

EFFECT OF NATURAL PRESERVATIVES ON THE ORGANOLEPTIC CHARACTERISTICS AND STORAGE STABILITY OF SMOKED *Heterotis niloticus*

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ABSTRACT

Improvement of consumer's palatability of Heterotis niloticus was investigated by value addition with natural spices and salt to improve its palatability and shelf-life. The major problem of H. niloticus is its poor taste, hence low demand and poor consumer's acceptability which affects the market value. The objective of this study was to improve the palatability and shelf-life of H. niloticus using selected natural spices. Heterotis niloticus, procured and treated with natural spices and salt were allowed to drain for one hour, subjected to charcoal fired smoking kiln for 12 hours, cooled and stored for 8 weeks at ambient temperature before microbial analysis. The result showed that treatment 1 had the best quality (37.7%), followed by control sample (34.7%), and treatment 2 (28.9%), while the least was treatment 3 (21.6%). Microbial analysis shows that no Salmonella, Coliforms and E. coli were detected from the samples after smoking. Staphylococcus aureus count ranged from 2×10^2 to 9×10^2 but it was not detected in treatment 1. Improvement of the consumers' palatability of H. niloticus and improved storage stability by spicing methods improved the poor taste of H. niloticus which has been discriminated despite excellent muscle quality.

Key words: *Heterotis niloticus*, organoleptic, spicing, microbial analysis

INTRODUCTION

Fish is an important source of food, income, employment and recreation for people around the world and it is a very important source of animal protein for both man and livestock in developed and developing countries (Emmanuel *et al.*, 2014; Amponsah *et al.*, 2016; FAO, 2014). Nutritionally, fish is an important and rich source of affordable protein characterized by a desirable composition of amino acids (Pasqualino *et al.*, 2016), vitamins A, B and D as well as minerals like calcium, iodine, selenium, zinc and iron (FAO, 2012). Besides its acceptance as a balanced source of animal protein and vitamins, fish also provides polyunsaturated fatty acids (PUFAs) and minerals necessary for optimal health (Allision, 2011).

However, nutritional benefits from fish and fish products are limited by its rapidly perishable nature and vulnerability to spoilage (Modibbo *et al.*, 2014; FAO, 2016). Spoilage in fish occurs because it is susceptible to microbial and enzymatic deterioration which results in quality reduction, especially in the absence of proper processing and storage techniques. Generally, during post-harvest period large amounts of fish spoil and waste due. This is not only due to lack of proper measure for processing and preservation, but also to the fact that normally not all the fishes caught are consumed and they are not transported to other places they are needed due to insufficient handling and transportation system.

In Nigeria and other countries in sub-Saharan Africa where fish offers about 40% protein intake of the people, *Heterotis niloticus* is one of the species that has great potentials for commercial aquaculture (Olatunde, 1989). The species has excellent flesh quality and good source of amino acids (Monentcham *et al.*, 2009), but is associated with low acceptability by consumers due to its poor taste. There has been great setback to consumers' demand of this fish which affects the market value. This results to *H. niloticus* being used mostly in many places for feed preparation instead of human consumption. Because of this problem it is barely known, cultured and consumed by fish consumers thereby reducing its market value. There is need to address the problems of poor taste and wastage by adding value to the fish to improve its consumer taste which would increase its demand, thereby attract better market value for the fish.

Also, in order to meet the complex and long chain of distribution as well as fish food security, it is important to ensure proper fish preservation using natural preservatives that can improve organoleptic properties of fish and also extend its shelf-life. Therefore, the use of natural preservatives like ginger, nutmeg and pepper in combination with sodium chloride which can inhibit microbial growth in smoked fish during storage becomes important (Deo, 1998). There is scanty of

information regarding the impact of natural preservatives and different storage times at room temperature on the microbial quality and palatability of smoked *H. niloticus*. This study was on the effect of selected natural preservatives on the changes in microbial population and quality of smoked *H. niloticus* during 8-week ambient storage. The general objective of this study was to improve the palatability of *H. niloticus* to fish consumers using selected natural spices and improve its shelf-life also.

