BACTERIOLOGICAL ASSESSMENT OF SMOKED FISH (CLARIAS SP) AROUND SHIRORO LAKE AREA OF NIGER STATE, NIGERIA

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ABSTRACT

Bacteria has been implicated in food poisoning and smoked fish is not an exception, since fish generally, is highly susceptible to spoilage, therefore this study evaluated the bacteria load in smoked fish from three major locations in Shiroro area of Niger State namely; Gwada, Kuta and Zumba.The smoked fish samples collected from these locations were smeared at both the gills and head regions of the fishs. The bacteria samples identified were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeroginosa, and Samonellatyphi,* which were common to all the three locations sampled, while only *Streptococcus feacalis*was only found to be present in both Kuta and Zumba location.The frequency of occurrence of these 68 bacteria samples isolated ranges from 8 – 20%, with *Bacilliussubtilis*having the highest occurrence and *Pseudomonas aeroginsa*having least occurrence. Out of the total 68 samples, 14 skin samples (20.6%) and 5 gills samples (7.4%) exceeded the acceptable limits of total mesophilic aerobic counts which were $10^6 - 10^7$ cfu/g. In the case of total coliform counts, 12 skin samples (17.6%) and 7 gills samples (10.3%) exceeded the acceptable limit which is 4.0 x 102, while in the case of *Staphylococcus aureus,* 4 skin samples (5.9%) and 2 gills samples (2.9%) exceeded the acceptable limit which is 10^3 cfu/g. Similarly 3 skin samples (4.4%) and 1 gill sample (1.5%) exceeded the acceptable limit of *Salmonella Oyphi*which is 10^4 cfu/g.

Keywords: Bacteria, Smoked (*Clariasspp*) fish , locations and recommended Values.

INTRODUCTION

Fish, according to Din et al; (2004) is the cheapest source of animal protein (and as such contribute about 40% animal protein in Nigeria (Eyo, 2001)), Thus fish has been the main food for human for many centuries and still constituent an important part of the diet in many countries (Ames et al; 1991). Larrsonet. al; (2004), outlined the health benefits of fish to include low cholesterol level, thereby reducing the risk of health diseases, it is important in the diet of pregnant women and also help in maintaining weight control, vital in the diet of malnourished infants suffering from kwashiorkor and other disorder including cancer. Eating fish show that, it is less tough, more digestible, when compared to beef, mutton, chicken and bush meat, making fish acceptable by infant and adult (Eyo, 2001).Fish is one of the most perishable of all staple commodities in the tropical climates of most developing countries, because of its protein constituent (Ames et.al, 2004) which Botta (1995) also reported, making fish prone to spoilage, Oyero (2001) citing Johnson and Clucas (1996), reported that after death the quality of fish can only remain wholesome only for a short time. Spoilage occurs mainly from within the fish (Ol Grady, 2003). At death the normal defense mechanism of fish stop working and a series of changes begins that cause spoilage. Firstly, the controlled biochemical processes that occur in all living animals to assist the digestion of food continue after death in an uncontrolled manner. The digestive enzymes attack the surrounding flesh which is sterile, Secondly, soon after death, the microorganism on the skin, slime and intestines multiply rapidly and spread into the soften flesh under the skin and around the belly. The spread into the flesh is more rapid if the fish has been damaged in anyway; also atmospheric oxygen can attack unsaturated oils in fatty fish such as Salmon and Mackerel (Price, 1998).According to Gram and Huss (2000), the common spoilage of fish includes species of mesophilic gram-positive, micro flora consisting of micrococcus, Bacillus and Coryneform found in freshwater fish. Bacteria genera commonly infecting meat, while it is been processed, cut, packaged, transported, sold and handle include: *Salmonelaspp,Shigellaspp, E.coli, B.proteus, S.aureus, Cl.welchii, B.cereus and Streptococci fecalis.* As these microorganisms colonize a piece of meat, they begin to break itdown, leaving behind toxins that can cause enteritis or food poisoning. The bacteria do not survive a thorough cooking of the meat, but several of their toxins and microbial spores do. (Wikipedia encyclopedia 2009).Food borne illness, also food borne disease and colloquially referred to as food poisoning is any illness resulting from the consumption of contaminated food. There are two types of food poisoning: they are food infection, which refers to the presence of bacteria which infect the body after consumption, Food intoxication refers to the ingestion of toxins contained within the food, including bacterially produced exotoxins, which can happen even when the bacteria that produce the toxins is no longer present or able to cause an infection.In order to avert the imminent spoilage of fish, fish can be processed into various products. The fish processing is a fairly wide field.

covering a large number of processing techniques, fish species, fish products, fish by-products and processing techniques. The basic functions of fish processing includes; Preservation of products, converting the raw materials to a desirable form, maintaining product quality assuring consumers safety and full utilization of raw material (Wheat and Lawson, 1985).

