Comparative Assessment of Potassium Sorbate and Sodium Metabisulphite on the Safety and Shelf Life of Smoked Catfish

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ABSTRACT

Forty-four sample of Catfish (*Clarias gariepinus*) were obtained from a fish pond in NIFFR divided into 11 portions of 4 each where 5 portions was treated with 1-5% Potassium sorbate respectively, the next 5 portions was treated with 1-5% Sodium metabisulphite (both are antimicrobial agents) prior to smoking and the last portion was not treated (it serve as control). They were later smoked and stored for 8-weeks at room temperature. Smoked samples were drawn after 0, 2, 4, 6, and 8 weeks for microbial, moisture contents and proximate analysis. All treated smoked samples were dominated with *Bacillus coagulans and Klebsiella ozanae* but negative for *E. coli and Streptococcus sp*. Unlike the Sodium metabisulphite 3% Potassium sorbate reduced the *Staphylococcus count* to 0 throughout the 8th week of storage. Potassium sorbate proved to be more efficient in controlling microbial quality and extending shelf life of smoked catfish.
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Key words: Potassium sorbate, sodium metabisulphite, Catfish, Quality and Safety.

INTRODUCTION

Fish is becoming increasingly important in the diet of the Nigerian as there is an increase awareness that regular red meat intake in adult above 40 years of age is not healthy. Fish constitutes 40% of animal protein intake in Nigeria at present (Olatunde, 1989). This is because fish are a cheap source of animal protein with little or no religious rejection of it, which gives it an advantage over pork or beef. Fish are a very perishable commodity, more than cattle, sheep, and poultry, and get spoiled very easily even in temperate climates. So unless it is disposed of quickly after capture, it must be preserved in some way. World fish production was estimated at 100 million tons in 1989, 15% of which was cured in one or another way. One third of the cured fish was smoked and about 20% of the smoked fish goes into international trade (Ward, 1995). Increasing consumer awareness of the nutritional value of seafood especially smoked fish has stimulated a strong demand for this product (Pigott and Tucker, 1990). To satisfy the consumer demand, it is necessary to produce good quality and safe smoked fish. Smoked fish and shellfish products can be a source of microbial hazards. Human infections may be caused by bacteria endogenous to fish. Bacterial pathogens, which may be transferred from fish to human beings include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastroenteritis), *Clostridium botulinum* type E (botulism), *Edwardsiella tarda* (diarrhea), *Leptospira interrogans* (leptospirosis), *Mycobacterium fortuitom marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *Salmonella sp*.(food poisoning), and *vibrio parahaemolyticus* (food poisoning) (Austin and Austin, 1989). Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and chemical preservatives like sorbates and sulphites. Sorbates are the most effective preservatives against a wide spectrum of food spoilage microorganisms; they include sorbic acid and potassium sorbate. They are among the safest, most efficient and versatile preservatives used in the food industry today. Sorbates are tasteless and odourless. Because they are non-toxic, they are used in a wide variety of foods, including cheese, yogurt, sour cream, bread, cakes, baking mixes, icing, beverages, margarine, fermented vegetables, fruit products, salad dressing, smoked and salted fish and mayonnaise. The antimicrobial activity of sorbates against molds, bacteria and fungi has been reported by researchers Sofos and Busta, 1993; Sofos, 2000). Also sulphites may be used as potassium bisulphite, sodium or potassium metabisulphite, sodium sulphite or sulphur dioxide on food. They are often used as preservatives in wines (to prevent spoilage and oxidation), dried fruits and dried potato products.

Sulphites also occur naturally in almost all wines. Sodium metabisulphite have been used in the preservation of fresh and frozen crustacean up to 150mg/kg in edible parts. (US FDA, 1978).

Considering the antimicrobial activity of Sorbates and Sodium metabisulphite this study was carried out to determine the microbial, organoleptic and nutritional quality changes of smoked catfish and to evaluate the effect of these antimicrobial agents at different concentration on the quality of smoked catfish during storage at room temperature since there are scanty information on this looking at smoked fish in Nigeria.

