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Microflora Associated With Processing and Storage of the White Catfish (*Chrysichthys nigrodigitatus*)

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ABSTRACT

Smoking of fish for preservation and taste developed early in the history of man. Smoked fish is part of the daily diet of most Nigerians and smoking of fish has been conducted by all manners of persons and in different kinds of conditions both hygienic and unhygienic resulting in health challenges in the form of food borne diseases. This study aimed at evaluating the quality of locally processed and stored smoked fish products sold in our markets for consumption, in order to highlight their health implications for the unsuspecting consumers. Quality deterioration (microbial load) of smoked white catfish (*Chrysichthys nigrodigitatus*) from Ekeonunwa market Imo state Nigeria, was studied for six weeks. The experimental fish was divided into two batches. One batch of 18 fishes with average weight of 700 g was resmoked weekly at 75°C. The second batch of 18 fishes weighing an average of 750 g was left unresmoked. Weekly samples of both unresmoked and resmoked fish were taken from different regions (body flanks, gill and gut) and analyzed in the laboratory. Total Plate Counts (TPC) for the bacterial organisms in both resmoked and unresmoked samples ranging between 1.56×10^5 and TNTC (too numerous to count) exceeded the range of specified microbiological limits recommended for fish and fishery products. Pathogenic organisms isolated included *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Aspergillus flavus*, *Penicillium varrucosum*, Yeast and Mucor. Both unresmoked and resmoked fish specimens were contaminated with these spoilage microbes but the microbial load of the resmoked samples were less than those of the unresmoked samples. Resmoking beneficially reduces microbial load of stored fish, increases the shelf/storage life but cannot totally eliminate pathogens. The presence of pathogens raises a public health concern.

Key words: White catfish, microflora, unresmoked, storage, public health

INTRODUCTION

Fish is a perishable commodity and its spoilage is as a result of both enzymatic and bacterial breakdown of the flesh, especially in the tropics where temperatures are high. There is therefore need to preserve fish in some way after capture to prevent loss due to spoilage and to provide an excellent "just caught flavor". Technically, smoking is the process through which volatiles from thermal combustion of wood penetrate meat and fish flesh (Simko, 1991). The quality of smoked product depends on the quality of the fish at the time of smoking, the nature of wood and the type of smoking procedure employed (Doe, 1998). Da Silva *et al.* (2008) examined the microbial safety and quality of smoked blue catfish (*Ictalurus furcatus*) steaks treated with antimicrobials and antioxidants during 6 weeks ambient storage. They found out that neither listeria, nor salmonella

was recovered from the smoked catfish steaks, also significant reductions ($p < 0.05$) in total plate counts were observed in all treated samples using 25% NaCl, 1% ascorbic acid and 3% sodium lactate with or without 5% rosemary extract and sorbic acid alone. The preservation of fish has been an integral part of every seafaring culture. Over the course of thousands of years of drying, salting and smoking fish, the technique has developed to a point where once common food has become a delicacy and there is need for corresponding concern for safety issues in smoked fish consumption (Riches, 2012). Fafioye *et al.* (2002) studied the fungal infestation of five traditionally smoked dried freshwater fish in Ago-Iwoye, Nigeria and isolated and identified eleven different fungal species of which *Aspergillus flavus* was the most frequently encountered fungi on the fish species. In many areas today fish is smoked for flavour and appearance and not strictly for preservation because the amount of salt and smoke used is not sufficient to prevent bacterial spoilage; as a result most food poisoning bacteria can and will grow under the conditions normally found in preparation and storage of smoked fish. *Clostridium botulinum*, the bacteria that may cause botulism, is the most harmful of these bacteria (Long, 2009).

Delay of microbial spoilage of fish may be achieved by introduction of different additives such as sodium lactate to inhibit *Listeria monocytogenes* and *Clostridium botulinum* and its toxin production; sodium chloride inhibiting the growth of *L. monocytogenes* and prevent the germination of *C. botulinum* endospore; Ascorbic acid utilized to prevent mold growth in smoked fish during storage; and Rosemary extract with ascorbic acid inhibits oxidative deterioration (USDA, 1999; Doe, 1998). The objectives of the study were to determine (1) The microbial load of unresmoked and resmoked white catfish stored at room temperature, (2) Physical quality changes of unresmoked and resmoked white catfish stored at room temperature and (3) A non toxic protocol of managing and increasing the storage life of smoked catfish.

MATERIALS AND METHODS

The investigation was carried out between August and September 2008 for a period of 6 weeks in the microbiology laboratory of the Federal Polytechnic Nekede, Imo State, Nigeria. The smoked fish samples used were purchased from smoked fish sellers at Ekeonunwa market, Owerri Imo State.

