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COMPARATIVE ASSESSMENT OF MICROBIAL LOAD OF DRIED FISH SOLD IN NGOR-OKPALA LOCAL MARKETS IN IMO STATE AND FEDERAL COLLEGE OF AGRICULTURE, ISHIAGU EBONYI STATE, NIGERIA

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Abstract
Microbial evaluation of dried fish samples from three local markets in Ngor-Okpala and Fisheries Department, Federal College of Agriculture, Ishiagu (FCAI) Ebonyi State were assessed using appropriate culture media in identifying isolates. The result revealed a total of nine (9) and seven (7) bacteria and fungi suspects respectively. Samples from Ngor-Okpala markets revealed four bacteria isolates (*Clostridium*, *Staphylococcus*, *Bacillus* and *Micrococcus*), while that from FCAI had eight (8) (*Salmonella*, *Proteus*, *Klebsiella*, *Staphylococcus*, *Bacillus, Micrococcus, Streptococcus* and *Pseudomonas*). Fungi isolates from Ngor-Okpala dried fish samples were *Geotrichum candidum,* while FCAI were *Phoma sorghina*, *Penicillium sclerotigenium*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor* and *Rhizopus stolonifer*. Heterotrophic bacteria mean counts from Ngor-Okpala was 2.5×10^5 cfu/ml, enteric coliform counts- 2.6×10^4 cfu/ml, while the fungi mean counts was 1.5×10^4 cfu/ml. The heterotrophic mean load isolates from FCAI was 1.8×10^5 cfu/ml, while the fungi mean counts was 1.7×10^4 cfu/ml. Sources of contaminations could be traceable to the waters where the fish were obtained, handlers, and sanitary conditions of the production, packaging, marketing or storage materials. The presence of these microorganisms in fish is detrimental to human health, and therefore precautions should be taken during processing, handling, storage, and transportation of fish.

Keywords: Comparative, assessment, microbial load, and dried fish

Introduction

Fish is an important part of a healthy diet, since it contains high quality protein but typically presents a low fat percent when compared to other meats. It can be eaten fresh, preserved (dried) or processed to form a much cherished delicacy that cuts across socio-economic, age, religions and educational barriers (Ayiba and Omeri, 2006). Fish generally have soft tissues and high amount of water which enhances its contemptibility to microbial contamination (Olayemi *et al.,* 2012). Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as good source of protein and other elements for maintenance of healthy body (Al-jufalli and Opera, 2006). It is regarded as healthier meat due to its high content of long chain poly-saturated fatty acids which are associated with improving health and preventing diseases at old age. Fish constitute 40% of animal protein intake (Oramadike *et al.,* 2010). In preserving fish by drying, water activity is lowered to the point where spoilage microorganisms is inhibited and the wood smoke add some microbial inhibitory substances like formaldehyde and alcohol (Okonta and Ekelemu, 2005). Smoke-dried fish is highly desirable because of its enhanced flavor and texture, in addition to the protection offered by drying against microorganisms (Sowumi, 2007).

Microbiological quality is of importance to public health as it directly relates to spoilage of fish and food poisoning (Oramadike *et al*., 2010). Fish spoilage usually triggered by microbial activities poses a dreaded challenge to fish farmers and fishermen due to severe economic losses associated with it (Buck *et al*. (2003). Food poisoning is a public health problem that could pose multi-dimensional challenges to agribusiness (Sivapalasingam *et al*., 2004; Oramadike *et al*., 2010). It could reduce work input due to hospitalization, reduced market demand for fish products and increased cost of treatment. Dried or smoked fish products could be contaminated due to poor handling during the smoking process or poor packaging, and since people can eat it even without cooking, the possibility of catching food poisoning increases. Some food borne infections have been traced to *Salmonella* spp, *Staphylococcus* spp, *Escherichia* spp and *Vibrio parahaemolyticus* (Yagoub

and Ahmed, 2009). The aim of this study is to isolate and compare the possible pathogenic microorganisms associated with dried fish sold in local markets in Ngor-Okpala Local Government Area of Imo State and that of FCAI Fish Production Unit, and highlight the risk involved in the consumption of dried fish bought from local markets.

Materials and Methods

Study area

Ngor-Okpala Local Government Area of Imo State is the study area and fish markets are located in the three main markets; Nkwo Ebi, Ekentta, and Orie Imo, each operate every eight days interval where different kinds of fish are sold. Ngor-Okpala is a Local Government Area in Imo State of Nigeria with its' headquarters in Umuneke. The people are industrious, engaged in many commercial activities like farming (farm cultivation, fish farming), trading, civil service work etc. They have a big river (Imo River) situated at Okpala-Aba road in Ngor-Okpala. This makes fish farming and selling (hawking) very common and rampant. Some hawkers display their goods (dried fish) in open trays, stalked on sticks or ropes, or hawk them about to sell. The second fish samples were obtained from the Fish Production Unit of the Fisheries Department of Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria as the second study area. Sizeable dried fish samples from each of the three markets in Ngor-Okpala were purchased from dealers and samples collected from the Fisheries Department were separately placed in sterile polythene bags. All were sent to the Pathology Laboratory for microbial investigation. All the media used were prepared according to the manufacturer's instruction. The microbial isolation of all the samples were carried out by 10-fold serial dilution according to standard protocol by Atlas *et al.* (1995) and inoculation done by surface plate method (Cheesbrough, 2006).

