The Effect of Different Processing Methods on the Nutritional Quality and Microbiological Status of Cat Fish (Clarias lezera)

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Abstract

This study assessed the effect of sodium citrate and black pepper (Piper guineense) on chemical, microbial and sensory characteristics of smoked catfish slices during six 6 week storage at ambient temperature. The fresh catfish were processed, soaked in the warm (45 ± 10°C) spice extracts for 10 minutes, drained and smoke dried. It was thereafter subjected to the following treatments: 1% Sodium citrate (B) 1% Black pepper (C) 1% Sodium citrate +1% Black pepper (D) while the control (A) sample was smoke-dried without soaking in any solution. The samples were analyzed using standard methods. Results of the proximate analysis of sample after 6 weeks storage showed the following; moisture content ranged from 10.12-19.42% at day 0 and 13.54-17.87%; protein content ranged from 60.52 to 69.30% and 63.68-69.13%; fat content ranged from 14.24 to 16.66% and 12.05-15.00%; ash content ranged from 3.42 to 5.48% and 3.71-5.95%. There was significant (p>0.05) reduction in the Peroxide Value (PV) and Thiobarbituric acid (TBA) values in comparison with the control. The samples total plate count ranged from 3.24 to 3,88 log10 Colony Forming Units (CFU)/g at day zero and increased to 6.24 log10 CFU/g. The result of general acceptability, however, shows that sample D was most acceptable. Using sodium citrate and black pepper singly and in combination have a potent antioxidant and antimicrobial effect more than smoking.

Keywords: Cat-fish sodium citrate; Black pepper; Microbial; Sensory characteristics

Introduction

Fish provides between 30% and 80% of the total animal protein intake of the coastal people of West Africa [1]. The amino composition of fish compared favourably well with egg, milk and meat. Catfish contain high amounts of unsaturated fatty acids, vitamins, proteins, minerals, and little or no saturated fat, and low in carbohydrates [2]. Catfish with about 1,250 species cultured by farmers are raised in freshwater. It is easily perishable and it’s loss in quality occurs rapidly after catch. Smoke-dried fish is an important ingredient in the Nigerian traditional diet and is relished for its appetizing taste and flavour [3]. The smoke produce from whole catfish and fillets, include:

- Production of volatile organic components like formaldehyde and phenols. Its heat effect resulting in reduced water activity of the fish allow better preservation and therefore increase fish availability to consumers [4].
- Organic salts of sodium acetate, lactate and citrate were shown to possess antibacterial activity against various food-borne pathogens like Staphylococcus aureus, Yersinia enterocolitica and Escherichia coli, Listeria monocytogenes and Clostridium botulinum, and to inhibit toxin production of Clostridium botulinum [5]. Pathogenic bacteria identified from whole catfish and fillets, include: Aeromonas sp., C. freundii, E. coli, H. alvei, K. pneumoniae, Listeria sp., P. shigellos,W. Proteus sp., S. aureus, and Vibrio sp. Infections from Salmonella's paratyphi B and Listeria monocytogenes infections had been associated with consumption of smoked fish [6].

The use of black pepper in combination with preservatives like sodium citrate can inhibit microbial growth in smoked fish during storage depending on the raw material, type of smoking, relative humidity, velocity, temperature, density, and composition of the smoke, and the time of smoking [7]. Also, lipids oxidation contributes to catfish spoilage by influencing the color, texture, nutrition, and safety, as well as the flavour of smoked fish.

Efiuvwewere BJO [8] reported that after subjected catfish to smoke Gram-positive bacteria like Bacillus, Staphylococcus and Streptococcus bacteria and spoilage molds Penicillium verrucosum, Aspergillus flavus and Achlya spp were the dominant microorganisms; while unsmoked fish samples treated with different concentrations of sodium benzoate had been reported to have reduced total viable count of Enterobacter, Escherichia, Serratia, Bacillus, Staphylococcus, Streptococcus, Penicillium, Aspergillus, and Achlya genera.

Food spoilage means alteration of the original nutritional value taste and flavour making it harmful to and unsuitable for consumption [9]. Among the factors which contribute to the spoilable are: degradation of protein, development of oxidative rancidity, vitamin degradation, enzymatic reaction, the action of microorganisms and most importantly water activity [10]. The rate of microbial spoilage depends upon the number of microorganisms present on the fish and the temperature at which the fish is kept. Rate of spoilage varies depending on the species.