Sample collection and methods: Freshly smoked white catfish was bought and divided into two parts tagged, unresmoked and resmoked samples (which was resmoked once every week in a hot air oven). Samples from the body, gill and gut were reduced to fine particles and diluted with 10 mL of distilled water, giving a final concentration of 1 g/10 mL. One microliter was added to 9 mL of distilled water in a sterile test tube to provide 10^{-1} dilution and 10^{-4} subsequently (Adegoke and Skura, 1994). Total bacterial counts were determined by inoculating 0.1 mL of the dilution 10^{-8} onto Nutrient agar, MacConkey agar and Blood agar by streak method and incubated at 37°C for 24 h while fungal counts were on Sabouraud and Dextrose agar incubated at room temperature ($25\text{-}26^{\circ}\text{C}$) for 2-4 days. All inoculation were done in triplicate.

Identification of isolates: Bacterial isolates were identified using colonial morphology, pigmentation, cell shape and gram staining reaction and biochemical test as described by Singleton (1999), Isu and Onyeagba (1998) and Cruickshank *et al.* (1982).

Gram staining: The method described by Cheesbrough (2002) was adopted to classify the organisms as gram negative or gram positive bacteria.

Biochemical test for the bacterial isolates: Several tests were carried out to identify the bacterial isolates.

Catalase test: The organism releases the enzyme catalase which catalyzes the release of oxygen from hydrogen peroxide $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$.

Coagulase test: This identifies *Staphylococcus aureus* which produces the enzyme coagulase.

Indole test: This identifies organisms that produce indole from utilizing amino acid from tryptophan e.g., strains of *E. coli*, *P. rettgeri*, *P. vulgaris* and *Providencia* species.

Oxidase test: This test was used to detect the presence of cytochrome oxide in micro organism differentiating *pseudomonas* and other gram negative rod like *Neisseria*, *Vibrio*, *Brucella* and *pasteurella* species.

Citrate utilization test: Enterobacteria is identified by this test which is based on the ability of the organism to use citrate as its only source of carbon.

Motility test: This was carried out in semi-solid agar medium and growth from the line of inoculation was recorded as evidence of motility.

Spore stain: These were identified by examination under oil immersion objective of stained heat fixed slides.

Identification of fungal isolates: This was carried out according to Cheesbrough (2004). Identification was based on colonial morphology, macroscopic and microscopical examination of the fungal growth on Sabouraud dextrose agar. The keys include-growth rates, general topography, surface texture pigmentation and pigmentation of the reverse side of the plates.

RESULTS

The results of total viable counts of bacterial isolates are presented in Table 1. which showed that at the end of the first week the total viable counts of bacterial isolates irrespective of the part of the body they are derived from or whether resmoked or unresmoked increased non-significantly ($p > 0.05$) over the initial number of isolates. The isolates from an initial value of 6 and 7 CFU g^{-1}

Table 1: Total viable count of bacterial isolates from unresmoked and resmoked catfish on MCA, NA and blood agar

Parts of fish	Treatment	Counts (CFU g^{-1})						
		No. of bacterial isolates	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Body (flanks)	Unresmoked	6	8	73	TNTC	TNTC	TNTC	TNTC
	Resmoked	7	8	15	80	185	245	250
Gut	Unresmoked	6	15	105	TNTC	TNTC	TNTC	TNTC
	Resmoked	7	12	20	110	260	290	320
Gill	Unresmoked	6	12	95	TNTC	TNTC	TNTC	TNTC
	Resmoked	7	10	17	90	210	270	300

TNTC: Too numerous to count, MCA: MacConkey agar, NA: Nutrient agar

Table 2: Total viable count of fungal isolates from smoked and resmoked catfish on SDA and lactophenol mount

Parts of fish	Treatment	Counts (CFU g ⁻¹)						
		No of bacterial isolates	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Body (flanks)	Unresmoked	5	6	65	255	TNTC	TNTC	TNTC
	Resmoked	5	7	60	135	TNTC	320	410
Gut	Unresmoked	5	5	65	250	TNTC	TNTC	TNTC
	Resmoked	5	6	55	100	TNTC	280	345
Gill	Unresmoked	5	6	75	255	TNTC	TNTC	TNTC
	Resmoked	5	7	60	135	TNTC	230	375

