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Physical and microbiological qualities of smoked *Oreochromis niloticus* sold at Jega, Nigeria

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ABSTRACT

To address public health implication of consumption of smoked fish, a study was conducted to determine the physical and microbiological quality of smoked Oreochromis niloticus sold in Jega Central market, Kebbi State Nigeria, between June and September 2016. A total of 108 samples of the smoked fish from three retail outlets in the market - Location A (main entrance), B (center of the market) and C (motor park)) within the metropolis were analyzed for their physical and microbiological qualities using the standard cultural method on general and selective media. Results revealed that Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia were present in the samples collected from the three different locations. The mean counts of the bacteria isolates found in the smoked fish samples in the three locations were A 1.5 x 10^6cfu/g, B 1.95 x 10^6cfu/g and C 2.45 x 10^6cfu/g. There was no significant difference (P>0.05) between locations on moisture contents of the collected samples but there was significant difference (P<0.05) among the locations in pH for the fish samples. Out of the 108 samples analyzed, 27 (25%) were positive for S. aureus while other species isolated were Klebsiella pneumonia12 (11%). The remaining 69 (64%) of the samples were negative for E. coli. The study shows that E. coli and other bacteria species are common contaminant of smoked fish in Jega Central market, and this may pose serious public health implications in the community.

Key Words: *Fish processing, Fish quality, Smoked fish and Bacteria isolates*

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I. Introduction

Fish is one of the main sources of animal protein foods available for human consumption (Itoua, 1989). Most of the catches come from oceans, seas, rivers and man-made ponds. It is a highly nutritious food which is about 60-80% water, 15-25% protein, 11-22% fat, 20% mineral and 1% carbohydrate. It is

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often cheaper than meat and is a rich protein source for both poor and wealthy people (Clarence *et al.* 2009). Smoking simply means a heating process that dries the fish to preserve it from spoilage (Koffi, 2007). Most dry fish consumed in Nigeria is smoked fish. The steps in smoking process are necessary not only for safe preservation but also to produce good flavor and aroma (Gallot, and Fremy 2006). Hence smoked fish are less prone to microbial spoilage than fresh fish. However, spoilage still occurs because of growth of microbes due to partial dehydration during smoking. Today smoking is the traditional and still primary method of preserving fish in Nigeria and Kebbi State in particular (Anon, 1998). Smoked fish products have wide acceptance today due to their accustomed taste and aroma as well as longer shelf life because of the combined effects of dehydration, antimicrobial and anti-oxidant activities of several smoked constituents mainly formaldehyde, carboxylic acid and phenols (Doe and Heruwati, 1988). It was reported that some factors contribute to the quality and safety of smoked fish products, and the rate of fish spoilage is affected by species, fat contents, fishing slaughter method, hygiene manipulation and postmortem handling (Huss *et al.* 1997). When fish is produced with reduced post-harvest losses and increasing the percentage of fish used for direct human consumption, food security is improved. Post-harvest losses caused by spoilage amount to about 10 to 12 million tons per year. It has been estimated that 20 million tons of fish in a year are discarded at sea which is another form of post-harvest loss (Emikpe *et al.* 2011). Spoilage of smoked fish is accompanied by a loss in value. The relative value of good, fair or poor-quality fish is a complicated matter and varies between countries and places.

One of the major risks involves the consumption of raw or undercooked fish that may be naturally contaminated by food borne pathogens and such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favorable conditions (FEHD, 2005). It is necessary to identify the potential point of contamination in aquaculture and the prevalence of microbiological quality of smoked fish and the efficient strategies to avoid the dissemination of microorganisms in the fish processing chain and to improve the quality and safety of the end product (Emikpe *et al.* 2011). Value chain is important for both aquaculture products, certification and agriculture (Siddique et al. 2015). Fish disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish (Emikpe *et al.* 2011). Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other foods. According to Hosein *et al.* (2008), seafood contributes a burden of disease to man being capable of transmitting many of the established food borne microbial infections and intoxications basically caused by bacteria. Bacteria have been reported to represent a major and important group of microorganisms because of their frequent occurrence and activities that may have a negative impact on fish quality (Emikpe *et al.* 2011). The presence of human pathogenic bacteria in fish and fish products may also be attributed to contamination during processing (Hosein *et al.* 2008; Emikpe *et al.* 2011). Several bacteria are, however, reported to cause infection and mortality in both fish and humans and these represent a particular hazard, caused either by handling infected fish on fish farms or in grocery stores or by the ingestion of raw or inadequately processed infected fish and/or contaminated fish products (Hosein *et al.* 2008). This study was designed to determine the microbiological and physical qualities of smoked tilapia fish sold at Jega township market.

