi on Fish Samples in the Study area

Fish/Location	*Aspergillus fumigatus*	*Aspergillus flavus*	*Aspergillus niger*	*Spiromyces minutes*	*Geotrichum albidum*
Tilapia					
Akungba-Akoko					
Okitipupa	+				
Akure		+			
Catfish					
Akungba-Akoko					
Okitipupa	+	+			+
Akure	+				
Herring					
Akungba-Akoko	+	+	+		
Akure				+	

+: fungal presence

Table 4. Moisture contents of smoked fish samples

Fish /Location	(%)
Tilapia	
Akungba-Akoko	12.30±1.01
Okitipupa	14.30±0.02
Akure	11.30±2.03
Catfish	
Akungba-Akoko	7.3±0.20
Okitipupa	6.8±1.05
Akure	7.5±2.20
Herring	
Akungba-Akoko	17.4±0.15
Akure	18.5±1.62

The microorganisms isolated in this study have been reported in some fish species. [4] isolated *Staphylococcus* sp. *E. coli* and *A. fumigatus* in smoked *Chrysichthys nigrodigitatus* while [3] reported the isolation of similar organisms from fresh shrimps sold at selected fish markets in southwest Nigeria. [22] reported that *Staphylococcus spp* has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus enterotoxin* which is resistant to gastrointestinal enzymes. *S. aureus,* a normal flora of human skin and mucous) membrane, is one of the most common causes of boils, impetigo and folliculitis and in some cases, bacteremia and infections of the bones and wounds [18]. *E. coli* and *Salmonella* are fecal borne pathogens and they could occur as a result of contamination from the handlers. Fish harvested from contaminated waters can harbour *Salmonella* sp [6]. [4] reported that *E. coli* caused diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections while *Salmonella* caused gastroenteritis and typhoid fever. The presence of *A. flavus* and *A. fumigatus* in the studied fish samples is of great health concern because of their mycotoxigenic potentials. [17] reported that *A. flavus* and *A. fumigatus* produced aflatoxins, which destroyed the liver and kidney in man resulting to death. The presence of these organisms in the fish could be as a result of handling processes during smoking and cross contamination during storage, or during sales of smoked fish.

There are serious safety concerns related to the consumption of raw fish and shellfish because of the presence of biological (bacteria, virus, parasites) and chemical (biotoxins) hazards. [8 and 20] reported that these hazards are present in fish and shellfish pre-harvest and are, therefore, difficult or impossible to control. However, several techniques exist in the prevention of the growth of pathogenic microorganisms during distribution and storage of processed fish. [20] observed that the hazards related to contamination, recontamination or survival of biological hazards during processing could be controlled by applying good manufacturing practice and good hygiene practice. Smoking at adequately high temperatures is capable of controlling microbial contamination in fish, although, the heat supplied might not be sufficient enough to kill all the microbial contaminants. While temperatures for hot smoking (>600°C) can inactivate vegetative microorganisms, the process produces a finished product with different sensory quality in some cold-smoked processed fish [13]. [25] recommended that the processed fish should be exposed to a drying temperature that will provide insufficient moisture content for the growth of micro-organisms.

A combination of smoking and treatments with antimicrobial agents and antioxidants have been found to retard microbial spoilage, extend shelf life, and enhance safety of smoked catfish. [16] reported low water activity, absence of mould, low microbial loads and better shelf-stability when smoked fish were treated with 25% NaCl and 1% ascorbic acid; 3% sodium lactate; 3% sodium lactate and 5% rosemary extract; and/or 5% sorbic acid. Also, irradiation has been found to significantly decrease the microbial populations of mesophilic aerobic bacteria, anaerobic bacteria, psychrophilic bacteria, lactic acid bacteria, and moulds and yeasts without adverse effects on chemical or sensory quality attributes of the product [11]. The effectiveness of electron beam irradiation and high pressure treatment for the sanitation of cold-smoked has also been studied. [21] reported that irradiation at 2 kGy kept the microbial population of smoked fish samples below 6 $\log_{10}$cfu/g after 35 days at 5°C, with negligible or very light changes in its odour. Also, pressurization at 450 MPa for 5 min kept the microbial population below 6 $\log^{10}$ cfu/g after 35 days at 5°C and did not alter odour, but affected negatively the visual aspect of smoked fish.

4. Conclusions

The pathogenic microorganisms implicated in the smoked fish samples are of public health importance. Therefore, fish processors, retailers or vendors should be educated to observe strict hygienic measures. Potable water should be used during processing of smoked fish and they should be properly cooked before consumption because many consumers often eat hawked smoked directly fish without further processing. Proper storage of smoked fish is also necessary because poor storage methods and unhygienic handling of the items are known to predispose dried fish to microbial contamination. The identified organisms are entirely preventable by practicing good sanitation and proper food handling techniques. To prevent an incidence of food contamination and intoxication in foods, there is a need to educate and advocate for the importance of environmental sanitation and good food handling practices, especially, proper hand-washing practices among fish handlers.

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