TNTC: Too numerous to count, SDA: Sabouraud dextrose agar

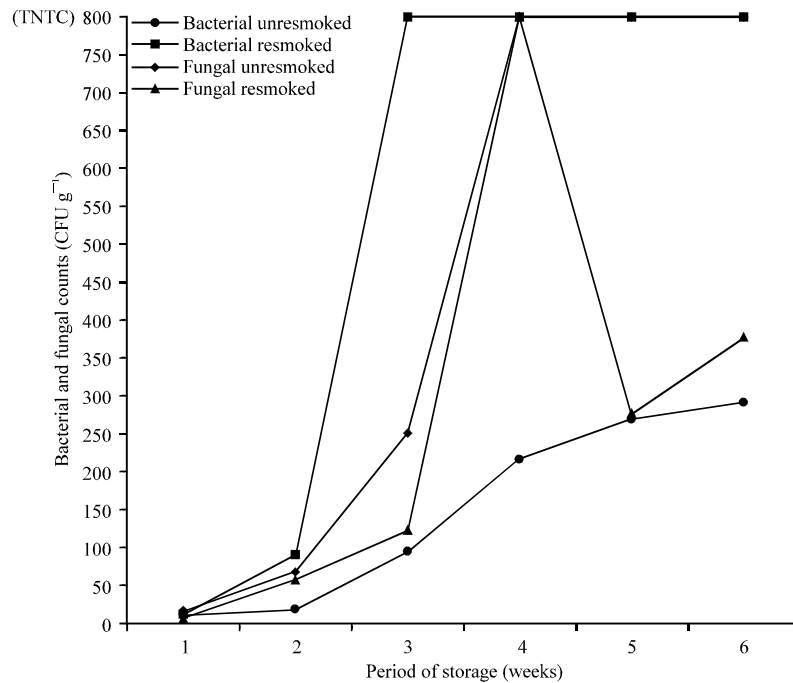


Fig. 1: Microflora (bacterial and fungal) counts from smoked and resmoked white catfish *Chrysichthys nigrodigitatus*. TNTC (800): The number scale 800 was assigned to TNTC to allow excel to recognise it and allow the graph to be plotted. In actuality TNTC exceeds 800 CFU g⁻¹ and is too numerous to count

for unresmoked and resmoked fish, respectively increased to a range of 8-15 CFU g⁻¹ for both categories of smoked fish. Significant increase in total viable counts of bacterial isolates was recorded at the end of week 2. From a bacterial isolate range of 8-15 CFU g⁻¹ in week 1 the bacterial isolates increased to a range of 15-105 CFU g⁻¹. Starting from week 3 to week 6, all unresmoked parts of the fish (body flanks, gut and gill) had exponential growth of bacteria too numerous to count (TNTC). The resmoked parts had a steady increase of isolates with time but never attained to TNTC values.

The total viable counts of fungal isolates are presented in Table 2 and shown in Fig. 1. At the end of week 1 there was no significant increase in fungal isolates ($p > 0.05$). From an initial fungal isolate value of 5 CFU g⁻¹ the value increased marginally to 6 or 7 CFU g⁻¹. Weeks 2 and 3 had

Table 3: Mean total viable count of both bacterial and fungal isolates from unresmoked and resmoked catfish

Microorganisms isolated	Treatment	Counts (CFU g ⁻¹)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Bacterial	Unresmoked	11.60 1.16×10 ⁵	91.00 9.10×10 ⁵	TNTC	TNTC	TNTC	TNTC
	Resmoked	10.00 1.00×10 ⁵	17.30 1.73×10 ⁵	93.3 9.33×10 ⁵	216.6 2.166×10 ⁶	268.3 2.683×10 ⁶	290 2.90×10 ⁶
Fungal	Unresmoked	15.60 1.56×10 ⁵	68.30 6.83×10 ⁵	253.3 2.533×10 ⁶	TNTC	TNTC	TNTC
	Resmoked	6.60 0.66×10 ⁴	58.30 5.83×10 ⁵	123.3 1.23×10 ⁶	TNTC	276.6 2.766×10 ⁶	376.6 3.76×10 ⁶

TNTC: Too numerous to count

significant increases in total counts of viable fungal isolates ($p < 0.05$). The growth increases ranged from 55-75 CFU g⁻¹; and 100-255 CFU g⁻¹ for weeks 2 and 3, respectively. From weeks 4-6, all the unresmoked fishes had fungal growths attaining the TNTC values. At the end of week 4 all resmoked fishes recorded TNTC values. This declined in weeks 5 and 6 to countable numbers ranging between 230-410 CFU g⁻¹.

Table 3 shows the mean total viable counts of bacterial and fungal isolates in the smoked fish. Mean bacterial isolate in the unresmoked fish ranged from 1.16×10⁵ CFU g⁻¹ in week 1 to TNTC in weeks 3-6. Bacterial isolates in resmoked fish ranged from 1.0×10⁵ CFU g⁻¹ (week 1) to 2.90×10⁶ CFU g⁻¹ (week 6). Fungal isolates ranged from 1.56×10⁵ CFU g⁻¹ (week 1) to TNTC (weeks 4-6). Resmoked fishes had fungal growth range of 0.66×10⁴ CFU g⁻¹ (week 1) to 3.76×10⁶ CFU g⁻¹ (week 6). Figure 1 illustrates the growth pattern of bacteria and fungi on the smoked fish over a period of 6 weeks in response to resmoking and no resmoking. The resmoked fish had a bacterial load of below 300 CFU g⁻¹. The resmoked fish also had a fungal load that was general below 400 CFU g⁻¹ except in week 4 when the fungal load was too numerous to count (TNTC). The bacterial and fungal load in the unresmoked fish reached the TNTC level in weeks 3 and 4, respectively.