MATERIAL AND METHODS

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 11 groups of 4 fish for each of the Catfish subjected to treatments. The treatments were as follows; (1) control (untreated samples); (2, 3, 4, 5 and 6) are treated with 1, 2, 3, 4 and 5% Potassium sorbate and 7, 8, 9, 10 and 11 are treated with 1, 2, 3, 4 and 5% Sodium metabisulphite for 5 minutes, A sample from each group were separated from each treatment and smoked. Smoking was done according to the methods 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 Analysis

A 25g representative sample (excluding the head and tail) of each fish sample was obtained aseptically to prepare serial dilution using 0.1% peptone water as diluents. Total bacteria counts and coliform counts were determined according to the method of Sneath *et. al*.(1986). *Faecal streptococci* and *E. coli* in samples were determined employing the methods described by speak (1984). *Staphylococcus aureus* counts in samples were determined by employing the method of Bennett (1984). 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

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 range of specified microbiological limits recommended by ICMSF (1986) for fish and fishery products is as follows: for the TPC, the maximum recommended bacterial counts for good quality products (m) is $5x10^5$ (5.7 log10 CFU/g) and the maximum recommended bacterial counts for marginally acceptable quality products (M) is 10^7 (7 log10 CFU/g). For *E. coli*, the m value is 11 (1.0 $\log^{10}$ CFU/g) and the M value is 500 (2.7 log10 CFU/g), and for *Staphylococcus*, m value is 10^3 (3 log10 CFU/g) (ICMSF, 1986). For all fish, the *Staphylococcus aureus* safety level is equal to or greater than 10^4 /g. In many cases, these levels represent the point at or above which the agency will take legal action to remove products from the market (FDA, 2001, Fish and Fishery Products Hazards & Controls Guidance manual).

Total Viable count (TVC), Coliform, Staphylococci and Fungi count in log CFU/g of fresh and smoked Catfish samples are shown in Tables 1 and 2. TVC of the fresh the control catfish was 6.60 log CFU/g but after the sample were subjected to treatments with 1-5% Sodium metabisulphite and 1-5% Potassium sorbate the TVC, Coliform, Staphylococcus and fungi count were reduced however, the reduction was higher in the treatment with Potassium sorbate also as the concentration is increases..

Smoking sharply reduced the total viable count (Table 1 and 2) in all samples, but the sample treated with 5% Potassium sorbate showed the greatest reduction and maintained a low level throughout 8 weeks of storage, especially on day 0 2.13 log CFU/g as shown in Table 2 while after 8-week storage the TVC was 4.60 log CFU/g. The TVC of the control samples were the highest throughout the period of storage where the sample were completely covered by mold after the 6^{th} week of storage; therefore, no further microbial analysis was conducted. The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the lowest microbial load and maximum shelf stability. Similar to TVC, the coliform count (of the smoked samples treated with 5% Potassium sorbate had the highest reduction of 0.93 log CFU/g on day 0 and remain the lowest of the treatments throughout the period of storage. 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 except for the sample treated with 1-2% Sodium metabisulphite which were above 3.0 log CFU/g in the eighth-week.