Recently, many researchers have evaluated the spoilage of seafood in general and fish in particular [11]. The effects of different processing and cooking methods on nutritional composition of different species of fish have been studied [12,13].

Different processing and drying methods have different effects on nutritional compositions of fish. The effects could be chemical and physical changes increase digestibility due to protein denaturation and reduction in the content of the mobilable compounds and polyunsaturated fatty acids. The quality of fish using different methods differs and the shelf life of fish dried in an electrically operated oven varies from that of fish dried using a smoking kiln [12].

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Processing and preservation methods using canning and freezing are hardly used in Nigeria due to cost non-availability of equipment and cold storage system rather traditional techniques such as salting, brining, sun-drying and smoking are in use and have enhanced fish availability to consumers. The heat and dryness associated with hot smoking reduces the water activity of the fish, pH thereby limiting microbial growth [13].

The use of smoke from smouldering wood for the preservation of perishable foods dates back to civilization [14] reported the various kinds of woods in the tropics are suitable for fish smoking process. Fish is often smoked in southern parts of Nigeria with the persistent rainfall and abundance of the semi-dried wood type in the region [13]. Effects of brining and smoking on the organoleptic attributes of the fish are important as the preservative effect [15]. The antimicrobial effect of smoking depends on temperature, humidity and density of the smoke, duration of smoking and concentration of active components in smoke preparations [16].

Nigeria’s high temperature, lack of processing and storage facilities can be responsible for the susceptibility of fish to damage and spoilage. There is therefore enormous waste through spoilage of both fresh and dried fish [17].

**Piper guineenses**

Piper Guineenses of pepper family is widely cultivated in Nigeria; it contains compounds like piperine and large amounts of β-caryophyllene which is reported to be anti-inflammatory. In Nigeria it is used in making stew where it adds pungent aroma. The peppers have preservative and anti-oxidant properties [18]. Brine concentration spices and brining time affects the texture of smoked fish [19].

**Sodium citrate**

Antimicrobial preservatives can be intentionally added as ingredients which inhibit microbial growth in catfish. Some preservatives used are acetic acid, potassium sorbate, sodium acetate, sodium chloride and sodium citrate. Antimicrobial property varies with quality and quantity of preservatives, and time of exposure [20].

Fernandez CF et al., [21] reported that shelf-life of fillets treated with 2% sodium lactate was extended from 4 to 7 days and aerobic plate counts and TBA values were lower (P<0.05) for fillets treated with 2% sodium lactate, compared to controls.

**Materials and Methods**

Fresh river catfish (Clarias lezera) were purchased from a fish farm, spices black pepper (Piper guineensis) and sodium chloride and dried (Mangifera indica) were purchased from Gboko market Benue state Nigeria.

**Sample preparation**

Fish samples were selected, washed, paper towel dried, measured with Vanier caliper, and weighed using Precision balance. They were eviscerated and re-washed in distilled water; water was drained by transferring to a sieving bowl. About 300 ml distilled water was added to 20 g each of ground black pepper and sodium chloride. Each mixture was boiled and refluxed for 5 minutes filtered hot through a sterile cheese-cloth (300 μm). The filtrate was cooled to room temperature (29 ± 1°C).

**Samples treatments**

Fish samples were soaked in warm (45 ± 1°C) spice extracts for 10 minutes, drained and smoke-dried by laying the fish samples over the smoking kiln at 365°F, uniform smoking was achieved by turning samples over at intervals of 15 minutes for 2 h after which they were cooled and weighed to a constant weight. The control sample was smoke-dried without prior soaking in extract solution. The smoked samples stored at -20°C and analysed at day one, (0), two, four and six weeks of storage.

**Shelf-life studies**

Smoke-dried fish was packaged in sterile low-density polyethylene bag, and sealed using a heat sealer (Poly-pack, U.K. Reg. No., 1004306). The samples were kept in cardboard waxed paper boxes and stored at room temperature (29 ± 1°C) for the shelf life study.

**Microbiological evaluation**

A 10 g representative sample from loin muscle of the fish was dissolved in sterile distilled water and serial dilutions (10-1-10-3) were made using sterile water as diluents. Samples were homogenized for 60 seconds using a Seward Stomacher Lab Blender at 40°C (Weber Science, Hamilton, NJ). Total plate count which is the total number of visible bacterial colonies, was determined by the Grid-Membrane Filtration method (GMFM) [23].

