EFFECT ON BRINNING ON THE MICROBIAL QUALITY AND SAFETY OF SMOKED CATFISH

Omojowo, Funso Samuel;* Omojasola, Patricia Folake,**, Kolawole Matthew Olatunji** Ngwu Emmanuel onyebuchi*, Oluborode Gabriel Bayode*; Adetunji Charles Oluwaseun***

jowosam@yahoo.com ; folakejasola@yahoo.co.uk ; tomak74@yahoo.com; ngwuemma@yahoo.com, tkayode2007@yahoo.com, charliguitar@yahoo.com

*NIFFR, P.M.B. 6006, NEW-BUSSA, NIGER STATE, NIGERIA.
** DEPT. OF MICROBIOLOGY, UNIVERSITY OF ILORIN, ILORIN. NIGERIA
***NIGERIAN STORED PRODUCTS RESEARCH INSTITUTE, ILORIN, KWARA STATE, NIGERIA.

ABSTRACT

This study was carried out to assess the microbial quality and safety of smoked catfish (*Clarias gariepinus*) treated with Sodium chloride (table salt) during 8-week storage at room temperature. Raw catfish steaks were subjected to the following treatments for 5 minutes prior to smoking: 5-25% table salt. The non-treated catfish served as control. The control and the fresh fish treated samples showed diverse microbial load. All treated smoked sample were negative for *E. coli and Streptococcus sp.* The treatment effectively reduced the TVC, Coliform, Staphylococcus and fungi after smoking and these low microbial counts was maintained until the end of the 8 weeks storage. Treatments with 20 and 25% salt proved best in terms of microbial reduction but organoleptically 5% treatments are acceptable to consumers. [New York Science Journal 2010;3(6):20-26]. (ISSN 1554 – 0200).

Key words: Table salt, storage, microbial load and smoked catfish

INTRODUCTION

Smoking of fish and/or meat products is one of the most ancient processing technologies. It has been for centuries used for preservation, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked for preservation (Ward, 1995). Hard curing by salting and smoking permits lengthy preservation by removing moisture, which is essential for bacteriological and enzymatic spoilage. Consumers are rediscovering the good taste of smoked seafood, including smoked catfish. To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products. Fish and fisheries products are among the most perishable commodities worldwide mainly due to microbial spoilage. About one-third of the world's food production is lost annually as a result of microbial spoilage. In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods (Lund *et al.*, 2000). Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella spp.*, and *Clostridium botulinum* (Heintz and Johnson, 1998). Omojowo and Ihuahi (2006) reported that smoked fish samples from 4 local Markets in Kainji Lake area of Nigeria were dominated by gram-positive bacteria, Potential pathogens, coagulase-positive Staphylococcus, and *Escherichia coli.* Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and brining. In certain instances, sodium chloride is added mainly as a flavoring and functional ingredient and hence in these cases the effect could be "indirect." Another reason that the antimicrobial effect of sodium chloride may be called indirect is that it reduces the water activity in many foods and thereby indirectly prevents microbial growth (Ravishankar and Juneja, 2000). The objectives of this study were to evaluate the effect of different concentration of table salt on the microbial, physical, organoleptic and nutritional quality of smoked catfish during 8-week storage at room temperature.

MATERIAL AND METHODS

Sample - Treatment

Fresh catfish (*Clarias gariepinus)* were obtained from a private Fish pond in National Institute for Freshwater Fisheries Research (NIFFR) Housing Estate, New Bussa, Niger State. The fish samples measuring 17-28cm in length and weighing 180-250g were transferred within 30 minutes to the laboratory in a sterile polythene bags and then

killed by severing the spinal cord with a sterile scalpel and aseptically eviscerated, washed and rinsed in sterile water. The fish samples were randomly chosen and divided into 6 groups of 5 fish samples and subjected to treatments. The treatments were as follows; (1) control (untreated samples); (2, 3, 4, 5, 6) with 5,10, 15, 20, and 25% Sodium chloride (table salt) for 5 minutes. A sample from each group were separated from each treatment and smoked. Smoking was done according to the methods described by Omojowo and Ibitoye (2005). After smoking and the fish were allowed to cool down and stored in different boxes. This was done to mimic commercial practices. The samples were drawn after two, four, six and eight weeks of storage; then subjected to analysis.

Microbiological and other Analysis

Total viable count (TVC), Coliform, Staphylococci and Fungi count were evaluated according to the methods described by Harrigan and McCance 1976; Speck 1984 and Sneath et. al., 1986). Moisture contents, fat and Crude protein were estimated as per AOAC (1980). All samples were done in duplicates. Sensory evaluation was carried out according to the method of Afolabi et. al. (1984). Statistical analysis was according to SAS, Institute, Inc, (1992) at $P < 0.05$.

