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EFFECTS OF HANDLING AND DISTRIBUTION ON THE MICROBIAL CONTAMINATION OF SOME FRESH FISH FROM TAGWAI DAM, MINNA, NIGER STATE, NIGERIA

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ABSTRACT
A study on the effects of handling and distribution on the microbial contamination of some fresh fish from Tagwai dam, Minna. Niger state, Nigeria was carried out. Samples from the fish skin, gills, fish net, fishing boat, fish basket, fisher man's hand and table were collected using swab stick and cultured in two different media; nutrient agar and Macconkey agar and on examination reveal the presence of four bacteria species namely *Staphlococcus aureus, Klebsiella pneumonea, Bacillus subtilis and Escherichia coli*. The highest colony count was obtained from the skin of fish samples transported to the laboratory without ice. The proximate composition of the fish samples which include crude protein, lipid, and moisture content were not affected directly by the handling and distribution but indirectly by the presence of the bacterial when consumed. Washing of the sample with one percent saline solution and preserved with ice showed a great reduction of the microbial growth. Bacterial growth in Macconkey agar revealed the presence of intestinal pathogens. These pathogens namely *Staphlococcus aureus, Klebsiella pneumonea, Bacillus subtilis and Escherichia coli,* in the fresh fish samples could pose a potential food poison to the consumers. It is recommended that better handling and distribution methods should be adopted to reduce or eliminate health risk to fresh fish consumers.

KEYWORDS: Handling, Distribution, microbial, characterization, load *etc.*

INTRODUCTION

Fish is regarded as a healthier meat option due to the high content of long chain polyunsaturated fatty acids (LCPUFAS), which are associated with improving health and preventing diseases of old age**,** (Kabahenda *et al.,* 2009). Fish is also a high protein product which makes it susceptible to rapid degradation by micro-organisms. In a few hours or days, depending on the species and the ambient temperature at which it is kept spoilage sets in. The general course of events leading to fish spoilage involves enzymatic actions (rigor mortis, autolysis and lipolysis), bacterial actions and fat oxidation (Huss *et al.,* 2004). Another major threat to the quality of fish products is how the products are handled. Fish is thus a product that needs proper handling and processing in order to preserve nutrients and its functional components that promote good health. Once fish dies, spoilage will set in, so when the fish is heavily bruised from the harvesting point to landing site without proper handling to preserve the freshness, spoilage continues rapidly, especially when there is no cold chain for fish meant for local consumption. Fish is thus a product that needs proper handling and processing in order to preserve nutrients and its functional components that promote good health. Thus fish is a highly perishable commodity and hence susceptible to high post-harvest losses if not properly handled and distributed (Kabahenda *et al*., 2009). In Minna, Niger State, the traditional fish processing methods include smoking, sun drying, salting, and deep-frying. The processing of fish is mainly done as a fall back to utilize fish, which cannot be sold in fresh form either because it is spoilt or has failed to go through the normal channel of distribution. A variety of fish species include Tilapia, catfish, clupeid and mud fish are smoked, sun dried and fried sometimes at the landing site. In Tagwai dam, Minna, fish are poorly handled, some die in the harvest gear, while others get mixed up with sand and other contaminating debris at the landing shores. In addition, poor storage and means of transportation for distribution of fish is a problem. The fish after harvest is washed with dam water without any cold chain and transport to their local market where the fish are spread on the table for sales allowing flies to perch on it. In such case deterioration sets in very fast in the light of the identified problems associated with fish handling in Tagwai dam a major source of fresh fish in and around Minna metropolis, this was aimed at assessing the effects of handling and distribution on nutritive quality of fresh fish sold in Minna, Niger state

MATERIALS & METHODS

Experimental procedures

Five pieces each of three species of fresh fish namely *Sarotherodon galilaeus, Tilapia zilli* and *Pellonula afzeliusi* were purchased from Tagwai dam. The net, hand, boat, basket and market table of the fisher man were swabbed at the landing site and market place respectively using sterilized swab stick to obtain a sample. Two set of samples

were then taken to the laboratories in the Department of Microbiology and Department of Water Resources, Aquaculture and Fisheries Technology for analyses. The first sets of samples were kept in ice in an ice box while the second set was kept without ice in an ice box.