Microbial Assessment

Ten grams of each sample were aseptically weighed and homogenized in 90ml of sterile distil water. Ten- fold serial dilutions of samples were done in 1% peptone water and plated out on three different culture media; Nutrient agar medium was used for isolation of aerobic mesospheric bacteria; MacConkey agar medium for isolating enteric bacteria, while Saboured dextrose agar for isolating heterotrophic fungi. Sub culturing was carried out using Nutrient broth for distinct colonies for confirmation of the isolates biochemically. MacConkey agar for coliforms. The plates were incubated at 35ºC for 18 hours. Pour plate method of Harrigan and McCance (1976) was used for the isolation. The pure isolates were identified using morphological and biochemical characteristics according to Bergy's manual of systematic bacteriology as described by Holt *et al.* (1994).

Results and Discussion

Microbial contamination is a regular phenomenon that is witnessed in fishing activities or industries due to the high nutritional values of the products. Dried fish samples from FCAI Fisheries department showed more rapid degradation than those bought from Ngor-Okpala markets (Table 1). The rapid degradation of the samples obtained from Fisheries Department was due to the high moisture content of the fish, an indication of insufficient exposure to heat during the drying process. Most farmers especially those living close to large number of consumers deliberately limit the time of heat exposure of the fish to avoid excessive loss of weight, size and the consequent low price tag of small sized fish products. High moisture content of dried fish products are reported to be an indication of retention of all the essential growth factors that favour microbial growth. In an earlier work, Frazier and Westhoff (1981) reported that the availability of nutrients is crucial to increase or decrease microbial numbers in any food during spoilage.

The maximum microbiological limit (total viable count) which separates the good quality fish from the bad quality is 5×10^5 cfu/g according to the International Commission of Microbiological Specification for Food (ICMSF, 1986). In this study, the microbial count and coliform were above the acceptable limit. This could be as a result of the anthropogenic activities in the environment or the duration of fish storage before sale.

According to the rule of International Association of Microbiology Society, fresh fish should possess neither *Vibrio* spp. nor *Salmonella* spp. The investigated samples were free from these pathogenic organisms. The presence of coliforms in the fish is a clear indication of environmental and faecal pollution, either from humans and /or from animals and poor handling practices (Eze *et al*., 2011). Most outbreaks of food poisoning associated with fish are derived from the consumption of raw or insufficiently heat-treated fish, which may be contaminated with bacteria from water environment. There is therefore need to enlighten consumers more on the adequate cooking of seafood before consumption.

Conclusion

In conclusion, this study has revealed that the level of microbial contamination of dry fish products could either be environmentally dependent or on the economic decisions of the fish farmers. It also revealed fish contamination as a public health problem. Eating uncooked dry fish could be hazardous and should be avoided.

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Table 1: Mean Microbial counts from fish obtained in Ngor-Okpala and FCAI

Parameter	Ngor-	Okpala	Fish		FCAI fish	
Medium oC	NA, 30	MA, 37	SDA, 37	NA, 30	MA, 37	SDA, 37
Sample 1 (cfu)	124	15	7	96	24	7
Sample 2 (cfu)	128	13	6	84	28	8
Sample 3 (cfu)	129	11	9	90	23	10
Mean	127.0	13.0	7.3	90	25.0	8.3
Dilution factor	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}
Volume of inoculum (ml)	0.5	0.5	0.5	0.5	0.5	0.5
MMC, cfu ml^{-1}	$2.5x10^5$	$2.6x10^4$	$1.5x10^4$	$1.8x\ 10^5$	$5x10^4$	$1.7\ x10^4$

MMC = Mean microbial count; cfu = colony forming unit; cfu ml^{-1} = colony forming unit per milliliter; NA= Nutrient agar, MA= MacConkey agar, SDA= Saboured dextrose agar

Table 2: Summary of Biochemical test in identifying bacteria pure culture

Gram stain result	Shape	Growth in air	Acid from glucose	Gas from glucose	Acid from lactose	Gas from lactose	Catalase	Oxidase	Indole	Coagulase	Urease	Citrate	Probable bacteria
+	R	-	+	+	-	-	-	-	-	-	-	-	*Clostridium* spp
+	C	+	+	-	+	+	+	+	-	+	-	-	*Staphylococcus* spp
+	R	+	+	-	-	-	+	+	-	-	-	-	*Bacillus* spp
+	C	+	-	-	-	-	+	+	-	-	-	-	*Micrococcus* spp
-	R	+	-	+	-	-	+	-	-	-	-	+	*Salmonella* spp
-	R	+	+	+	-	-	+	-	+	-	+	+	*Proteus* spp
-	R	+	+	+	+	+	+	-	-	-	+	+	*Klebisiella* spp
+	C	+	+	+	-	-	+	+	-	-	-	-	*Streptococcus* spp
-	R	+	-	-	-	-	+	+	-	-	+	-	*Pseudomonas* spp

Interpretation: + positive test; - negative test; R rod – shaped; C cocci – shaped.

The load of aerobic mesophilic bacteria of Ngor- Okpala fish samples of 2.5 x 10 5cfu/ml is higher than the FCAI fish samples whereas the enteric bacteria count and fungi count from FCAI samples were higher than that of Ngor-Okpala fish. Comparatively, the result revealed the nature of human activities in relation to keeping the fish, environmental factors surrounding the fish and other intrinsic factors of sampled f ish, Tables 1 and 2.