

The Anti-Oxidative and Anti-Fungal Effects of Fresh Garlic (*Allium sativum*) on the Shelf-Life of Hot Smoked Catfish (*Clarias gariepinus*, Burchell, 1822)

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Abstract: The efficacy of fresh garlic, *Allium sativum* at 10g/kg, 30g/kg and 50g/kg on smoked catfish was conducted in an experiment. Its antioxidative, antifungal and seasoning effects were observed on smoked catfish, *Clarias gariepinus* in the laboratory of Department of Fisheries, Lagos State University, Nigeria. The fish samples were eviscerated, cleaned and dipped in 15% brine for 10 minutes. They were treated with fresh garlic paste, smoked at 80 - 85°C for 6 hours, cooler, stored at room temperature (25 - 30°C) for 28 days and were subjected to physical, chemical, microbial and sensory evaluation. From the result of analysis, samples treated with garlic paste had lower microbial load compared to the control samples and were not visibly covered by moulds during a 28-day storage period. The antioxidative activity of garlic was evident from the lower thiobarbituric acid (TBA) and peroxide values of the treated samples relative to the untreated samples. Results of panel evaluation showed a general preference for garlic-treated samples.

Key words: Garlic • *Clarias gariepinus* • Thiobarbituric acid • Peroxide • Organoleptic • Microbial count

INTRODUCTION

Fish is a major source of food for human providing significant portion of the protein intake in the diets of a large proportion of the people, particularly so in the developing countries. Fish is a cheap source of animal protein with little or no religious rejection, which gives it an advantage over pork or beef [1]. It is less tough and more digestible compare to beef, chicken and mutton. Fish has higher levels of essential sulphur-containing amino acids such as cysteine, methionine and lysine, which are limiting in some legumes and most cereal diets [2].

Fish is a major source of animal protein in Nigeria; it is readily available in most market as fresh, smoked, dried, canned, chilled or frozen. Fish muscle contains four basic nutrients in varying proportions; water 70 - 80%, protein 16 - 25%, lipid 1 - 5% and vitamins [3].

However, in spite of the valuable nutrients derived from fish, it is a highly perishable commodity than cattle, sheep and poultry as it get spoiled very quickly after capture, unless it is disposed off quickly after capture [4]. Fish flesh contains the nutrients necessary to support the growth of wide range of micro-organisms at death. The post-harvest changes; (sensory, autolytic, bacteriological changes and lipid oxidation) affect the

quality of fish as food. This alteration in component continues when left unchecked until the fish becomes unwholesome and unfit for consumption or can be prevented through preservation [5].

In Nigeria, only a negligible proportion of the fish caught in rivers and lakes is market fresh. A greater portion is preserved by smoking and sun drying [1, 6]. Fish smoking is mostly done at artisanal level by women in riverside communities whose main economic activity is fish processing and marketing. The reasons for fish smoking are varied, but in Nigeria, the process has proven relevant to prolonging shelf-life, enhancing flavour, storing for lean season and increasing protein availability to people throughout the year [4].

Smoking is the process through which volatile substances from combustion of wood penetrates fish or meat flesh [7]. The phenomenon is based on incomplete combustion followed by thermal disintegration or pyrolysis of high molecular mass organic compound to yield compound of lower molecular mass which becomes volatile at the smoking temperature. While the woods used for smoke generation are composed of cellulose, hemicelluloses and lignin, the compounds reputed to be of most importance in smoke flavouring are produced from the pyrolysis of lignin fraction [8]. Heat generated as a result of smoking dehydrate, inhibits bacterial

growth, retard enzymatic actions, add aroma, taste and colour on processed fish, but its quality can deteriorate during storage due to lipid-oxidation and microbial growth. While lipid-oxidation is responsible for reduction in nutrient quality as well as changes in flavour [5], microbial contamination could precipitates public health concern and economic loss in term of fish spoilage. Thus, suitable agents possessing both anti-oxidative and anti-fungal properties may be useful to compliment smoking technique for maintaining fish qualities. Food preservatives and anti-oxidants are used to prolong the shelf-life of food by killing micro-organisms or controlling their growth in food. They also preserve by preventing or retarding the oxidative deterioration of food [4].

Synthetic anti-oxidants such as Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) have been prohibited in many countries of the world because of their undesirable effect on the enzymes of the liver and lungs [9]. This has paved way for the use of natural anti-oxidants such as spices in the prevention of rancidity in smoked fish [10].

Spices are edible plant substances that possess anti-oxidative, antiseptic and bacteriostatic properties [11]. They are added to food to delay the onset of deterioration, such as rancidity. They also function as seasonings to the food as well as impact flavour [12]. Garlic (*Allium sativum*), is one of the mostly used natural ingredient to enhance flavour in food. It has a wide spectrum of actions, which include: antibacterial, antifungal and anti-oxidative. It also has a beneficial effect on cardiovascular and immune system of human [9].