LABORATORY ANALYSIS

Microbial determination

Microbial colonies counts were done using a manual colony counter after incubation on the skins and gills of the fish samples at the landing site, in the ice box with ice and in the ice box without ice. For identified coliform bacteria in Macconkey agar, colonies of each suspected bacteria species were cultured in freshly prepared nutrient, starch, Simmon citrate agar and Triple Sugar Iron agar to obtain a pure isolate. Morphological and biochemical characterization of the isolated bacteria was done according to the method of pure culture technique as described by Oyeleke and Manga (2008). Their characters were compared with known taxa of Barrow and Feltham (2004).

Statistical analysis

Data obtained were analyzed using S. P. S. S. 16.0 version. The results of the samples were subjected to one way analysis of variance (ANOVA) at 5% probability level. Multiple parameters means comparison of treatments was according to Duncan multiple range tests.

RESULTS & DISCUSSION

The tables (1-12) below show the microbial count obtained from the cultured samples of the three species of the fresh fish at the landing site, fish with ice and fish without ice in two separate media, Nutrient agar and Macconkey agar.

TABLE 1: Microbial, colony count(X10 CFU/ML) on the skin of *Pellonula afzeliusi* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.58X10^{2b}$	$1.1X10^{c}$	$2.18X10^{2a}$
2	$1.41X10^{2b}$	$5.0X10^{c}$	$2.1X10^{2a}$
3	$1.66X10^{2b}$	$1.1X10^{c}$	$1.78X10^{2a}$
4	$1.16X10^{2b}$	$5.0X10^{c}$	$1.76X10^{2a}$
5	$1.48X10^{2b}$	$5.0X10^{c}$	$1.56X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other.

TABLES 2: Microbial, colony count(X10 CFU/ML) on the skin of *Pellonula afzeliusi* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.18X10^{2b}$	$2.6X10^{c}$	$1.6X10^{2a}$
2	$1.7X10^{2b}$	$3.3X10^{c}$	$1.63X10^{2a}$
3	$1.08X10^{2b}$	$1.0X10^{c}$	$2.03X10^{2a}$
4	$7.5X10^{b}$	$2.8X10^{c}$	$2.05X10^{2a}$
5	$8.3X10^{b}$	$1.5X10^{c}$	$1.35X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 3: Microbial, colony count (X10 CFU/ML) on the gill of *Pellonula afzeliusi* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$9.6X10^{b}$	$1.5X10^{c}$	$1.65X10^{2a}$
2	$5.6X10^{b}$	$8.0X10^{c}$	$1.25X10^{2a}$
3	$1.66X10^{2b}$	$6.0X10^{c}$	$1.81X10^{2a}$
4	$7.2X10^{b}$	$5.0X10^{c}$	$2.01X10^{2a}$
5	$5.6X10^{b}$	$3.0X10^{c}$	$1.56X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 4: Microbial, colony count(X10 CFU/ML) on the gill of *Pellonula afzeliusi* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$9.5X10^{b}$	$4.0X10^{c}$	$1.31X10^{2a}$
2	$9.1X10^{b}$	$3.1X10^{c}$	$1.15X10^{2a}$
3	$1.16X10^{2b}$	$3.5X10^{c}$	$1.63X10^{2a}$
4	$6.8X10^{b}$	$2.1X10^{c}$	$9.0X10^{a}$
