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MICROFLORA ASSOCIATED WITH PROCESSING AND STORAGE OF THE WHITE CATFISH (*CHRYSICHTHYS NIGRODIGITATUS*)

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ABSTRACT

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 700g was resmoked weekly at 75°C. the second batch of 18 fishes weighing an average of 750g 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 (Table III) 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). Pathogenic organisms isolated included Escherichia coli, Staphylococcus aureus, Lysteria monocytogenes, Bacillus cereus, Aspergillus flavus, Penicillium varrucossum, 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 can not totally eliminate pathogens. The presence of pathogens raises a public health concern.

INTRODUCTION

Fishes are a very 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 et al, (1998). 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 et al, 1998). The objective of the study is: 1. To determine the microbial load of unresmoked and resmoked white catfish stored at room temperature. 2. To determine physical quality changes of unresmoked and resmoked white catfish stored at room temperature. 3. To determine a non toxic protocol of

managing and increasing the storage life of smoked catfish.

MATERIALS AND METHODS

This study was carried out with samples obtained from dried fish sellers at Ekeonunwa market, Owerri, Imo state of Nigeria.

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 10ml of distilled water, giving a final concentration of 1g/10ml. 1ml was added to 9ml 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 0.1ml of the dilution 10^{-3} onto Nutrient agar, Macconkey agar and Blood agar by streak method and incubated at 37°C for 24 hours while fungal counts were on sabouraud and Dextrose agar incubated at room temperature ($25-26^{\circ}\text{C}$) 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 Monica Cheesbrough, (2004) was adopted to classify the organisms as gram negative or gram positive bacteria.

Biochemical Tests 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.rettger*, *P.vulgans* 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 *Neisseri*, *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 Cheesborough (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

Results of the bacterial and fungal isolates present in the unresmoked and resmoked catfish *Chrysichthys nigrodigitatus* are represented below.

TABLE I: Total viable count of bacterial isolates from unresmoked and resmoked catfish on MAC, NA and blood agar.

Parts of fish	Treatment	No of bac. Isolates	Week I	Week II	Week III	Week IV	Week V	Week VI
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

TABLE II: Total viable count of fungal isolates from smoked and resmoked catfish on SDA and Lactophenol mount.

Parts of fish	Treatment	No of bac. Isolates	Week I	Week II	Week III	Week IV	Week V	Week VI
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

TABLE III: Mean total viable count of both bacterial and fungal isolates from unresmoked and resmoked catfish

Microorganisms Isolated	Treatment	Week I Cfu/g	Week II Cfu/g	Week III Cfu/g	Week IV Cfu/g	Week V Cfu/g	Week VI Cfu/g
BACTERIAL	UNRESMOKED	11.6	91	TNTC	TNTC	TNTC	TNTC
		1.16×10^5	9.1×10^5				
	RESMOKED	10	17.3	93.3	216.6	268.3	290
		1.0×10^5	1.73×10^5	9.33×10^5	2.166×10^6	2.683×10^6	2.90×10^6
FUNGAL	UNRESMOKED	15.6	68.3	253.3	TNTC	TNTC	TNTC
		1.56×10^5	6.83×10^5	2.533×10^6			
	RESMOKED	6.6	58.3	123.3	TNTC	276.6	376.6
		$.66 \times 10^4$	5.83×10^5	1.23×10^6		2.766×10^6	3.76×10^6

Figure I : micro flora (bacterial and fungal) counts from smoked and resmoked white catfish *chrysichthys nigrodigitatus*

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 (FDA, 2001). Total

plate counts (TPC) for the bacterial organisms in both resmoked and unresmoked samples (Table III) 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 I and figure I). Table I 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 (Tables I and II). 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 travellers diarrhea (ETEC) also called "Delhi belly". *Baccillus 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 (Abalaka and Elegbade 1981).

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 processor 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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