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Effect of preservatives on microbial safety and quality of smoked catfish (*Clarias gariepinus*) during ambient storage

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ABSTRACT
The microbial safety and quality of smoked catfish (Clarias gariepinus) treated with antimicrobials and antioxidants were examined during six weeks storage at temperature (30-33)^{0}C. Eleven pre-smoking treatments were applied: 25% sodium chloride (NaCl) and 1% ascorbic acid for 1h; 25% NaCl and 1% ascorbic acid for 30 min; 3% sodium lactate for 30min 3% sodium lactate and ginger (Zingiber officinale) extract for 30min; 5% sorbic acid for 30min; 5% sorbic acid for 1h; 3% sodium lactate and aidan (Tetrapleura tetraptera) extract for 30min; 3% sodium lactate and Ethiopian pepper (Xylopia aethiopica) extract for 30min; ginger (Zingiber officinale) extract for 30min; Ethiopian pepper (Xylopia aethiopica) extract for 30min and aidan (Tetrapleura tetraptera) extract for 30min. The samples were smoked, cooled and packed for analysis at 0, 2, 4 and 6 weeks of ambient storage. No Salmonella and coliforms were recovered from the smoked samples and there was significant reductions in total plate count in treated and control samples after cooking and smoking. The microbial counts results were, however, within the ICMSF limits, for acceptable microbial quality. There were no significant changes of pH and peroxide values (PV) fluctuated within samples during the 6 weeks storage without refrigeration.

Keywords: *African mud catfish, preservatives, microbial safety, smoking, storage, physicochemical.*

INTRODUCTION

Catfish including African mud catfish (*Clarias gariepinus*), *Heterobranchus longifilis*, and *Heterobranchus bidorsalis* are highly nutritious food commodities with wide consumer acceptance. In Nigeria, catfish accounts for about 80% of aquaculture production. However, catfish like any other fish species, could result in significant economic loss due to its perishable nature, if adequate preservation techniques are not adopted (Clucas and Ward**,** 1996).

Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf life of fish in general including freezing, chemical preservation, salting, and smoking (Nickelson *et al*., 2001). Up to 70% of the total fish catch in developing countries is preserved by smoking (Clucas and Ward, 1996). Smoking usually extends the shelf life of fish due to the reduced moisture content and effects of imparted phenolic compounds (Efiuvwevwere and Ajiboye, 1996). In addition, during hot smoking, high heat results in direct microbial destruction (Nickelson *et al*., 2001). Another shelf life-promoting strategy involves salting with sodium chloride or curing with chemical preservatives (Ravishankar and Juneja, 2000).Common food preservatives used for catfish include antibacterial and antifungal agents such as lactic acid (Fernandes *et al*., 1998), sodium benzoate (Efiuvwevwere and Ajiboye, 1996), sodium lactate and sorbic acid and antioxidants such as ascorbic acid to slow down lipid oxidation (Antonia da Silva *et al*., 2008). Despite various types of smoking processes and added preservatives, subsequent microbial population change and storage stability were also determined by fish type, the quality of fish at smoking and post-smoking storage conditions. Microbial flora distribution in smoked fish products

varies largely, depending on the quality of fish at the time of smoking, the smoking temperature and duration, the salt content , and the drying time (Nickelson *et al*., 2001). For hard-smoked products with high heat input, relatively heat-stable organisms such as *Bacillus, Micrococcus* and Yeasts predominate. Additionally, microbial pathogens such as *Listeria monocytogenes*, *Salmonella*, and *Clostridium botulinum* type E may present safety hazards in smoked fish products (Heinitz and Johnson, 1998)

The impact of chemical preservatives, and different storage times at room temperature on the microbial quality, pH and peroxide values of the smoked catfish (*Clarias gariepinus*) has not been reported. This study was embarked upon to give information on the effects of some natural and synthetic preservatives on the changes in microbial population and quality of smoked African mud catfish during 6-week ambient storage. It would also be a guide in designing HACCP for smoked African mud catfish.

MATERIALS AND METHODS

Materials

Fresh catfish (*Clarias gariepinus*) weighing 350 - 500g were collected from a private catfish processing fish farm (Azemor Agribiz Ltd) in Oyo state, Nigeria. All preservatives used were food grade and were purchased from local markets

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Pre-smoking sample treatments

The samples were stunned using 160g of salt to 40kg of fish, dressed and randomly divided into twelve groups. Except for control (T_{12}), the other eleven of the groups were subjected to the following soaking treatments using preservative solutions: 25% sodium chloride (NaCl) and 1% ascorbic acid for 1h (T_1); 25% NaCl and 1% ascorbic acid for 30min (T_2); 3% sodium lactate for 30min (T_3); 3% sodium lactate and ginger extract for 30min (T_4); 5% sorbic acid for 30min (T_5); 5% sorbic acid for 1h (T_6); 3% sodium lactate and aidan (*Tetrapleura tetraptera*) extract for 30min (T_7); 3% sodium lactate and Ethiopian pepper extract for 30min (T_8); ginger extract for 30min (T_9); Ethiopian pepper extract for 30min (T_{10}); aidan extract for 30min (T_{11}).