DISCUSSION

The result of the study revealed that all is not well with the quality of smoked dry white catfish (*C. nigrodigitatus*) sold in Nigerian markets. The isolation of pathogenic and spoilage organisms such as *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aspergillus flavus* etc., raises public health concerns about safety in consuming smoked fish products from our markets and cause a high rate of spoilage leading to shorter shelf/storage life of the product (Ramos, 1999). Tainting of fish with these organisms is attributed mainly to poor handling by processors and traders who expose smoked fish to unsanitary conditions. *E. coli* is often implicated in gastroenteritis associated with poor handling of food. In addition to chemicals found in smoke, smoked fish should be treated with some bacteriostatic and, or bacteriocidal agents like sodium chloride and sorbate etc. to eliminate or reduce the effect of microorganisms as contaminants on smoked fish products. Omojowo *et al.* (2009) compared the salts of metabisulphite and sorbate with concentrations of 1-3% on the safety and shelf life of smoked catfish. They reported that both are good in reducing the *E. coli* and *Streptococcus* sp. This finding should be communicated to fish processors to improve handling of smoked fish. Total Plate Counts (TPC) for the bacterial organisms in both resmoked and

unresmoked samples (Table 3) ranging between 1.56×10^5 and TNTC (too numerous to count) exceeded the range of specified microbiological limits recommended for fish and fishery products by ICMSF (1986). The ICMSF protocol recommends a maximum bacterial count of 5×10^5 for good quality product and a maximum count of 10^7 for marginally acceptable quality products. The good effects of resmoking on the shelf life of the smoked fish by inhibiting growth of spoilage organisms was demonstrated (Table 1, Fig. 1). Table 1 revealed that bacterial growth in all the resmoked regions of the fish never attained the TNTC level, unlike growth in the unresmoked corresponding samples which all reached the TNTC growth ceiling. This shows that resmoking inhibited bacterial growth more than fungal growth (Table 1, 2). The fact that hot-smoking followed by resmoking inhibited the growth of bacteria (Fig. 1) points to the beneficial preservation effect of elevated heat application to the shelf life of stored smoked fish, this assertion is supported by the work of Lindstrom *et al.* (2003) who observed that Commercial hot-smoking processes employed in five Finnish fish-smoking companies provided reduction in the number of spores of nonproteolytic *C. botulinum* of less than 10^3 . The pattern of growth of microorganisms in Fig. 1 shows a lag phase in growth within the first two weeks following initial hot-smoking of the fish and by the third week the unresmoked fishes experienced exponential growth in bacterial and fungal organisms. Nwachukwu and Ezeronye (2004) observed that fish dried at temperatures between 90-120°C were better irrespective of brining. This was evidenced by a drastic reduction in microbial growth on stored fish smoked and dried at these temperatures. The unexpected exponential fungal growth in week 4 in the resmoked fish reaching the TNTC level was believed to be due to improper resmoking in terms of the amount of heat delivered and the exposure time to the heat. Some of the fungal species like *Aspergillus flavus*, *mucor* sp., recovered from smoked catfish in Ekeonunwa market Owerri were also found on smoked catfishes from Oja-Oba market, Ago-Iwoye Ogun state, Nigeria (Fafioye *et al.*, 2002). This shows that the processing environment and market environment for smoked fishes in different parts of Nigeria are comparable and any method or technology developed for better fish handling and processing may have general application and comparable success level. Some of the diseases caused by these microbes are listeriosis manifesting as meningitis, abortion and pre-natal septicemia affecting mostly immuno-compromised individuals, pregnant women and infants *E. coli* causes life threatening epidemic gastroenteritis in humans e.g., travelers diarrhea (ETEC) also called "Delhi belly". *Bacillus cereus* produces toxins that cause a disease that is more an intoxication than a food borne infection. Also *S. aureus* is known to cause enterotoxigenicity due to the production of enterotoxin and also known to cause *Staphylococcus* food poisoning which is a major type of food intoxication.

CONCLUSION

It was noted that stored smoked dried *C. nigrodigitatus* sold in Ekeonunwa market, Owerri Imo state, was contaminated with pathogenic and spoilage organisms even when resmoked. This finding has both storage and public health implications. It is noted that fish processors and vendors should improve handling hygiene and consumers should also cook smoked fish properly to minimise early spoilage and possible health hazards.

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