**Bacterial isolation**

The isolation of heterotrophic bacterial counts was carried out by homogenizing ten (10) gms of each of the fish portions from head, skin and tissue aseptically into 90 ml of sterile peptone water. Tenfold serial dilutions of the fish solution (homogenate) were prepared and carried out accordingly. One milliliter (1 ml) of the diluents was poured on nutrient agar and consequently incubated at 30°C for 48 hrs. The means of duplicate colony counts were calculated and used to compute the number of heterotrophic bacteria from the different portions.
Representative colonies were sub-cultured into freshly prepared nutrient agar for purification. They were then transferred into nutrient agar slants for storage and further analysis after which they were identified.

### Bacterial identification

Biochemical tests carried out were Gram reaction, Catalase, Coagulase, Indole test, Urea and Citrate utilization, Glucose, lactose, sucrose and manitol while cultural appearances were observed on MacConkey Agar.

### Sensory evaluation

Trained panel lists were made to evaluate the effect of smoking and preservative spices on the organoleptic attributes of the catfish. The descriptive 5-point hedonic scale was used for the sensory attributes (taste, texture, aroma, colour and general acceptability) of the samples at 0.05 level of significant. The parameters were assessed using ranking scores like extremely (5), like moderately (4), neither like nor dislike (3), dislike moderately (2) and dislike extremely (1) respectively.

### Statistical analysis

All measurements were carried out in triplicates and subjected to tests. All microbial counts were converted into base-10 logarithms of colony forming units per g of sliced catfish samples (log, CFU/g). Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software of SAS Institute [24]. Differences among the mean values of the various treatments were determined by the Least Significant Difference (LSD) test, and the significance was defined at p<0.05. The differences which are equal to or more than the identified LSD values are considered statistically significant.

### Results and Discussion

The average length of the fish was 30 cm while their average weight was 103 g (fresh weight)

Proximate Composition of Sliced Catfish during Six Weeks Storage

The results of proximate composition are shown in Tables 1-4. Generally, significant (p<0.05) differences existed between the samples in almost all the parameters.

#### Moisture content

In Table 1, the initial moisture content at day 0 in the sliced catfish ranged from 10.12 to 19.42% with sample B having the lowest value while sample A had the highest value. However, samples B, C and D showed significantly (p<0.05) lower moisture content values with the storage time, when compare with the control sample this might be due to the loss of water during smoking [25] coupled with the addition of spices applied. By the end of the storage time, significant (p<0.05) differences were observed in the moisture content between the control (15.13%) and each of sodium citrate (NaC) and Pg-treated samples B and D which exhibited lower values of 13.54 and 14.64% respectively while highest value (17.87%) was obtained in sample C.

The moisture content of samples treated with sodium citrate was 13.54%, which was lowest value of all treatments at the end of 6th week storage. There was no significant (p>0.05) different in the moisture content of all treatments throughout 6 weeks of storage except samples A and C which showed significant (p<0.05) different at the last week of storage.

#### Protein content

In Table 2, the initial protein content at day 0 in the sliced catfish ranged from 60.52 to 69.30% with sample A having the lowest value while sample B had the highest value. By the end of the storage time, significant (p<0.05) differences were observed in the protein content between the control (69.13%) which had the highest value and each of NaC and Pg-treated samples B, C and D which exhibited lower values of 65.54, 63.66 and 65.54% respectively. The increase observed in the protein content of the smoked catfish could be attributed to an increase in the dry matter content per unit of weight following sample dehydration during smoking in agreement to the finding of [26]. Storage time appeared not to have affected (p>0.05) the protein content of smoked catfish.

#### Fat content

The initial fat content is shown in Table 3. At day 0 in the sliced catfish ranged from 14.24 to 16.66% with sample C having the least value while sample A had the highest value. By the end of the storage time, significant (p<0.05) differences were observed in the fat content between the control (12.05%) which had the least value and each of NaC and Pg-treated samples B, C and D which exhibited lower values of 15.00, 13.93 and 14.34% respectively. There was significant (p<0.05) difference in the fat content of the samples among the treatments. The significant increase observed in the protein content of the smoked catfish could be attributed to loss of moisture and an increase in the dry matter content per unit of weight following sample dehydration. The reduction in the fat content on the 2nd week could be due to a sampling...
problem, since on the 6th week of storage most of these samples showed results similar to that of the day 0.