Smoking and storage

After soaking treatments, all the catfish belonging to both treatment and control groups were loaded into the smoking kiln and slowly cooked for about 5h with charcoal at 160^{o}C chamber temperature. After cooking, the fire was extinguished and the fish left in the chamber overnight. The following morning, charcoal was added and smoking continued at a temperature of 180^{o}C chamber temperature for 8h.

After smoking and cooling, all catfish of each treatment group and control were packed in polythene bags, sealed and kept in paper boxes at ambient (30-33^{o}C) temperature. Samples were subjected to microbial, pH and PV analyses on 0, 2, 4 and 6 weeks of storage.

Microbial analysis

A swab of the skin and gut of the freshly treated catfish samples were taken with sterile swab stick and 1g representative sample was obtained aseptically from the loin muscle of the smoked catfish samples. The samples were grounded and serial dilutions (10^{-1} -10^{-4}) of the homogenized samples were made using sterile distilled water.

Total Plate Count (TPC)

This was done using the pour plate method of Harrigan and McCance (1976). One millilitre of the serially diluted samples was taken in duplicates and plate count agar was poured at 40^{o}C on the plates. The samples and the medium were properly mixed, allowed to set and incubated at 35^{o}C for 24h. The number of colonies on the plates was counted.

Staphyloccoccus sp. Count

Manitol salt agar was used to enumerate the number of *Staphylococcus* colonies. The plates were incubated at 35^{o}C for 24h and bright yellow colonies were counted. The bright yellow colonies were sub-cultured for 24h and coagulase test was carried out to confirm the presence of *Staphylococcus aureus*.

Coagulase Test

A small amount of the colony was introduced into blood plasma, formation of clots or coagulation of plasma showed the presence of *Staphylococcus aureus*.

Salmonella count

Samples for detection of salmonella were plated out on Salmonella-Shigella Agar. The plates were incubated at 35^{o}C for 24h. Black colonies showed the presence of *Salmonella sp*.

***Escherichia coli* count**
This was done using Eosine Methylene Blue Agar at 35°C for 24h. Colonies with green metallic sheen were counted as *E. coli*

Coliform count
This was also done using Eosine Methylene Blue Agar at 35°C for 24-48h. The samples were first inoculated into lactose broth for 48h. The production of gas in the Durham's tubes showed the presence of coliforms. The samples showing gas production were plated out and counted.

Yeast and mould Counts.
This was done by plating out serially diluted samples on Potato Dextrose Agar (PDA) at room temperature (30- 35°C) for 48-72h.

Determination of pH
This was determined in duplicate using a digital pH/temperature meter. Ten grams (10g) of fresh sample was blended with 30ml of distilled water while 5g of smoked sample was blended with 30ml of distilled water for 1min. The homogenate was allowed to stay for 5mins and the readings were taken.

Determination of Peroxide value (PV)
The PV was determined in duplicate using titrimetric method. 30 ml glacial acetic acid-chloroform solution (3:2 v/v) was added to 5g of extracted fat and swirled. Excess potassium iodide (0.5ml of KI solution) was added to react with the peroxides and iodine was liberated. After 1 min., 30 ml of H_2O was added. Then the solution was titrated with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) using 0.5ml of 1% starch as indicator, until the blue color disappeared. The peroxide value was calculated by multiplying ml of $Na_2S_2O_3$ 33 by normality and by 1000 then divided by grams of catfish sample (AOAC, 1995).

Statistical analysis
The statistical analysis was done by using mean, correlation coefficient equation, chi-square and t-tests.