Table 1: Microbial Load of Catfish Treated With Sodium Metabisulphite (Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
B/4 Smoking	TVC	6.60 ^{a}	5.95 ^{b}	5.48 ^{c}	5.46 ^{c}	5.24 ^{d}	5.10 ^{d}
After „	TVC	4.59 ^{b}	4.10 ^{cd}	4.16 ^{cd}	4.21 ^{d}	4.12 ^{cd}	4.01 ^{c}
2nd week	TVC	6.04 ^{c}	4.48 ^{c}	4.56 ^{cd}	4.70 ^{d}	4.68 ^{d}	4.21 ^{e}
4th „	TVC	6.52 ^{a}	5.20 ^{b}	5.17 ^{b}	5.11 ^{b}	5.06 ^{b}	5.00 ^{b}
6th „	TVC	7.35 ^{b}	6.69 ^{c}	6.68 ^{c}	6.60 ^{c}	6.51 ^{c}	6.32 ^{d}
8th „	TVC	Mouldy	7.79 ^{b}	7.66 ^{bc}	7.61 ^{c}	7.63 ^{c}	7.67 ^{bc}
B/4 smoking	Coliform	4.60 ^{a}	4.46 ^{b}	4.44 ^{b}	4.40 ^{b}	4.43 ^{b}	4.39 ^{b}
After „	Coliform	3.54 ^{b}	2.24 ^{c}	2.19 ^{c}	2.20 ^{c}	2.18 ^{cd}	2.06 ^{d}
2nd week	Coliform	4.10 ^{c}	2.55 ^{d}	2.43 ^{d}	2.20 ^{e}	2.04 ^{f}	2.10 ^{ef}
4th „	Coliform	4.43 ^{a}	2.60 ^{b}	2.48 ^{b}	2.30 ^{c}	2.33 ^{bc}	2.28 ^{c}
6th „	Coliform	5.17 ^{b}	2.98 ^{c}	2.84 ^{cd}	2.76 ^{d}	2.76 ^{d}	2.59 ^{d}
8th „	Coliform	Mouldy	3.51 ^{b}	3.50 ^{b}	3.47 ^{b}	3.39 ^{bc}	3.22 ^{c}
B/4 smoking	Staph.	4.55 ^{a}	4.55 ^{b}	4.26 ^{c}	4.31 ^{c}	4.34 ^{c}	4.20 ^{c}
After „	Staph.	3.17 ^{b}	2.10 ^{c}	1.80 ^{d}	1.25 ^{e}	0.38 ^{f}	0.0 ^{f}
2nd week	Staph.	5.06 ^{c}	1.75 ^{d}	1.68 ^{d}	1.60 ^{d}	0.41 ^{e}	0.0 ^{f}
4th „	Staph.	5.32 ^{c}	1.95 ^{d}	1.73 ^{e}	1.48 ^{f}	0.80 ^{g}	0.0 ^{h}
6th „	Staph.	5.52 ^{c}	2.70 ^{d}	2.49 ^{e}	2.03 ^{f}	1.10 ^{g}	0.0 ^{h}
8th „	Staph.	Mouldy	3.73 ^{a}	3.56 ^{b}	2.74 ^{c}	1.06 ^{d}	0.0 ^{e}
B/4 smoking	Fungi	4.52 ^{a}	4.00 ^{b}	3.92 ^{b}	3.55 ^{c}	3.46 ^{c}	3.30 ^{d}
After „	Fungi	3.11 ^{b}	2.04 ^{c}	2.10 ^{cd}	2.15 ^{cd}	2.20 ^{d}	2.18 ^{d}
2nd week	Fungi	5.28 ^{c}	3.21 ^{d}	3.19 ^{d}	3.19 ^{d}	3.14 ^{de}	3.05 ^{e}
4th „	Fungi	5.41 ^{c}	3.73 ^{d}	4.00 ^{e}	3.70 ^{d}	3.65 ^{de}	3.54 ^{e}
6th „	Fungi	5.70 ^{a}	4.43 ^{b}	4.36 ^{bc}	4.24 ^{c}	4.18 ^{cd}	4.04 ^{d}
8th „	Fungi	Mouldy	6.10 ^{a}	6.09 ^{a}	5.96 ^{ab}	5.88 ^{b}	5.80 ^{b}

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

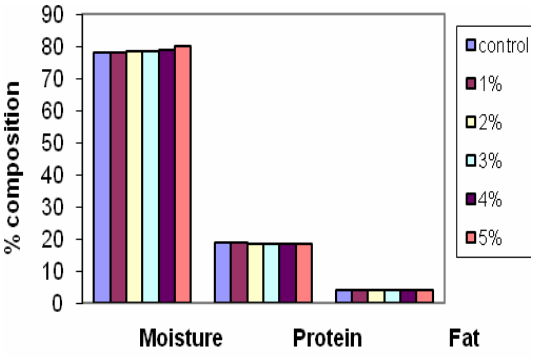

Figure1. Proximate Composition of Fresh Catfish Treated with Sodium metabisulphite