II. Materials and Methods

Study Area

The study was conducted in Jega, a Local Government Area located between Latitude 12° 13' 19.88" N and Longitude 4° 22' 46.67" E of Kebbi State in the Sudan savanna ecological zone, Northern Nigeria. Jega is characterized by erratic and scanty rainfall that last for about five months. The climate of the area is semi-arid with the average rainfall of about 550mm-650mm per annum. The relative humidity ranges from 21-48% and 51-83% during dry and rainy seasons, respectively. The temperature average is between 41^{0}C during dry season and 25-41^{0}C during rainy season.

Sample Collection

A total of one hundred and eight (108) smoked samples of *Oreochromis niloticus* of average weight were randomly collected from three (3) different locations (location A: main entrance, B: center of the market and C: motor park) in Jega township market, Jega Local Government of Kebbi State. Twelve (12) smoked

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samples of *Oreochromis niloticus* were randomly collected weekly from each of the three selling points in the market for a period of three (3) weeks. The samples collected were aseptically wrapped in sterile polythene bags and taken to the laboratory for analysis of microbiological population. Portion of the tissue of all the samples was cut, weighed and labeled accordingly. All the purchased samples were kept in cooler containing ice blocks to arrest the growth of microbes and analyzed within four (4) hours of collection.

Microbiological Analysis
Spoilage bacteria in the smoked fish samples were determined. The total viable bacteria count (TVC) of each sample was estimated using the method of Collins *et al.* (1989a). The frequency of occurrence of bacteria on the smoked fish was also determined.

Isolation and Serial Dilution
Isolation of bacteria was carried out using the standard cultural methods on general and selective media. Serial dilution of the fish samples was varied between 10^1 and 10^5. The diluents used were buffered pepton water (BPW 0 x 01D CM0509, Typical pepton 10g/L sodium chloride 5g/L disodium phosphate 3.5g/L potassium dehydrogen phosphate 1.5g/L, pH 7.2<0.2 at 25^0C, (Bashingstoke Hampshire, LTD, England). Nine milliliters of sterile water were poured aseptically into five tubes each and 1 ml of the original cut fish sample were added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was also taken and introduced into the third test tube and mixed very well. The crushed sample was therefore diluted from 10 -1 to 10-5 for each fish sample.

Enumeration of Total Bacteria Counts
The method described by Collins *et al*. (1989b) for estimating bacteria counts was used to enumerate the total viable counts of the isolates. Countable plates showing 1 to 32 colonies were selected and counted. The mean colony count on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (CFU/ g).

Physical Qualities of Smoked Fish
The physical qualities examined were moisture content and pH of the smoked fish.

Statistical Analysis
Statistical analysis was carried out using SPSS Version 16.0. The procedure for the generalized linear model (Proc-GLM) was used for the analysis of variance. Means of the microbial loads of the three independent locations were separated using the Tukey test.

III. Results

Microbiological Population of Fish sold at Jega Central market
The result for microbiological population of fish sold at Jega central market is presented in Table 01. There was significant difference (P<0.05) between the three locations in microbiological population. There was no significance difference (P>0.05) between locations on moisture content of the collected samples but there was a significant difference (P<0.05) between locations on pH.

Table 01. Microbiological population of fish sold at three locations in Jega Central market

Location	TVC (cfu/g)	TVC	Moisture	pH
A	1.507 x 10^6	150.70^a	34.24	7.06^a
B	1.947 x 10^6	194.70^b	37.22	6.55^c
C	2.44 x 10^6	244.90^C	35.85	6.68^b
SE		5.773	1.333	0.008

Morphology and Biochemical Characteristics of Isolates
The morphology and biochemical characteristics of isolates from smoked fish sold at Jega Central market are presented in Table 02. The results indicate the presence of some microorganisms from isolate of the samples.

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Table 02: Morphology and biochemical characteristics of isolates from smoked fish sold in Jega Central market

Isolate	Cell morphology	Gram stain	Methyl red	Voges	Vogesproskauser	Citrate	Indole	Catalase	Coagulase	Lactose	Maltose	KIA/TSI	Probable organism
Week 1													
A	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
B	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
C	Rod	-	-		+	+	-	+	+			A/AG	*Klebsiella*
D	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
E	Cocci	+	N/A		N/A	N/A	N/A	+	+			N/A	*S. aureus*
F	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
G	Cocci	+	N/A		N/A	N/A	N/A	+	+			N/A	*S. aureus*
Week 2													
A	Rod	-	-		-	+	-	+	+			A/AG	*Klebseilla*
B	Rod	-	-		-	+	-	+	+			A/AG	*E. coli*
C	Cluster	+	N/A		N/A	N/A	N/A	+	+			A/AG	*S. aureus*
D	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
E	Chain	+	N/A		N/A	N/A	N/A	+	+			N/A	*S. aureus*
F	Rod	-	-		-	+	-	+	+			A/AG	*Klebseilla*
G	Rod	-	-		-	+	-	+	+			A/AG	*E. coli*
Week 3													
A	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*
B	Rod	-	-		-	+	-	+	+			A/AG	*Klebseilla*
C	Rod	-	+		-	-	+	+	+			A/AG	*E. coli*

KIA/TSI = Kligler Iron Agar/Triple Sugar Iron Agar.