5	$7.5X10^{b}$	$1.0X10^{c}$	$1.O3X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 5. Microbial, colony count (X10 CFU/ML) on the skin of *Sarotherodon galilaeus* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.64X10^{2b}$	$5X10^{c}$	$2.00X10^{2a}$
2	$1.60X10^{2b}$	$5X10^{c}$	$1.90X10^{2a}$
3	$1.58X10^{2b}$	$1.0X10^{c}$	$1.80X10^{2a}$
4	$1.41X10^{2b}$	$1.3X10^{c}$	$2.03X10^{2a}$
5	$1.43X10^{2b}$	$1.0X10^{c}$	$2.06X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 6: Microbial, colony count(X10 CFU/ML) on the skin of *Sarotherodon galilaeus* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.0X10^{2\,b}$	$1.6X10^{\,c}$	$1.83X10^{2\,a}$
2	$1.03X10^{2b}$	$1.5X10^{c}$	$1.76X10^{2a}$
3	$1.41X10^{2b}$	$1.5X10^{c}$	$2.26X10^{2a}$
4	$1.11X10^{2b}$	$6.1X10^{c}$	$1.40X10^{2a}$
5	$9.6X10^{b}$	$2.3X10^{c}$	$1.45X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 7: Microbial, colony count(X10 CFU/ML) on the gill of *Sarotherodon galilaeus* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.21X10^{2b}$	$1.5X10^{c}$	$1.2X10^{2a}$
2	$1.08X10^{2b}$	$5X10^{c}$	$1.2X10^{2a}$
3	$1.23X10^{2b}$	$1.1X10^{c}$	$1.53X10^{2a}$
4	$1.03X10^{2b}$	$5X10^{c}$	$1.36X10^{2a}$
5	$8.6X10^{b}$	$5X10^{c}$	$1.16X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 8: Microbial, colony count (X10 CFU/ML) on the gill of *Sarotherodon galilaeus* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$7.8X10^{b}$	$2.1X10^{c}$	$2.60X10^{2a}$
2	$4.5X10^{b}$	$1.0X10^{c}$	$1.10X10^{2a}$
3	$1.05X10^{2b}$	$2.3X10^{c}$	$2.03X10^{2a}$
4	$7.6X10^{b}$	$1.3X10^{c}$	$1.06X10^{2a}$
5	$8.1X10^{b}$	$2.3X10^{c}$	$1.23X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 9: microbial, colony count(X10 CFU/ML) on the skin of *Tilapia zilli* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.23X10^{2b}$	$1.0X10^{c}$	$2.00X10^{2a}$
2	$1.33X10^{2b}$	$3X10^{c}$	$1.96X10^{2a}$
3	$9.6X10^{b}$	$6.0X10^{c}$	$2.06X10^{2a}$
4	$1.03X10^{2b}$	$1.0X10^{c}$	$1.46X10^{2a}$
5	$1.03X10^{2b}$	$5.0X10^{c}$	$1.90X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 10: Microbial, colony count (X10 CFU/ML) on the skin of *Tilapia zilli* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$1.03X10^{2b}$	$6.0X10^{c}$	$1.70X10^{2a}$
2	$1.10X10^{2b}$	$3.OX10^{c}$	$2.13X10^{2a}$
3	$1.0X10^{2b}$	$1.1X10^{c}$	$1.76X10^{2a}$
4	$1.13X10^{2b}$	$3.0X10^{c}$	$1.66X10^{2a}$
5	$7.3X10^{b}$	$5.0X10^{c}$	$1.73X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 11: microbial, colony count (X10 CFU/ML) on the gill of *Tilapia zilli* in Macconkey agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$9.1X10^{b}$	$6.0X10^{c}$	$1.63X10^{2a}$
2	$8.0X10^{b}$	$5.0X10^{c}$	$1.26X10^{2a}$
3	$7.0X10^{b}$	$1.1X10^{c}$	$1.26X10^{2a}$
4	$8.6X10^{b}$	$1.0X10^{c}$	$1.28X10^{2a}$
5	$1.08X10^{2b}$	$5.0X10^{c}$	$1.26X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