RESULTS AND DISCUSSION
This study assessed the effects of smoking and preservatives on microbial load, pH and PV attributes of African mud catfish (*Clarias gariepinus*), during six-week ambient storage. The smoking was able to reduce the total plate count of day 0 smoked samples The total plate count for fresh samples ranged between 52 x 10^2 CFU/g to 150 x 10^3 CFU/g and 4 x10^2 CFU/g to 146 x 10^2 CFU/g for day 0 smoked samples. *Salmonella*, the pathogenic microorganism examined, for fresh was 1 x 10^2 CFU/g and found only in T_7. The smoking process was sufficient to destroy this organism in this study as found in previous study (Antonia da Silva *et al*, 2008). The low level of contamination in fresh and the lack of post-processing contamination as shown in Table 1 indicated general good sanitary conditions of the plant. A combination of preservative treatment and smoking resulted in variations of microbial levels. No coliforms and *E. coli* were found in any of the samples. The pathogenic organisms, *Staphylococcus aureus* counts were below 1x10^2 and *Salmonella* were not isolated from any of the smoked samples. No clear relationships were ascertained for duration of soaking treatments and the use of preservatives alone or in combination with others. Nonetheless, the microbial populations for all the smoked catfish observed in this study *E. coli*, *Salmonella*, *Staphylococcus* and Coliform, were within the recommended limits for good quality fish product according to ICSMF, (1986). All pH values in the catfish samples were between 6.0 and 7.0, a level not inhibitory to microbial growth as presented in Fig.1. The pH is an important factor that affects microbial growth and spoilage of foods (Jay, 1998), and may help explain the observed differences in the effects of antimicrobial and antioxidant treatments on microbial population. The initial PV of the control and treated fresh samples ranged from 1.90 to 6.50 milliequivalents (mEq) peroxide/kg fats. The PV of the smoked samples ranged between 1.10 and 13.0 mEq peroxide/kg fat, the lowest value was observed in sample treated with sodium lactate and ginger extract and highest in the sample treated with sodium lactate and Aidan extract. PV fluctuated during the six weeks storage as seen in Fig.2. A similar result was observed by (Antonia da silva *et al*, 2008). Hence, due to the fluctuations observed between and within treatment groups for PV in the study, the parameter provided limited value in predicting the microbial safety and quality of smoked African mud catfish during storage.Understanding the microbial and physicochemical attributes of the African mud catfish products helps to establish a generic Hazard Analysis and Critical Control Points (HACCP) plan to be used for this species of catfish. By evaluating the presence or absence of target food-borne pathogens, microbial population loads, and physicochemical attributes of the smoked catfish

throughout process and storage, important parameters could be used to establish critical control points.

TABLE1: MICROBIAL POPULATIONS IN FRESH CATFISH (*CLARIAS GARIEPINUS*)

Microbial populations	Mean value of microorganisms CFU/g											
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
TPC	55x103	19x103	90x102	87x103	45x103	52x102	50x103	91x103	150x103	30x103	15x103	11x103
Salmonella	ND	ND	ND	ND	ND	ND	1x 102	ND	ND	ND	ND	ND
Staphylococcus	ND	3x 102	2x 102	9x 102	2x 102	2x 102	ND	ND	ND	ND	ND	ND
Coliforms	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast and mould	1x 101	1x 101	1x 101	1x 101	ND	1x 101	3x 101	6x 101	4x 101	7x 101	3x 101	5x 101

T_1 = Samples treated with 25% sodium chloride (NaCl) and 1% ascorbic acid for 1h
T_2 = Samples treated with 25% NaCl and 1% ascorbic acid for 30min
T_3 = Samples treated with 3% sodium lactate for 30min
T_4 = Samples treated with 3% sodium lactate and ginger (*Zingiber officinale*) extract for 30min
T_5 = Samples treated with 5% sorbic acid for 30min
T_6 = Samples treated with 5% sorbic acid for 1h
T_7 = Samples treated with 3% sodium lactate and Aidan (*Tetrapleura tetraptera*) extract for 30min
T_8 = Samples treated with 3% sodium lactate and Ethiopian pepper (*Xylopia aethiopica*) extract for 30min
T_9 = Samples treated with Ginger extract for 30min
T_{10} = Samples treated with Ethiopian pepper extract for 30min
T_{11} = Samples treated with Aidan extract for 30min
T_{12} = Control