MATERIALS AND METHODS

Sample Collection

Four table-sized fish samples of *H. niloticus* with a total weight of 12.7 kg were purchased from the artisanal fishermen at Otuocha market in Anambra-East Local Government Area of Anambra State. It was conveyed down to Nnamdi Azikiwe University Research Farm in an ice-filled plastic bowl covered with a sack bag to prevent deterioration.

Sources of Spices and Other Materials

Some spices including Ginger (*Zigiber officinalis*), Nutmeg (*Myristica fragrans*), Cameroon pepper (*Capsicum chinense*) and common salt (Sodium chloride) were obtained from a local market (Eke Awka) in Awka, Anambra State. Other materials like charcoal were equally sourced locally from Eke Awka while smoking kiln was used in the smoke drying of the fish samples.

Sample Preparation

The collected fish samples were scaled, gutted and washed severally and then chunked into 48 pieces as each spice were replicated into three places. The three spices used; were g grinded into powder using grinding machine (Manual Multi-function Grinder Machine, model JR 10). The spices were placed in 100 g cup and 50 g each was weighed using a sensitive scale (Atom A 122 Electronic kitchen digital weighing scale, model SF: 400A). Each spice was added 10 g of salt and mixed with half a liter of water. The fish samples were then soaked in the prepared spice extract for 1 h, placed on smoking trays, tagged and allowed to drain water and placed into the smoking kiln. The fourth sample soaked in 10 g of salt served as the control.

Fish Processing

The treated samples were replicated three times as well as the control. The drying of the fish samples was done using a charcoal fired smoking kiln. They were subjected to heat and allowed to dry for 12 h with turning at intervals to achieve a uniformly dried product. The fish samples were cooled, removed from smoking kiln and tagged as: salt only: Control, Ginger + salt: Treatment 1, Nutmeg + salt: Treatment 2, Cameroon pepper + salt: Treatment 3.

Organoleptic Evaluation

Subjective analysis was used for organoleptic characteristics analysis, in which staff and students of Nnamdi Azikiwe University, Awka were used to carry out the tests. Five members of the panel were selected for each parameter like flavour, texture, appearance and taste trained on the rudimentary aspects of organoleptic characteristics and how to apportion mark to each parameter. The fish samples were given out with questionnaires for the panel members to feel and taste the fish products and score based on how it appealed to the taste, texture, appearance and flavour.

Pre-Storage Microbial Analysis of the Samples

Initial microbial sample isolate

One gram (1 g) representative sample was obtained aseptically from the loin muscle of the smoked *H. niloticus* 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 milliliter of the serially diluted samples was taken in duplicates and plate count agar was poured at 40 °C on the plates. The samples and the medium were properly mixed, allowed to set and incubated at 35 °C for 24 h. The number of colonies on the plates was counted.

Staphylococcus sp. count

Manitol salt agar was used to enumerate the number of *Staphylococcus* colonies. The plates were incubated at 35 °C for 24 h and bright yellow colonies were counted. The bright yellow colonies were sub-cultured for additional 24 h 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 *S. aureus*.

Salmonella count

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

Escherichia coli count

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

Post Ambient Storage Microbial Analysis

After 8 weeks in storage at ambient temperature, the different treated samples of the smoked *H. niloticus* were subjected to microbial load analysis.

Sample Preparation

One gram (1g) of each of the treated fish samples and their replicates were weighed out aseptically and introduced into 10 ml of sterile peptone water in a test tube, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. A known volume (0.1ml) of appropriate dilutions (10^{-2} and 10^{-3}) of the sample were pour plated in sterile plates of Nutrient agar (NA) plates and MacConkey agar plates for the cultivation of bacteria. All cultured plates were incubated at 37°C aerobically for 24-48 h. Developed colonies were counted to obtain total viable count and coliform counts respectively. Discrete colonies were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods.

Characterization and Identification of Bacteria

Identification of the bacterial isolates was done by the observation of colonial characteristics, Gram reaction and biochemical tests (Cheesbrough, 1984). The characterization of the isolates was performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Oxidase test, Coagulase test, Motility test, Indole test, Methyl Red and Voges proskauer test as described by Bergey and Holt (1994).