RESULTS AND DISCUSSION

Microbial Analysis

A study for the absence and presence of the target food borne pathogens such as *Salmonella, Staphylococcus, and E. coli* is required to evaluate microbial safety of smoked catfish. The Total Viable count (TVC), Coliform, Staphylococci and Fungi count in log CFU/g of fresh and smoked samples plated on selective and non-selective media are shown in Tables 1. The total viable count (TVC) of the fresh non-treated (control) Catfish was 6.60 log CFU/g but after the sample were subjected to treatments with table salt the TVC reduction was highest in 25% (5.02 log CFU/g and least in 5% (5.54 log CFU/g). Similarly, Coliform count was reduced from 4.60 log CFU/g in the control to 3.34 log CFU/g in 25% and least was 4.11 log CFU/g in 5% salt concentration. In the same vein, Staphylococci count was reduced from 4.55 log CFU/g in the control to 3.0 log CFU/g in 25% and least in 5% (4.0 log CFU/g). In addition, Fungi count was reduced from 4.52 log CFU/g (control) to 3.62 log CFU/g in 25% and least in 5% (4.21 log CFU/g. Smoking sharply reduced the total viable count in all samples but the sample treated with treated with 25% concentration had the best reduction of 2.13 and 4.60 log CFU/g on day 0 and at the end of eighth week of storage. The TVC of smoked control (untreated) samples were the highest throughout the period of storage and the sample were even completely covered by mold after the 6th week of storage; therefore, no further microbial analysis was conducted. The results obtained were similar to those reported by Goktepe and Moody (1998) where aerobic plate counts in raw catfish fillets were 4.03 log CFU/g prior to brining and 3.61 log CFU/g after brining. Similar to TVC, the coliform count as shown in (Table 1) of the smoked samples treated with 25% sodium chloride had the best output of 0.98 log CFU/g on day 0. This results is comparable with synthetic antimicrobial agents like Potassium sorbate, Citric acid and Sodium metabisulphite which microbiological properties were reported earlier (Omojowo *et. al.*, 2009a, Omojowo *et. al.*, 2009b).

Significant increases in coliform population of all samples occurred after 4 weeks of storage. Coliform count of all treated samples was less than 3.0 log CFU/g throughout the 8-week storage. In the control samples, the Coliform population of the control sample showed 5.17 log CFU/g on the 6th week while the sample was completely covered by mold on the 8th week of storage. This result was similar to that reported by Virginia, (2002) where the Coliform in the control sample showed 2.6 log CFU/g on the 4th week and the sample was completely covered by mold on the 6th week of storage hence the sample was not analyzed on the 6th week. The high coliform count recorded in this report may be due to contamination from the animal manure used in fertilizing the ponds at one time or the other. In the Staphylococcus population, the smoked sample treated 20-25% Sodium chloride reduced the Staphylococcus count to 0 and remained 0 until the end of 8th week storage (Table1). The isolation of *Staphylococcus* in smoked samples on day 0 may be attributed to post processing contamination. The population of the Fungi reduced in all the treatments and at the end of the 8-week storage time however, the control samples were high throughout the period of storage and were even completely covered by mold at the end of the 8-week storage. It is of interest to observe that in spite of the slightly reduced moisture contents (from 2nd to 6th week) in almost all the samples microbial load still increases dramatically. This

suggests that one single factor may not account for these microbial changes. Cross contamination, pH, purity of preservatives are among other factors that can influence microbial changes.

The TVC of the most of the treated samples were all below $5x10^5$ CFU/g to the 6th week which is below m in a three-class attribute plan and signifies good quality. Low levels of coliform bacteria were detected and the pathogens *Staphylococcus aureus* counts were below 10^3 in all the treated samples till the 6th week. The control however, has TVC higher than $5x10^5$ CFU/g in the second week and higher than the recommended limit 7.0 log CFU/g (ICMSF, 1986) after the 4th week. In addition the Coliform count already exceeded 10^3 even immediately after smoking. This finding is of concern as a result of the associated public health implications. For example, generally, hot smoked fish are consumed in the tropics with little or no further processing/cooking; thus, they fall into the high-risk category of foods (ICMSF, 1986). Hence there is a need for the use of appropriate percentage of choice antimicrobial agent.

Visual Observation

The actual external colour of smoked Catfish varied from dark to very dark grayish brown. There were generally, no major difference between the control and most of the treated samples except for the sample treated samples appear slightly darker than the control in this other; 25% > 20% > 15% > 10% > 5%. Generally, the external colour of the treated samples did not change during the eighth week of storage. However, in the 8th week there was profuse growth of moulds in the control.