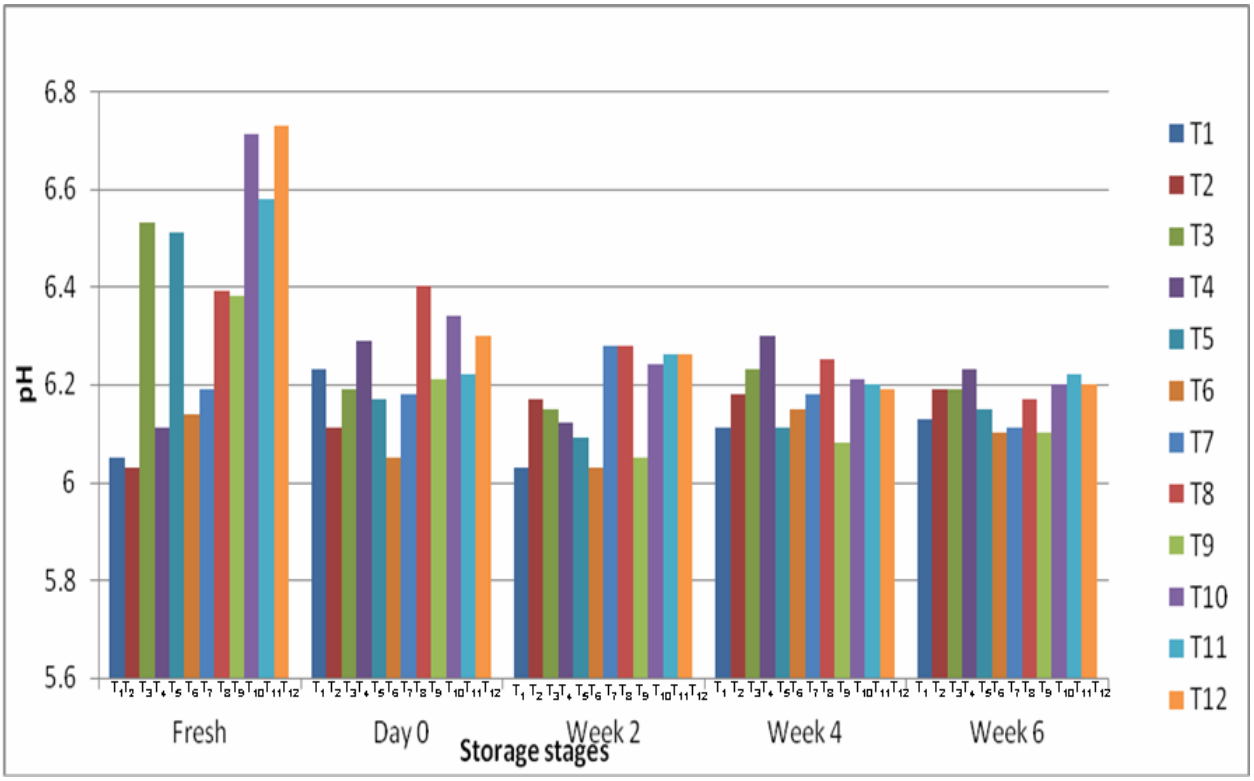

Fig 1:Changes in pHof fresh and smoked catfish during storage as affected by preservation treatments

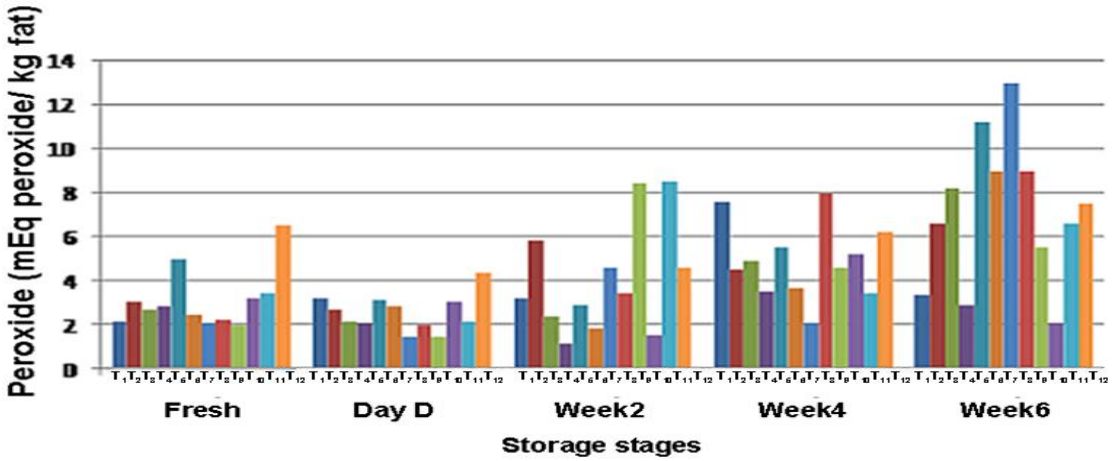

Fig 2: Changes in PV of fresh and smoked catfish during storage as affected by preservative treatments

CONCLUSION

This study demonstrated the effectiveness of cooking and smoking in controlling microbial population and pathogens in African mud catfish and peroxide value provide limited value in predicting the microbial safety and quality of smoked catfish during 6 weeks ambient storage.

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