RESULTS

Organoleptic Properties of *Heterotis niloticus*

The organoleptic properties of treated and smoked *Heterotis niloticus* are shown in Table 1. The overall performance shows that treatment 1 had the highest value (38.5%) in terms of flavour, while the least (23.1%) acceptable flavour was recorded by treatment 3, treatment 2, and the control had 30.8% acceptability in flavour. The result shows that the flavour of treatment 1 differed from that of treatment 3 and no difference was observed in flavour of treatment 2 and control. The control sample had the best appearance (38.5%) while treatment 3 had least (15.4%) accepted appearance. The result shows slight difference in the appearance of treatment 1 (36.9%) and control (38.5%) samples, but differs greatly from treatment 2 (26.2 %) and

treatment 3 (15.4%) samples. The texture of the control sample (38.5%) and treatment 1 (36.9%) were better when compared with that of treatment 3 (24.6%). The taste parameter showed that treatment 1 had 38.5 % acceptability followed by control and treatment 2 (30.8%), while the least was 23.1% acceptability in treatment 3. However, there were no differences recorded in treatment 2 and control samples, while major difference exist between treatment 3 and other treatments.

Microbiological Analysis

The result of the microbial analysis of *H. niloticus* treated with some selected natural preservative and smoke-dried before storage for eight weeks at ambient temperature showed reduced total plate count of day zero (0) for the samples. The total plate count for freshly smoked samples ranged from 34×10^2 to 35×10^3 CFU/g for day zero samples (Table 2). *Salmonella*, the pathogenic microorganism examined, for freshly smoked fish prior to storage was not detected in any of the samples. No coliforms and *E. coli* were found in any of the treated samples after smoking. The pathogenic organisms, *S. aureus* counts ranged from 2×10^2 to 9×10^2 CFU/g. Nonetheless, the microbial populations for the entire treated smoked fish sample observed in this study before storage were within the recommended limits for good quality fish product according to ICMSF (1986).

Table 3 shows the mean value of the total viable count (TVC) of bacterial load in *H. niloticus* after storage in ambient room temperature for a period of 8 weeks with a total viable count ranged from 3.4×10^5 CFU/ml in treatment 1 to 7.8×10^5 CFU/ml in control sample. These are above the recommended WHO Standard (1.0×10^3 Cfu/ml).

Table 2: Microbial population in freshly smoked preservative treated *H. niloticus*

| Microbial Population | Mean value of microorganism CFU/g | | | |
|----------------------|-----------------------------------|------------------|------------------|------------------|
| | Treat-ment 1 | Treat-ment 2 | Treat-ment 3 | Control |
| TPC | 35×10^3 | 12×10^3 | 34×10^2 | 25×10^3 |
| Salmonella | ND | ND | ND | ND |
| Staphylococcus | ND | 3×10^2 | 9×10^2 | 2×10^2 |
| Coliforms | ND | ND | ND | ND |
| E.coli | ND | ND | ND | ND |

TPC - total plate count, CFU - colony forming unit, ND - not detected, Control - salt treated, Treatment 1 - ginger + salt, Treatment 2 - nutmeg + salt, Treatment 3 - pepper + salt

Table 1: Sensory scores of smoked *H. niloticus* with natural preservatives

| Preservative | Overall acceptability | Percentage (%) perception of respondent | | | |
|--------------|-----------------------|---|---------|-------|---------|
| | | Appearance | Texture | Taste | Flavour |
| Control | 34.7 | 38.5 | 38.5 | 30.8 | 30.8 |
| Treatment 1 | 37.7 | 36.9 | 36.9 | 38.5 | 38.5 |
| Treatment 2 | 28.9 | 26.2 | 27.7 | 30.8 | 30.8 |
| Treatment 3 | 21.6 | 15.4 | 24.6 | 23.1 | 23.1 |

Control: Salt treated, Treatment 1: Ginger + salt, Treatment 2: Nutmeg + salt, Treatment 3: Pepper + salt.