MATERIALS AND METHODS

Smoked fish of *Clarias spp* were purchased at random from three market locations in the vicinity of Shiroro Lake, namely; Gwada, Kuta and Zumba. These fishes were collected from each location on a weekly basis and were in triplicates given a total of 9 (Nine) samples (i.e, August to September, 2011) with the assigned values of T_1, T_2, and T_3 respectively(T– Treatment) making up a grand total of seventy two (72) samples (though only 68 samples were eventually used).These samples were afterward transported in a clean polythene bags to the laboratory for bacteria analysis. The smoked fish samples were then analyzed at two treatment levels; i.e (a) skin and (b) Gills and afterward examined quantitatively for standard plate count, Coliforms, Staphylococcus and Salmonella count respectively. All the procedures for the preparation of culture media, bacteria count isolation, cauterization and identification of bacteria isolates used were according to Manga and Oyeleke (2008), and Chessbourgh (2000).

CULTURE MEDIA PREPARATION:Media used for culture preparation included Nutrient (NA) Mac conkey Agar (MCA), Manitol salt Agar (MSA), Salmonella/Shigella Agar (SSA). One gram of fish sample was introduced into a test tube containing 9ml of sterile distilled water and mixed thoroughly. Ten folds of diluted samples were plated on Nutrient Agar, Mac conkey Agar, Manitol salt Agar and Salmnella / Agar for the enumeration of total viable bacteria, coliforms, staphylococci and salmonella/shigella respectively.

BACTERIAL COUNTS AND ISOLATED:These media were then incubated at 37^{0C} for 24 – 48hours. At the end of incubation, colonies that developed on the plates were counted and recorded as colony forming unit per milliliter (Cfu/ml) of isolated colonies which were then repeatedly subculture on fresh media to obtain pure culture.

CHARACTERIZATION AND IDENTIFICATION OF BACTERIA:Characterization of bacteria isolates was carried out as described by Cowen (1974). The colonial morphology of the isolates were examined and the characteristic colonies were identified using microscopic techniques and biochemical test

EXPERIMENTAL DESIGN:The parameters obtained after the quantitative examination were subjected to computation and analysis of variance (ANOVA) test. The experimental design used was completely randomized design. The smoked fish samples supplied from three (3) treatment locations (T_1, T_2 ,T_3) and each treatment were replicated three times.

RESULTS

Based on table 1: The following bacteria isolates were conspciously found on both the skin and gills of the smoked fish.*Bacilliussubtilis* , *Staphylococcus aureus* , *Staphylococcus epidermis* , *E.coli* , *Pseudomonas aeroginosa*, *Salmonella oyphi* and *Streptococcus feacalis*. From this result it shows that *Bacilliussubtilis*had the highest frequency occurrence of 20%, followed by three bacteria isolates of *S.aureus*, *E.coli*and *Salmonella tyhpii*, all had the next frequency of occurrence after *B.subtilis*with 16%, then followed by *S.epidermis*and *Strep.feacalis* with 12% occurrence and finally *Pseudomonas aeroginosa*had the least occurrence of 8%.