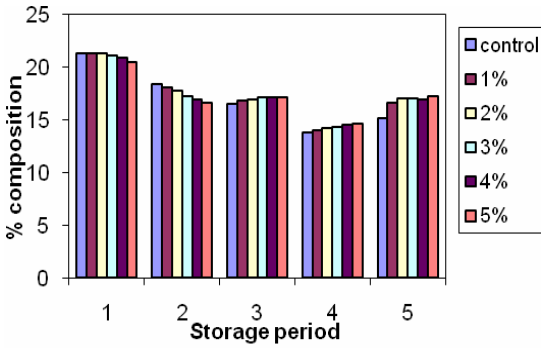

Figure 2. Moisture contents of Smoked Catfish Preserved with Sodium metabisulphite

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

Table 2: Microbial Load Of Catfish Treated With Potassium Sorbate (Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
B/4 Smoking	**TVC**	6.60 [a]	5.48 [b]	5.46 [b]	5.42 [b]	5.12 [d]	5.07 [e]
After „	**TVC**	4.59 [b]	3.61 [c]	3.50 [c]	3.47 [c]	3.10 [d]	2.04 [e]
2nd week	**TVC**	6.04 [c]	4.14 [d]	4.06 [d]	3.98 [d]	3.65 [e]	2.72 [f]
4th „	**TVC**	6.52 [a]	5.00 [b]	5.01 [b]	4.84 [c]	4.30 [d]	3.43 [e]
6th „	**TVC**	7.35 [b]	5.71 [c]	5.68 [c]	5.50 [d]	4.71 [e]	3.90 [f]
8th „	**TVC**	Mouldy	6.72 [b]	6.64 [b]	6.35 [c]	6.21 [c]	4.54 [d]
B/4 smoking	**Coliform**	4.60 [a]	3.95 [b]	3.76 [c]	3.74 [cd]	3.61 [cd]	3.58 [d]
After „	**Coliform**	3.54 [b]	1.55 [c]	1.40 [cd]	1.32 [d]	1.24 [d]	0.93 [e]
2nd week	**Coliform**	4.10 [b]	1.72 [cd]	1.88 [d]	1.61 [c]	1.55 [c]	1.10 [e]
4th „	**Coliform**	4.43 [c]	2.08 [d]	2.00 [de]	1.76 [ef]	1.62 [f]	1.27 [g]
6th „	**Coliform**	5.17 [a]	2.50 [b]	2.42 [b]	2.23 [c]	2.11 [c]	1.92 [d]
8th „	**Coliform**	Mouldy	2.81 [b]	2.42 [c]	2.54 [c]	2.50 [c]	2.20 [d]
B/4 smoking	**Staph.**	4.55 [a]	3.88 [b]	3.74 [bc]	3.71 [c]	3.74 [bc]	3.65 [c]
After „	**Staph.**	3.17 [b]	0.40 [c]	0.32 [c]	0.0 [d]	0.0 [d]	0.0 [d]
2nd week	**Staph.**	5.06 [a]	0.60 [b]	0.45 [b]	0.0 [c]	0.0 [c]	0.0 [c]
4th „	**Staph.**	5.32 [b]	1.0 [c]	0.84 [c]	0.0 [d]	0.0 [d]	0.0 [d]
6th „	**Staph.**	5.52 [c]	1.60 [d]	1.25 [d]	0.0 [e]	0.0 [e]	0.0 [e]
8th „	**Staph.**	Mouldy	2.10 [a]	1.80 [b]	0.0 [a]	0.0 [a]	0.0 [a]
B/4 smoking	**Fungi**	4.52 [a]	4.12 [b]	4.02 [b]	4.03 [b]	3.71 [c]	3.28 [d]
After „	**Fungi**	3.11 [b]	1.21 [c]	1.22 [c]	1.05 [d]	0.46 [e]	0.0 [f]
2nd week	**Fungi**	5.28 [c]	1.73 [d]	1.84 [d]	1.55 [e]	0.54 [f]	0.0 [g]
4th „	**Fungi**	5.41 [c]	2.59 [d]	2.61 [d]	1.92 [e]	0.62 [f]	0.0 [g]
6th „	**Fungi**	5.70 [d]	3.36 [e]	3.25 [ef]	2.14 [f]	1.26 [g]	0.22 [h]
8th „	**Fungi**	Mouldy	3.78 [a]	3.61 [b]	2.57 [c]	1.42 [d]	0.36 [e]