Population and Species of Microorganism Identified

Table 03 shows the population and species of microorganisms identified from isolates in smoked fish from Jega Central market. Three species of microorganisms *viz E. Coli, S. aureus* and *Klebsiella* sp. were detected from location A in week 1 and 2, in location B, *E. Coli* was detected in week 2 of sample collection and in location C, *E. Coli* was detected in week 1 and 3 of sample collection. *S. aureus* was only detected in week 3 at location B. *Klebsiella* sp. were detected in location A week 3 and location C week 2.

Table 03. Population and species of microorganism identified from smoked *Oreochromis niloticus* sold in Jega Central market and their mean count

Location	Week	Mean total viable count of microbial species (cfu/g)					
		E. coli	Presence of *E. coli*	*S. aureus*	Presence of *S. aureus*	*Klebsiella*	Presence of *Klebsiella*
A1	1	6.8×10^5	+	NG	-	NG	-
A2	2	8.4×10^5	+	NG	-	NG	-
A3	3	NG	-	NG	-	4.4×10^5	+
B1	1	NG	-	NG	-	NG	-
B2	2	1.02×10^6	+	NG	-	NG	-
B3	3	NG	-	5.4×10^5	+	NG	-
C1	1	9.8×10^5	+	NG	-	NG	-
C2	2	NG	-	NG	-	2.0×10^6	+
C3	3	1.5×10^6	+	NG	-	NG	-

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IV. Discussion

The isolation of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* is indication of faecal contamination and this is in agreement with the report of Fraizer and Westhoff (1995) and Dike-Ndudim *et al.* (2014) that microbial flora on fish depends on the microbial contents of the water in which they lived in, has proven that water sources in Jega especially the streams and rivers from where these fishes were obtained are contaminated with the these organisms. The pH of the fish samples determine the rate of growth of bacteria isolate and the growth pattern of these isolate and the prepared pH range in which they grow well among the mean pH of the samples collected for the three weeks which are 7.06, 6.55 and 6.68 and final analysis result is 0.008 and best pH for bacteria growth is pH 6.55 so bacteria can grow in low pH and there is significant difference ($P<0.05$) between locations on pH of the samples collected and the bacteria grow well. These result shows that the smoked fish in Jega Central market are contaminated with *S. aureus, E. coli and Klebsiella* sp. The presence of these organisms on fish may have resulted from the contamination from post processing and during storage. The bacteria can also be transferred to the processed smoked fish by the processors (Kerr *et al.* 1993). Based on the bacteriological evaluation of the smoked fish collected from different locations in the market 69 (64%) were negative for *E. coli* other species isolated in this study are *S. aureus* 27 (25%) and 12 (11%) of the sample were positive for bacteria. *E. coli* is a food borne bacteria pathogen that is ubiquitous in nature and shows the ability to persist in the processing and storage environment for prolonged time. Population and species of microorganism were *E. coli, S. aureus* and *K. pneumonia*. *E. coli* was detected from location A in week 1 and 2 of sample collection and in location B. *E. coli* was detected in week 2 of sample collection and in week 1 and 3 of sample collection *S. aureus* was only detected in week 3 at location B. *Klebsiella* sp. were detected in location A week 3 and location C week 2. After analyzing the mean moisture content of the collected samples for the three weeks with the mean total viable count (cfu/g) for the first and the third week as $1.51x10^6$, $1.94x10^6$ and $2.45x10^6$. There was no significant difference ($P>0.05$) between the locations on moisture content of the collected samples from Jega central market. In addition, the isolation of these microorganisms from the smoked fish indicates partial dehydration during smoking.

V. Conclusion

Results obtained from this study demonstrated the presence of *S. aureus, E. coli* and *Klebsiella* sp. in smoked fish sold at Jega Central market Kebbi State, Nigeria. The presence of this pathogen on smoked fish is an indication that the hygiene and safety of such smoked fish is compromised. The study recommends the use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture. Suitable processing and handling should be treated as important control measure to minimize or eliminate the hazard associated with *S. aureus, E. coli* and *Klebsiella* sp.

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