Ash content

In Table 4, the initial ash content at day 0 in the sliced catfish ranged from 3.42% to 5.48% with sample A having the lowest value while sample B had the highest value. By the end of the storage time, significant (p<0.05) differences were observed in the ash content between the control (3.71%) which had the least value and each of NaC and Pg-treated samples B, C, and D which exhibited lower values of 5.95, 4.23 and 4.92% respectively. The significant increase observed in the mineral content of the samples could be attributed to an increase in the dry matter content per unit of weight following sample dehydration, the addition of NaC, Pg and smoke during the smoking process. In addition, the ash content of samples treated with NaC was higher than that of all other treatments. Storage time had no significant effect on ash content of smoked catfish.

Lipid oxidation assessment of sliced catfish during six weeks storage

Peroxide value (PV): The highly unsaturated fatty acids found in fish lipids are very susceptible to oxidation. The primary oxidation products are the lipid hydroperoxides which are odour- and flavourless, thus the PV is not related to sensory quality of the fish. The result of peroxide value also called primary lipid oxidation products [27] is shown in Figure 1. The initial PV at day 0 in the sliced catfish analysed ranged from 5.73 to 7.36 meq/kg with sample D having the least value while sample A had the highest value. All the PV values obtained were significantly (p<0.05) increased with the storage time. However, samples B, C and D showed significantly (p<0.05) lower PV values with the storage time, when compared with the control sample. By the end of the storage time, significant (p<0.05) differences were observed in the PV between the control (9.23) and each of NaC and Pg-treated samples B, C and D, which exhibited lower values of 6.08, 5.97 and 6.15 meq/kg respectively. PV of Pg-treated samples was also significantly lower than that of NaC-treated sample. Storage time has a significant effect on the PV for each of the control and treated samples, nonetheless the PV in all samples were below the acceptable level of 10-20 meq/kg fish fat [28]. This might be due to the extended storage and high temperature exposure. A low PV as observed in this study during the storage can indicate both an early phase of autoxidation and a late stage of oxidized product, where most hydroperoxides have been broken down in dried fish [29]. Connell [30] reported that the PV (by iodometric titration) should not be above 10-20 meq/kg fish fat.

Fatty fish are of course, particularly vulnerable to lipid oxidation which can create severe quality problems such as unpleasant (rancid) taste and smell, and also it may produce alterations in texture, color, and nutritional value, even on storage at sub-zero temperatures [27,28]. The various reactions involved in the lipid oxidation are either non-enzymatic or catalyzed by microbial enzymes or by intracellular or digestive enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature [28].

Thiobarbituric acid (TBA): The result of thiobarbituric acid value is shown in Figure 2. The initial TBA values (mg MA per kg of fish sample) at day 0 in the sliced catfish analysed ranged from 0.14 to 0.24 with sample B having the least value while sample A had the highest value. All the TBA values obtained were significantly (p<0.05) increased with the storage time. However, samples B, C and D showed significantly (p<0.05) lower TBA values with the storage time, when compared with the control.

By the end of the storage period (6th week), NaC-treated sample B achieved significant (p<0.05) lower TBA value of 1.04 in comparison with the control-treated sample, which attained a higher level of 1.73. Also, Pg-treated sample C showed significantly (p<0.05) lower TBA value (0.33 meq/kg) when compared with the control and samples B and D. TBA assay is a widely used indicator for the assessment of degree of lipid oxidation. The result of TBA assay corroborated that obtained by the PV.
Significant effect of storage time on TBA values had been verified by Williams SK [21]. It is possible that effects of different treatments on lipid oxidation in fish products may be dependent on a variety of factors including the extent of microbial growth, packaging method, and storage time. It has been proposed that the maximum level of TBA value indicating the good quality of the fish (frozen, chilled or stored with ice) is 5 mg malonaldehyde/kg, while the fish may be consumed up to the level of 8 mg malonaldehyde/kg. Schormuller [31] reported that TBA above 10 µmol MDA-equiv per 1 kg fish will probably have a drastic reduction of the initial TPC.

Microbiological evaluation of sliced catfish during six weeks storage

**Total plate count (TPC):** The result of total plate count value is shown in Figure 3. The initial TPC at day 0 in the sliced catfish analysed ranged from 3.24 to 3.88 log_{10} CFU/g with sample C having the least value while sample A had the highest value. This indicated that dipping of the sliced catfish in the different treatments solutions resulted in drastic reduction of the initial TPC.