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

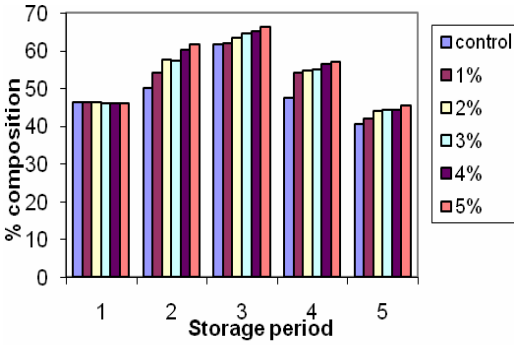

Figure 3. Protein composition of Smoked Catfish Preserved with Sodium metabisulphite

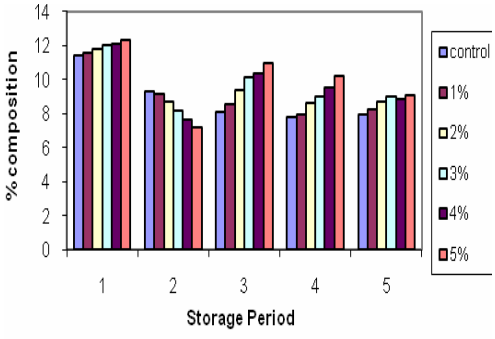

Figure 4. Fat composition of Smoked Catfish Preserved with Citric Acid

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

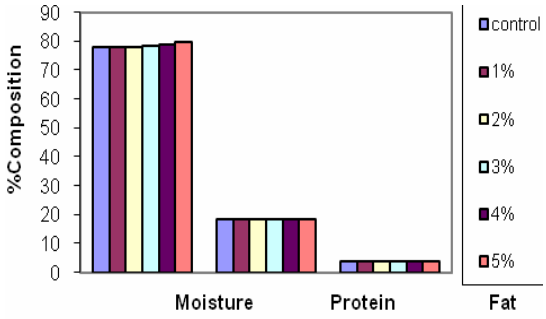

Figure 5. Proximate Analysis of Fresh Catfish Treated with Potassium sorbate

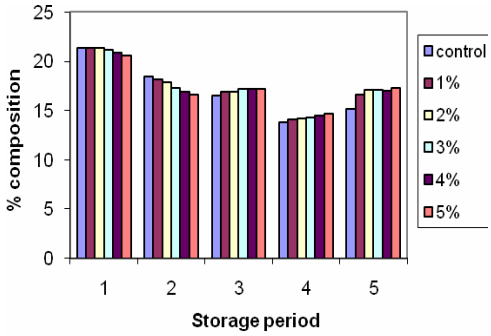

Figure 6. Moisture Contents of Smoked Catfish Preserved with Potassium sorbate

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

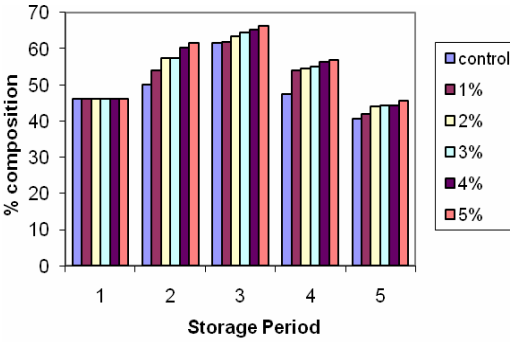

Figure 7. Protein Composition of Smoked Catfish Preserved with Potassium sorbate

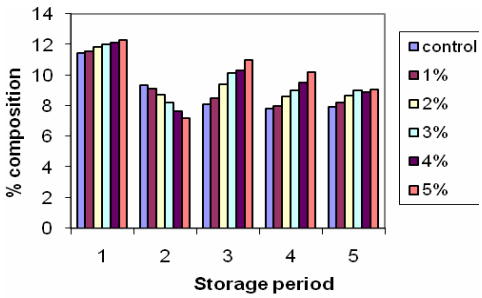

Figure 8. Fat composition of Smoked Catfish Preserved with Potassium sorbate

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

In the control samples, the Coliform population was 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. 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. Furthermore, the smoked sample treated with 3-5% Potassium sorbate had no Staphylococcus count throughout the period of storage while only 5% Sodium metabisulphite was able to reduce the Staphylococcus count to 0 and remained 0 until the end of 8th week storage. Generally, Potassium sorbate showed the lowest count throughout the 8th week of storage.