TABLE 1: MICROBIAL LOAD OF CATFISH TREATED WITH SODIUM CHLORIDE (Log10)

	Microbial group	Control	5%	10%	15%	20%	25%
B/4 Smoking	TVC	6.60 ^{a}	5.54 ^{b}	5.50 ^{b}	5.48 ^{bc}	5.32 ^{c}	5.02 ^{d}
After „	TVC	4.59 ^{b}	4.16 ^{c}	3.48 ^{d}	2.30 ^{e}	2.21 ^{ef}	2.13 ^{f}
2nd week	TVC	6.04 ^{c}	4.58 ^{d}	3.92 ^{e}	3.45 ^{f}	3.21 ^{g}	2.02 ^{g}
4th „	TVC	6.52 ^{a}	5.02 ^{b}	5.00 ^{b}	4.21 ^{c}	4.06 ^{c}	3.00 ^{d}
6th „	TVC	7.35 ^{b}	6.07 ^{c}	5.80 ^{d}	5.52 ^{e}	5.28 ^{f}	4.11 ^{g}
8th „	TVC	Mouldy	7.05 ^{a}	6.94 ^{a}	6.77 ^{b}	6.20 ^{c}	4.60 ^{d}
B/4 smoking	Coliform	4.60 ^{a}	4.11 ^{b}	4.04 ^{b}	4.00 ^{bc}	3.84 ^{c}	3.35 ^{d}
After „	Coliform	3.54 ^{b}	1.95 ^{c}	1.80 ^{c}	1.61 ^{d}	1.02 ^{e}	0.98 ^{e}
2nd week	Coliform	4.10 ^{c}	1.97 ^{d}	1.70 ^{e}	1.71 ^{e}	1.24 ^{f}	1.12 ^{f}
4th „	Coliform	4.43 ^{a}	2.03 ^{b}	1.94 ^{bc}	1.86 ^{c}	1.59 ^{d}	1.30 ^{e}
6th „	Coliform	5.17 ^{b}	2.48 ^{c}	2.31 ^{d}	2.20 ^{de}	2.07 ^{e}	1.97 ^{e}
8th „	Coliform	Mouldy	2.91 ^{a}	2.86 ^{ab}	2.71 ^{b}	2.46 ^{c}	2.27 ^{d}
B/4 smoking	Staph.	4.55 ^{a}	4.00 ^{b}	4.00 ^{b}	3.95 ^{b}	3.88 ^{b}	3.00 ^{c}
After „	Staph.	3.17 ^{b}	1.60 ^{c}	1.70 ^{d}	0.92 ^{e}	0.0 ^{f}	0.0 ^{g}
2nd week	Staph.	5.06 ^{c}	1.47 ^{d}	1.30 ^{e}	1.10 ^{f}	0.0 ^{g}	0.0 ^{g}
4th „	Staph.	5.32 ^{d}	2.46 ^{e}	1.52 ^{f}	1.30 ^{g}	0.0 ^{h}	0.0 ^{h}
6th „	Staph.	5.52 ^{d}	3.60 ^{e}	3.20 ^{f}	1.70 ^{g}	0.0 ^{h}	0.0 ^{h}
8th „	Staph.	Mouldy	4.20 ^{a}	3.30 ^{b}	2.30 ^{c}	0.0 ^{e}	0.0 ^{e}
B/4 smoking	Fungi	4.52 ^{a}	4.21 ^{b}	4.20 ^{b}	4.17 ^{b}	3.90 ^{c}	3.62 ^{d}
After „	Fungi	3.11 ^{b}	2.00 ^{c}	1.65 ^{d}	0.60 ^{e}	0.51 ^{e}	0.48 ^{e}
2nd week	Fungi	5.28 ^{c}	2.75 ^{c}	2.70 ^{c}	1.80 ^{d}	1.54 ^{e}	1.50 ^{f}
4th „	Fungi	5.41 ^{c}	3.14 ^{c}	3.08 ^{c}	2.90 ^{d}	2.78 ^{d}	2.57 ^{e}
6th „	Fungi	5.70 ^{d}	3.56 ^{e}	3.40 ^{ef}	3.26 ^{fg}	3.12 ^{gh}	3.05 ^{h}
8th „	Fungi	Mouldy	4.13 ^{a}	4.05 ^{ab}	3.91 ^{bc}	3.80 ^{c}	3.62 ^{d}

Means in the same rows with different superscript are significantly different ($p < 0.05$).