By the 6th week of storage, however, TPCs in sliced catfish for all of the different treatments were still below 6 log_{10} CFU/g, while that of control attained a count of 6.24 log_{10} CFU/g, which is in close proximity to the maximal recommended limit of 7 log_{10} CFU/g for TPC in raw fish [34]. Emborg [35] reported a maximum shelf life of t 20 days for whole salmon (Salmo salar) stored at 2°C, the variations in the antimicrobial effect of the spices applied singly and in combination on the catfish confirmed a significant decrease in TPCs, along with shelf life extension of all the samples during the storage. The discrepancy of sodium lactate (NaL) effects on the microbial growth in fish products may be dependent on a variety of factors including the concentration of NaL used, the dipping time, the species of fish, the type of fish product, the degree of microbial contamination, and as well as the storage condition.

A little antimicrobial effect was also confirmed in both shrimp (Penaeus spp.) and cat-fish fillets after dipping for 30 min into 2% sodium lactate when compared with the control samples [36]. The comparatively lower shelf life of smoked sliced catfish (control) versus treated catfish can be attributed to the postharvest handling condition which involved higher bacterial contamination in sliced fish either from handling, processing tables and knives or from the fish viscera during preparation. Indeed, the initial microbial contamination, storage and packaging condition (aerobic storage, vacuum-packed, or packed under modified atmosphere) as well as the storage temperature can play the major role in determining the shelf lives of fishery products. At the point of sensory rejection, the common number of total spoilage bacteria in aerobically stored fish products is typically 7-9 log_{10} CFU/g [29,36]. Nevertheless, standards, guidelines, and specifications often use much lower total microbial counts as indices of acceptability [29].
The dominant bacteria identified by 4 weeks and of 6 weeks for all samples were *Staphylococcus aureus*, *Klebsiella* spp. Especially *Klebsiella aerogonos* or *Klebsiella pneumonia*.

From 0-4 weeks the cultural characteristics and biochemical tests in Table 5 showed all samples had colonies that were pinkish in color, catalase, coagulase and Gram positive which could be indicative of *Staphylococcus aureus*, while by 6 weeks storage time, *Klebsiella* spp were identified as non-motile rods that were negative to indole and Gram test.

### Sensory evaluation of sliced catfish during six weeks storage

The result of sensory evaluation of the catfish is presented in Table 6. There was significant (p<0.05) difference in the sensory attributes of all the samples assessed this might be due to variations among individuals in responding to the same level of stimuli like colour, taste and sensitivity to chemical stimuli. There was no significant difference (p>0.05) in the colour of samples C and D and were also most preferred for colour. Sample D was most preferred compared to others in terms of aroma, texture, taste while sample A was least preferred for the same sensory attributes. The result of general acceptability, however, shows that sample D was most acceptable.

### Conclusion

From this study, sodium citrate and black pepper can be used as preservatives in smoked catfish without adversely affecting quality in terms of lipid oxidation, microbial quality, potency antioxidant, color, and nutritional quality throughout 6 weeks of storage. Dipping of sliced catfish in sodium citrate and black pepper singly and in combination reduced the microbial load in smoked catfish. There was significant (p>0.05) reduction in the PV and TBA values in comparison with the control. The antimicrobial effect of the spices applied singly and in combination on the catfish showed a significant decrease in TPCs, along with shelf life extension of all the samples during the storage. The catfish samples exhibited shelf stability and the microbiological load fell within acceptable level stipulated by microbiological standards. This indicates that the samples were safe for consumption throughout the period of storage.

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### Table 6: Sensory Evaluation of Sliced Catfish during Six Weeks Storage.

| Sample | Colour | Aroma | Texture | Taste | General Acceptability |
|--------|--------|-------|---------|-------|-----------------------|
| A      | 3.93d ± 0.38 | 3.59d ± 0.52 | 3.62d ± 0.57 | 3.96d ± 0.66 | 3.96d ± 0.66 |
| B      | 4.29c ± 0.72 | 3.73c ± 0.84 | 3.62c ± 0.95 | 4.38c ± 0.86 | 4.52c ± 0.63 |
| C      | 4.37d ± 0.85 | 4.93b ± 0.49 | 4.77b ± 0.52 | 4.57b ± 0.74 | 5.74b ± 0.84 |
| D      | 4.36a ± 0.87 | 5.40a ± 0.72 | 4.89a ± 0.64 | 5.89a ± 0.85 | 5.88a ± 0.73 |

Values are means ± SD of triplicate determinations. Means with different superscripts within each column are significantly (p<0.05) different. Key: A=Control, B=1% Sodium citrate, C=1% Black pepper, D=1% Sodium citrate+1% Black pepper.
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