The isolation of *Staphylococcus* in smoked samples on day 0 may be attributed to post processing contamination. However, *Staphylococcus* was killed by the treatments 3-5% Potassium sorbate. Fungi counts were also reduced in all the treatments and at the end of the 8-week storage time; however, the sample treated with 5% Potassium sorbate showed 0 counts till the 4th and 6th weeks of storage. The control samples were high throughout the period of storage and the sample was even completely covered by mould at the end of the 8-week storage. This result were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the minimum fungal load during storage and presence of profuse mould growth after day 8 in the control.

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 bacterial contamination of hot smoked fish just out of the smokehouse is usually below 10^3 per gram (Doe, 1998). 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 *S. aureus* counts were below 10^3 in all the treated samples till the 8th week except samples treated with (1-2% Sodium metabisulphite). 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, 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.

BACTERIAL ISOLATES

All treated smoked sample were negative for *E. coli and Streptococcus sp*. However, 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 sample (control) were dominated by the following organisms *B. coagulans,* (about 70% of the isolates) while the remaining being *S. aureus,* and *Streptococcus sp*. The treated sample showed the microbial load in the following pattern; 1% and 2% potassium sorbate of the fish samples contains the following spp *B. coagulans, S. aureus, K. ozanae, A. candidus and A. nidulan* while in 3% and 4% potassium sorbate treated samples have the following isolates *B. coagulans, K. ozanae and A. nidulan while* 5% treatment have only *B. coagulans.* While 1-4% Sodium metabisulphite treated samples *have* following isolates *B. coagulans, S. aureus, , A. candidus, A. nidulan and A. flavus while* 5% treated sample have all except *S. aureus and A. flavus.*

Proximate Analysis

The proximate analysis of raw and Smoked catfish are presented in Figure 1 to 4 There were no significant ($p \leq 0.05$) differences in Protein (18.3 – 20.2% and 17.8 - 18.6%), Fat (2.6 – 3.0% and 3.9 – 4.30%), and Moisture contents (73.4 - 77.0% and 78.2 - 79.4%) of the samples subjected to different treatments. The moisture content of fresh sample was 78.2%. In the treatments the moisture contents ranged from 78.2 - 79.4%. Moisture content of catfish decreased sharply after the smoking process and this decrease was due to loss of water during smoking (Asiedu et al., 1991). Also the study reveals that the average protein content increases after smoking, and increases till the 4th week and later decreases till the end of the 8th week of storage. There was an inverse relationship between the moisture and protein content in the smoked samples. The initial increase in protein content in smoked fish and 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.

These results 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 1-5%. This increase may be due to the effects of the preservatives which slow down autolysis in the fish muscles and consequently slow down the protein break down.

CONCLUSION AND RECOMMENDATION

This study has reveals that the samples treated with Potassium sorbate before smoking showed the greatest reduction and maintained a low level throughout the 8th weeks of storage. Hence, Potassium sorbate can be used as a choice preservative in smoked catfish without adversely affecting quality in terms of lipid oxidation, color, microbial and nutritional quality. The use of 3% Potassium sorbate as a choice antimicrobial agent is hereby recommended since it has been found to keep smoked fish in wholesome state for 8th week, reducing the TVC to 6.35 log CFU/g, the Coliform to 2.64 log CFU/g, Staphylococcus count to 0.0s and Fungi to 2.57 log CFU/g at the end of 8th week storage. This will ensure prolonged shelf life and safe consumption of smoked fish of ICMSF standard of smoked fish quality.

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