Results from Table 3 showed the bacteria count ranked highest for Kuta location with values of $4.0X10^5$(Skin) and $3.5x10^5$(Gills). On the other hand, coliform count ranked highest for zumba with values of $2.8x10^4$(Skin) and $3.1x10^4$(Gill). *Staphylococcus aureus*count was highest for Zumba location with values of $2.0x10^4$(Skin) and $1.8x10^4$(Gill), while lastly the *Salmonella sp*count ranked highest for Zumba location with values of $1.8x10^4$ and$2.3x10^4$, Values of *Staphylococcus aureus*were significantly different from each other (P>0.05), though the values of Zumba location for *S.aureus*were not significantly different (P <0.05). Base on the recommended values (RV); 10^6-10^7 , 4X 10^{-2} , 10^3 and 10^4 , 4 samples of skin representing 17.4%, with the highest values from Kuta exceeded the recommended values of total bacteria count of 10^6-10^7. 5 samples from the coliform count representing a value 21.7% likewise exceeded the recommended value of $4x10^{-2}$, These samples also came from the Kuta location. For the *Staphylococcus aureus* count, the highest values for both skin and gills were recorded at zumba location, exceeding the recommended values of 10^3 by 9.1% as shown in Table 4.

TABLE 1: SHOW THE MORPHOLOGY AND BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES

Sample code	Gram Rxn	Cell Shape	Catalase	Coagulase	Starch hydrolysis	Motility	Citrate utilization	Urease	MR	VP	H2S production	Glucose	Fructose	Sucrose	D mannitol	Lactose	SUSPECTED BACTERIA
T_1R_1A	+	Rods	+	-	+	-	+	-	-	-	-	+	+	-	-	-	Bacillus subtilis
T_1R_1B	-	Rods	+			+	+	-	-	-		+	+	-		+	E. coli
T_1R_1C	+	Cocci	+	+	-			+	-	+	-	+	+			+	S. aureus
T_1R_2A	+	Cocci	+	-	-	-	-	+	-	+	-	+	+		-	+	S. epidermis
T_1R_2B	-	Rods	-				-	+	-	-	-	+	+		+	+	Pseudomonas
T_1R_3A	-	Rods	+	-	-	-	-	+				+		+		+	E. coli
T_1R_3B	+	Cocci	+	-	-	-	-	+		+	-	+	+		-	+	S. epidermis
T_1R_4A	-	Rods	+	-	-	+	+	+			+	+	+	-	-	+	Salmonella
T_1R_4B	+	Rods	+		-	-	+	+				+	+	-	+	+	Bacillus subtilis
T_2R_1A	+	Cocci	+	+	-	-	-	+	-	+	-	+	+			+	S. aureus
T_2R_1B	+	Rods	+	-	+	-	-	+	+	-	-	-	+	-	+	+	Bacillus subtilis
T_2R_2A	+	Rods	+	-	+	-	+	+	-	-	-	+	+	-	+	-	Bacillus subtilis
T_2R_2B	+	Cocci	+			+	+	-	+	-		-	-			+	Strepti. Feacalis
T_2R_3A	-	Rods	+			+	+	-	-	-	-	+				+	E. coli
T_2R_3B	-	Cocci	+			+	+	+	-	-	-	+	-			+	Strepti. Feacalis
T_2R_4A	-	Rods	+	-	-	+	-	+			+	+	+	-		+	Salmonella
T_2R_4B	+	Cocci	+	+	-		-	+	-	+	-	+	+			+	S. aureus
T_3R1A	-	Rods		-	-	-	-	+		-	-	+	+	-	+	+	Pseudomonas
T_3R1B	+	Cocci	+	-	-	-		+		+	-	+	+	-	-	+	S. epidermis
T_3R_2A	-	Cocci	+	+	-	-	-	+		+	-	+	+	-	-	+	S. aureus
T_3R_3B	-	Rods	-		-	-	-	+			+	+	+	+		+	Salmonella
T_3R_3A		Rods	+		-	-	-	+	-		+	+	+	-		+	Salmonella
T_3R_3B	+	Cocci	+	+			-	+	-	+	-	+	+			+	S. aureus
T_3R_4A	+	Rods	+		+		+	+	-	-	-	-	+		+	-	Bacillus subtilis
T_3R_4B	-	Rods	+	-	-	+	+	+				+		+		+	E. coli
T_3R_4C	+	Cocci	+			+	+	-		-		+	-			+	Strepti. Feacalis

+ = positive (indicates presence of bacteria)
- = Negative (indicates absence of bacteria)

TABLE 2: FREQUENCY OF OCCURRENCE BACTERIA IN SMOKED (*Clariasspp*) FISH ANALYSED

BACTERIA ISOLATES	PERCENTAGE FREQUENCY
Bacilliussubtilis	20%
Staphylococcus aureus	16%
Staphylococcus epidermis	12%
Escherichia coli	16%
Pseudomonas aeroginosa	8%
Salmonella typhii	16%
Streptococcus feacalis	12%