BACTERIAL ISOLATES

All treated smoked sample were negative for *E. coli and Streptococcus sp*.
The control and the fresh fish treated samples showed the following bacteria flora *Bacillus coagulans, B. cereus, Klebsiella ozanae, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, and Streptococcus sp,* while the fungi isolated include *Penicillium verrucosum, Aspergillus niger, A. candidus, A. flavus and A. nidulan* while the smoked untreated catfish sample (control) were dominated by the following organisms *B. coagulans*, (about 70% of the isolates) while the remaining being *S. aureus*, and *Streptococcus sp*. Smoked untreated sample also showed bacteria load above except that *Streptococcus sp* was not isolated in the sample. The treated sample showed the microbial load in the following pattern; 5% and 10% Sodium chloride (salt) contains the following isolates *B. coagulans, S. aureus, K. ozanae, A. candidus, Sporendonema epizoum, and P. verrucosum* while 15% *have the isolates* of 5 and 10% *above except A. candidus* while 20-25% salt treated sample have *B. coagulans, K. ozanae and S. epizoum.*

Proximate Analysis

The proximate analysis of raw and smoked samples are presented in Figure 1-4. There were no significant ($p \geq 0.05$) differences in Protein (17.8 - 18.6%), Fat (3.9 – 4.30%), and Moisture contents (78.2 - 79.4%) of fresh samples respectively subjected to different treatments. The moisture content of the fish samples decreased sharply after the smoking. This decrease was due to loss of water during smoking (Asiedu et al., 1991). The fat content of raw fish samples increased significantly due to loss of moisture and an increase in the dry matter content per unit of weight following sample dehydration. There was an inverse relationship between the moisture and protein content in the smoked samples. There was increase in the protein contents till the 4th week and later began to decline throughout the storage period. The initial increase in protein content in smoked fish till the 4th week may be due an increase in the dry matter content per unit of weight following sample dehydration during smoking and reduction in the moisture contents during the early part of the storage before autolysis becomes pronounced. However, this result shows that storage time causes a decrease in the protein content of smoked catfish which agreed with earlier work of Ufodike and Obureke (1989) where there was decrease in crude protein of preserved *Oreochromis niloticus.* These workers attributed the decrease to hydrolysis of protein during the process of autolysis in the fish muscle. However, the treated samples show some corresponding higher value of protein more than the control especially as the concentration of the preservatives increases from 5-25%. This increase may be due to the effects of the salt preservatives effects which slow down autolysis in the fish muscles and consequently slow down the protein break down.

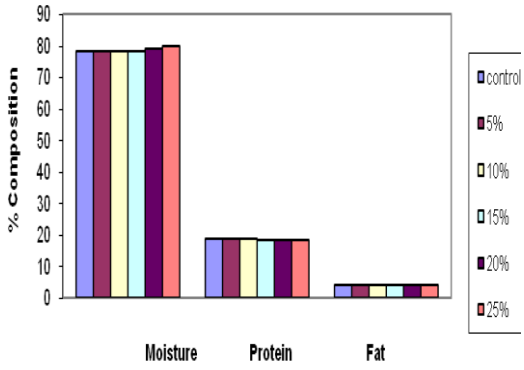

Figure 1. Proximate analysis of Fresh Catfish Treated with Sodium Chloride

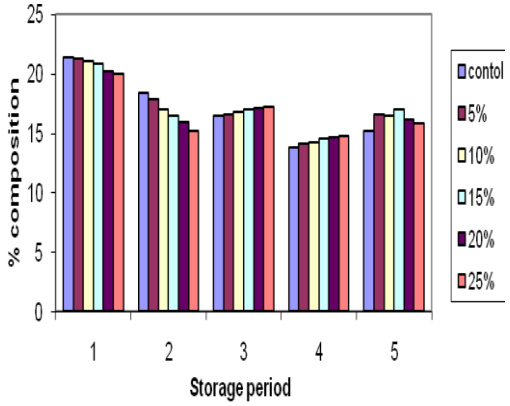

Figure 2. Moisture Contents of Smoked and Stored Catfish Treated with Sodium chloride

Note, in x-axis 1= Day 1, 2= 2$^{n\,d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

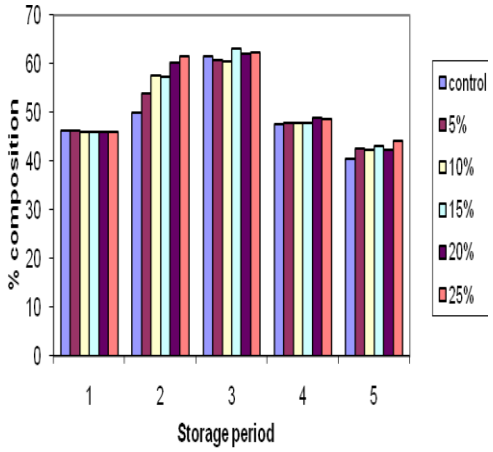

Figure 3. Protein composition of Smoked and Stored Catfish Treated with Sodium chloride