TABLES 12 Microbial, colony count **(**X10 CFU/ML) on the gill of *Tilapia zilli* in nutrient agar

S/N	LANDING SITE	FISH WITH ICE	FISH WITHOUT ICE
1	$8.6X10^{b}$	$2.1X10^{c}$	$1.43X10^{2a}$
2	$6.5X10^{b}$	$1.8X10^{c}$	$1.50X10^{2a}$
3	$8.6X10^{b}$	$1.1X10^{c}$	$1.46X10^{2a}$
4	$6.6X10^{b}$	$1.0X10^{c}$	$1.10X10^{2a}$
5	$4.3X10^{b}$	$8.0X10^{c}$	$1.10X10^{2a}$

Values with different superscripts in a column are significantly different ($p<0.05$) from each other

The numbers of the colonies in all fresh fish samples were analyzed and mean recorded. The result showed a significant difference ($P<0.05$) between the fish at the landing site, fish with ice and the fish transported to the laboratory without ice. The trend in variation at various points in the two media ranged from fish with ice to fish at landing site and highest in fish without ice in all the species. It also was observed from the table that the Macconkey has a high number of colony count on the skin and gill ranging from $2.06x10^2$ to $2.18x10^2$ and $1.53x10^2$ to $2.60x10^2$ respectively, than nutrient agar ranging from $1.5x10^2$ to $2.26x10^2$ and $1.5x10^2$to $2.03x10^2$ respectively of all the species that was transported through market to the laboratory without ice The results further revealed that there was more contamination on the fish transported through market to the laboratory without ice. This could be attributed to the exposure to air and other environmental contacts such as the hand, basket and market table that were also contaminated because the microbial count suggested contamination. Higher microbial counts in the skin and gill of the fresh fish transported without ice to the Laboratory comparatively to the skin and gill landing site and transported fish with ice may be attributed to improper handling and distribution. The high *Escherichia coli* Table 13 in all the samples may be due to its ubiquity nature as it could be found in almost all the environment including human skin, water and air during handling and distribution. The result corroborated that of Majeed and Macrae (1991) who observed that most bacteria flora associated with spoilage of fish were gram negative rod bacilli which include *Bacillus subtilis* and *Escherichia coli*. The total bacterial count on the fish samples rarely indicated the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health condition of consumers of such contaminated fish. (Gram *et al.*, 2000). The presence of coliform bacteria such as *Bacillus subtilis, E.coli, klebsiella pneumonia* and *Staphylococcus aureus* as revealed in the samples were the pathogens associated with food poison which fish is one. These pathogens also are majorly responsible for fish spoilage. This finding is in agreement with the previous finding by Gram and Huss (2001) who reported that these organisms were the major causes of microbial spoilage of fresh fish after capture. The presence of *Klebisella spp* in the fresh fish samples is an indication that the dam is fecal contaminated (Walderhaug, 1992). The presence of *Staphlococcus aureus* a normal flora of skin and mucous membrane of human can be attributed to human contact during handling and distribution (Dalgaard *et al*., 2006). *Staphylococcus aureus* produces a variety of extra cellular enzymes and toxins that have been found to be responsible for food poisoning and can rapidly develop resistance to many antimicrobial agents and pose the therapeutic problems (Thrower, 2000). However, the two media used served for different purpose, Macconkey agar, a differential medium, was used to identify intestine bacterial while nutrient agar, multipurpose medium was mostly used to determine microbial load. The four bacteria isolated from Macconkey agar, have different ways of affecting man when consumed with food or in fish. Like *E. coli,* this organism causes gastroenteritis in human. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is dominant species found in faeces. Symptoms of *Bacillus sp* diarrheal type of food poisoning mimic those of *clostridium perfringens* food poisoning. The onset of watery diarrhea abdominal cramps and pain occurs 6 to 15 hours after consumption of contaminated fish or any other type of food. The symptoms of this type food poisoning parallel those caused by *Staphylococcus aureus* food borne intoxication (Dalgaard *et al*., 2006) *Klebisella sp* is enteric (intestinal) bacteria also, it has been suspected of causing acute and chronic gastro intestinal disease. The organisms may be recovered from natural environments such as forests and fresh water as well as from farm produce (vegetable) where they reside as normal micro flora.

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TABLE 13: morphological and biochemical characterization of the isolated bacteria

Samples	G.stain	Catalase	Coagulate	Citrate	H_2S	INDOLE	Methyl red	VP	SHR	SUGAR FERMENTATION			ORGANISMS
										L	G	S	M	
Gill and skin	-R	+	-	+	+	-	+	-	-	+	+	+	-	*Klebsiellia pneumonia*
Gill and Skin	-R	-	-	+	-	+	+	-	-	+	+	+	-	*Escherichia coli*
Skin	+C	+	+	-	-	-	-	-	-	-	-	+	-	*Staphylococcus aureus*
Skin	+R	+	-	+	-	-	-	+	+	-	-	+	-	*Bacillus subtilis*

+R =Gram positive rod
+C= Gram positive cocci
S= Sucrose Sugar
Vp=Voges Proskauer test

SHR= Starch hydrolysis test.
L =Lactose sugar
M = Mannitol sugar
+= Positive (there is reaction)

-R=Gram negative rod
G =Glucose sugar
H_2S= Hydrogen sulphide production test
- = Negative (No reaction).

They may be recovered from the stools of healthy individuals with no disease symptoms. Acute gastro enteritis is characterized by two or more of the symptoms of vomiting, nausea, fever, chills, abdominal pain and watery (dehydrating) diarrhea occurring after injection of such contaminated fish or other food.

The organisms can also occur in soils used for crop production and shellfish harvesting water and therefore, may pose a health hazard (Walderhaug, 1992).

In conclusion, the presence of these bacterial isolates in the fresh fish samples is indicative of public health risk in contacting diseases associated with these organisms. The presence of large number of bacteria greater than10^6 organisms/ml in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health. Lack of proper handling and distribution facilities after harvest and insanitary conditions during processing are the major sources of contamination identified in this study. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be ensured. In view of these findings, it is therefore recommended that good hygienic condition should be maintained in during handling and distribution of harvested fresh fish. Also the use of salt solution to wash the fresh fish after harvest and preserve with ice so as to maintain a cold chain during distribution should be strictly adhered to as it showed a well reduced contamination and also capable of inhabiting survival of mesophilic Bacteria.

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