Table 3: Total viable count (TVC (CFU/ml)) of treated ambient stored *H. niloticus*

| Treatments | 10 ⁻² dilution | 10 ⁻³ dilution |
|-------------|---------------------------|---------------------------|
| Control | 1.42 × 10 ⁵ | 7.8 × 10 ⁵ |
| Treatment 1 | 7.2 × 10 ⁴ | 3.4 × 10 ⁵ |
| Treatment 2 | 1.28 × 10 ⁵ | 7.5 × 10 ⁵ |
| Treatment 3 | 9.6 × 10 ⁴ | 4.2 × 10 ⁵ |

CFU - colony forming unit (WHO's standard 1.0 × 10⁵ CFU/ml),
Control - salt treated, Treatment 1 - ginger + salt,
Treatment 2 - nutmeg + salt, Treatment 3 - pepper + salt

DISCUSSION

The result of organoleptic properties of spice treated smoked *H. niloticus* in this study shows improved end product and a prolonged shelf- life in *H. niloticus*, this may not be unconnected to the fact that the spice treatment and the smoking process seem sufficient to have destroyed microbes. The absence of visible mould mass under ambient condition observed in this study indicates the effectiveness of spices as anti-fungal agents and resulted in the extended shelf-life of spice treated samples; this agrees with the report of Kumolu-Johnson *et al.* (2015) that ginger extract was effective in reducing microbial load in the stored fish sample. Also, the low level of contamination in freshly processed products and the lack of post-processing contamination as shown in Table 2 indicated general good sanitary conditions at point of collection and processing. A combination of preservative treatment and smoking result might have equally contributed to this low record in variations of microbial levels.

Furthermore, the microbial results observed in this study are in agreement with the studies of Kumolu-Johnson and Ndimele (2011) in which it was observed that there was a reduction in microbial proliferation and lipid oxidation in the samples treated with ginger paste, Ikeme and Bhandary (2001) and Sallam *et al.* (2004) in which ginger and garlic paste were effective in retarding the development of oxidative rancidity in mackerel, (*Scomber scombrus*) and chicken sausage, respectively. These results also indicate that ginger which is natural spice clearly has anti-fungal properties that can compare with synthetic antimicrobial agents like potassium sorbate, citric acid and sodium metabisulphite (Omojowo *et al.*, 2008). Kiin-Kabari *et al.* (2011) showed that *Piper guinensis*, *Myristica monodora* and *Xylophia aethiopicum* had chemical preservative and antioxidant properties which have effective preservation potential for smoked-dried fish during storage by retarding growth of microorganism and thus extend the shelf- life of fish. These antioxidants could be part of the reason for low microbial count after 8-week storage which makes the fish to remain within the acceptable limits recorded by International Commission on

Microbiology safety for food and for good fish product (ICMSF, 1986). Similarly, Oluborode *et al.* (2015) reported that the microbial analysis of fish samples (*Clarias gariepinus*) treated with 3 spices , ginger, garlic and combination shows that total viable counts (TVC) as well as bacterial species were not detected in the fish samples after smoking. This corroborates the result of this study. Furthermore, the result of this study confirmed report of Ihuahi *et al.* (2006) that *Clarias gariepinus* treated with mixture of pepper and garlic paste was more stable than untreated samples and have more acceptable organoleptic properties.

CONCLUSION

This study demonstrated the effectiveness of natural spice addition in improving consumer's palatability and controlling microbial population and pathogens in *Heterotis niloticus* by predicting the microbial safety and quality of smoked *H. niloticus* for 8 weeks ambient storage. The study shows that ginger plus salt is an efficient and effective spice for pre-treating fish (*H. niloticus*) before smoking for production of high quality, palatable and safe smoked-dried products. It was also evident from this study that the application of ginger plus salt and good/hygienic handling procedures adopted greatly improved consumer's palatability of the fish (*H. niloticus*) and control microbial population. Hence ginger plus salt is recommended for pre-treatment of fish (*H. niloticus*) prior to processing especially when consumer's palatability and safety is desired.

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