TABLE 3: BACTERIALOGICAL STATUS OF THE FISH SAMPLES (Cfu/g^{-1})

A(location)	B	C	D	E	F	G
$T_{1(Gwada)}$	23	Skin	$2.1x10^5$	$1.5x10^4$	$1.1x10^{49}$	$1.2x10^4$
		Gills	$1.5x10^5$	$0.9x10^4$	$0.8x10^{46}$	$1.2x10^4$
$T_{2(Kuta)}$	23	Skins	$4.0x10^5$	$2.9x10^4$	$0.9x10^{46}$	–
		Gills	$3.5x10^5$	$1.0x10^4$	$0.2x10^{46}$	
$T_{3(Zumba)}$	22	Skin	$2.2x10^5$	$2.8x10^4$	$2.0x10^{49}$ –	$1.8x10^4$
		Gills	$2.5x10^5$	$3.1x10^4$	$1.8x10^{49}$	$2.3x10^4$

A=Treatment B = No of Samples C = Sampling area D = Total bacteria count E = Coliform count F = *Staphylococcus aureus*count.
G = *Salmonella* count.

TABLE 4: COMPARISON OF THE BACTERIOLOGICAL STATUS OF THE SMOKED FISH SAMPLES (Cfu/g) WITH THE RECOMMENDED VALUES, INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATION FOR FOOD. (ICMSF)

A	B	C		D		E		F		G	
				No.	%	No.	%	No.	%	No.	%
T_1(Gwada)	23		Skin	4	17.4	3	13.0	2	8.2	1	4.3
			Gill	2	8.7	3	13.0	0	0.0	0	0.0
T_2(Kuta)	23		Skin	6	13.0	5	21.7	0	0.0	1	4.3
			Gill	2	8.7	2	8.7	0	0.0	0	0.0
T_3(Zumba)	22		Skin	4	9.2	4	18.2	2	9.1	1	4.5
			Gill	1	4.5	2	9.1	2	9.1	1	4.5

A = Treatment, B = No. of Samples, C = Sampling Area, D = Samples exceeded recommended value of total bacteria count, E = Samples exceeded recommended value of coliform, F = Samples exceeded recommended value of Staphylococcus count. G = Samples exceeded recommended value of Salmonella count.

DISCUSSION

The result obtained from table 1 agrees with report of Wikipedia encyclopedia (2009), which reported similar bacteria that could be found in meat to include *Bacilliussubstilis, Staphylococcusaureus , Streptococcus feacallis, Pseudomonas aeroginosa, Salmonella typhii , E.coli and S.epidermis.* The frequency of occurrence of bacteria (in Table 2) like *Staphylococcus spp, E.coli*and *Bacilliusspp*found in smoked fish also agrees with the finding of Heek (2005) that found similar occurrence of bacteria from market survey of Tanzania, Liston and Matches (1986), reported that handling of fish can induce or increase the population of bacteria particularly Bacillius and Staphylococcus spp. This could explain why in this study there was the incidence of bacteria even after the smoked fish had initially been subjected to High temperature (smoking) techniques preservation.

The presence of *Salmonella typhii*in these studies is an indication of feacal contamination which could have resulted poor handling or contamination of the environment with feacal substances. The highest occurrence of *Bacilliussubtilis*in this study could suggest to the fact that this bacteria can survive even in very harsh condition, this agree with finding of Huss (1994), who reported that *Bacilliusspp*are spore formers which does allow them to survive in unfavorable condition even when the organism is no longer living.

RECOMMENDATIONS

After all that have been discovered in the course of this study is to ensure that smoked fish are free of bacteria, the following practices are recommended:

Sewage contamination should be prevented by adequate disposal of sewage.

After smoking of fish, the market women should avoid displaying them opening in the markets for sale as they can easily get contaminated by exposing them to air. They could be displaced in show glasses.

Smoked fish marketers should be educated about personal hygiene involved in handling of smoked fish. This will help to reduce bacteria cross contamination of smoked fish in the markets.

Before smoking, fish should be properly dried after washing. This critical step allows a pellicle, or glaze to form on the skin to keep moisture in and contaminant out.

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