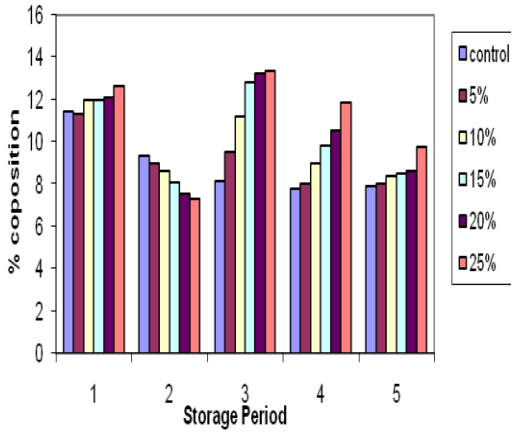

Figure 4. Fat composition of Smoked and Stored Catfish Treated with Sodium Chloride

Note, in x-axis 1= Day 1, 2= 2nd Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

ORGANOLEPTIC ASSESSMENT

The quality of the smoked fish (both treated and untreated) was evaluated immediately after smoking and after storage for 8th week on taste, flavour, texture, appearance and overall acceptability. The fish flesh overall score was given to both untreated (control) and the one of various treatment using a hedonic scale of 1- 5 fish scoring less than 2 being regarded as unacceptable. Table 2 summarizes the taste panel results. From the result, the trend of scores, for the overall acceptability of freshly smoked catfish was scored as follows: 5 > C > 10% > 15% > 20 % > 25% while on the 8th week the trend is 5% > 10% > 15%> 20 % = 25% while the control were not tasted since it was covered with mould indicated by the asterisk (**).

N.B. The panelist were made of people with no formal training in fish assessment representing the ordinary consumers outside that needs no training before deciding the acceptability of fish in the markets.

TABLE 2. ORGANOLEPTIC ATTRIBUTES OF FRESHLY SMOKED AND 8TH WEEK STORED CATFISH TREATED WITH SALT

Treatment		Taste	Flavour	Texture	Appearance	Overall-acceptability
CONTROL		**4.6**	**4.3**	**4.6**	**4.7**	**4.6**
FRESHLY SMOKED -	**5 %**	5.0	4.9	4.9	5.0	5.0
	10 %	2.9	3.4	3.6	3.7	4.0
	15 %	2.2	2.3	3.0	2.7	2.4
	20 %	1.9	1.9	1.8	1.7	1.9
	25 %	1.2	1.3	1.5	1.6	1.2
CONTROL (8TH WK)		**	**	**	**	**
8TH WEEK OLD	**5%**	4.3	4.5	4.1	4.0	4.4
	10%	4.0	3.6	3.8	3.8	3.3
	15%	2.0	3.0	2.2	2.9	2.0
	20%	1.0	1.0	1.0	1.0	1.0
	25%	1.0	1.0	1.0	1.0	1.0

CONCLUSION AND RECOMMENDATION

Though, 25% concentration of Sodium chloride (table salt) showed the greatest reduction of TVC, Fungi, and even Staphylococcus population to 0. However, organoleptic study has reveals that the samples treated with 5% and 10% Salt are preferred by the consumers. On the 8th week 5% preference is on the category of LIKE and above and thus preferred above 10% concentration. This 5% concentration was able keep the fish to ICMSF (1986) standard of good quality till the 6th week by reducing the TVC from 7.35 in the control to 6.07 log CFU/g. It also reduced the coliform in the control from 5.17 log CFU/g to 2.48 log CFU/g. Also in the staphylococcus count the reduction is from 5.52 log CFU/g in the control to 3.60 CFU/g. Also the Fungi count was reduced from 5.70 log CFU/g in the control to 3.56 log CFU/g. The Control samples were covered with moulds on the 8th week. Hence no further analysis was carried out on it. Hence, 5% Sodium chloride (Salt) may be used as a preservative in smoked fish without adversely affecting quality in terms of color and organoleptic quality for a period of 6 weeks.

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E-mail: jowosam@yahoo.com, G.S.M:08073536126

Correspondence to:
Omojowo Funso Samuel,
National Institute for freshwater Fisheries Research (NIFFR). P.M.B. 6006, New-Bussa, Niger-State, Nigeria.

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