Most of the previous studies on anti-oxidative and anti-fungal effects of garlic have been done using chicken. This study is perhaps one of the first few studies, particularly in Africa, South of the Sahara, where the anti-oxidative and anti-fungal effects of fresh garlic paste on the shelf-life and keeping quality of hot smoked catfish (*Clarias gariepinus*) was studied. The objectives of this study is to determine the effects of fresh garlic at different concentrations on the qualities, acceptability and shelf-life of hot smoked *Clarias gariepinus*.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Department of Fisheries, Faculty of Science, Lagos State University from March, 2010 to September, 2010.

Fish Handling: 160 fresh catfish *Clarias gariepinus* used for the experiment were obtained from Lagos State University hatchery complex. They were stunned, eviscerated and washed in clean water to remove blood. The mean weight and length of the fish was 110 ± 12 g and 21.50 ± 3.60 cm respectively. The whole fish were then immersed in 15% brine solution for 10 minutes, drained and divided into four (4) batches (for the 3 treatments and control) and labeled. Each batch was made up 40 fish specimens.

Garlic Treatment: Fresh soft-neck garlic (*Allium sativum* var. *sativum*) was bought from a local market in Oke-Odo, Abule-Egba, Lagos state, Nigeria. The outer coats were removed, cloves peeled and crushed finely using a kitchen blender (model MX-X61-W, National, Japan). The quantity of fresh garlic paste required to give a particular treatment concentration was measured into a clean bowl. The fish specimens in that batch were then thoroughly mixed with the garlic paste until most of the garlic paste has been used up. The garlic paste application was done for five (5) fish specimens at a time for even distribution of the garlic paste. Fish samples in batch A (control) were not treated with garlic paste while the fish samples in batches B, C and D were treated with garlic paste at 10g/kg, 30g/kg and 50g/kg of fish respectively.

Fish Smoking: Each batch was demarcated with wooden rod on the wire gauze placed on the top of the smoking kiln and smoked with tropical hardwood. Initial smoking temperature was low to prevent surface drying of the fish samples. A temperature of about 80°C was maintained and the samples were removed after 6 hours of smoking. Smoked samples were cooled for 30 minutes, packaged in bulk and stored at room temperature of (25 - 30°C) for 28 days. Samples were subjected to visual observation, chemical, microbiological and sensory evaluations.

Thiobarbituric Acid Reactive Substance (Tba-Rs)

Determination: The oxidative stability of smoked samples was measured by Thiobarbituric Acid Reactive Substance according to Association of Official Analytical Chemist [13]. 10g of the sample was macerated in 10cm³ of water for 2 minutes and washed into a distillation flask with 47.5cm³ water. 2.5cm³ of 4M Hydrochloric Acid (HCl) was added to bring the pH to 1.5. The flask was heated by an electric mantle until 50cm³ of distillate is collected after 10 minutes. 5cm³ of the distillate was pipette into a

glass stoppered tube and filtrated with TBA reagent (0.2883gm/100cm of 90% glacial acetic acid). The test tube was cooled in water for 10 minutes and the absorbance measured against the blank at 538nm using UV-VIS spectrophotometer (model UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg sample.

Peroxide Value: The oxidative stability of smoked samples was also measured using titrimetric determination of the amount of peroxide and hydroperoxide group (the initial products of lipid oxidation) according to [13]. To 10gm of sample was added 1gm powdered potassium iodide (KI) and 20cm³ solvent mixture (2vol glacial acetic acid + 1 vol chloroform) placed in boiling water for 30 seconds. The content was then poured into a flask containing 20cm³ of potassium iodide (KI) solution (5%) and titrated with 0.002N sodium thiosulphate using starch as indicator. Peroxide was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\text{Peroxide value (meq/kg)} = \frac{(S - B) (N) 1000}{\text{Unit of sample}}$$

Where

B = Titration of Blank

S = Titration of Sample

N = Normality of Sodium thiosulphate.

Microbiological Analysis: Mould counts were determined according to standard procedures described by Fawole and Oso [14]. Fish samples (10 g) were homogenized with

90 ml of sterile peptone water (1 g/l) in a laboratory homogenizer (AM-5 Ace homogenizer, Nihonseiki, Japan) and serial dilutions were prepared. Then 0.1 ml of each dilution was spread with a bent sterile glass rod on duplicate plates of pre-poured and dried standard plate count. After 48-h incubation at 25°C, colonies were counted and results were expressed as log₁₀ CFU/g of fish sample.

Organoceptive Assessment: Subjective evaluation of the product quality was carried out in accordance with method outlined by Poste *et al.* [15] by panel of 5. Coded samples accompanied by questionnaires were presented to the panelists. Quality attributes studied include taste, texture, colour, rancidity and general comment. The hedonic scale used was from 1 - 5, where a score of 5 was “excellent” and a score of 1 was “very poor”.

Statistic Analysis: Analysis of Variance (ANOVA) was applied to the treatment values obtained using SPSS for Windows version 15.0. Differences between means were determined by the least significant difference test and significance was defined at p < 0.05.

RESULTS

The Oxidative stability of the smoked catfish samples *Clarias gariepinus* measured using Thiobarbituric Acid (TBA) reaction and peroxide values during the 28 days storage period are as showed in Figures 1 and 2 respectively. They revealed a general increase in (TBA) and peroxide values with time. However, the values of the control sample

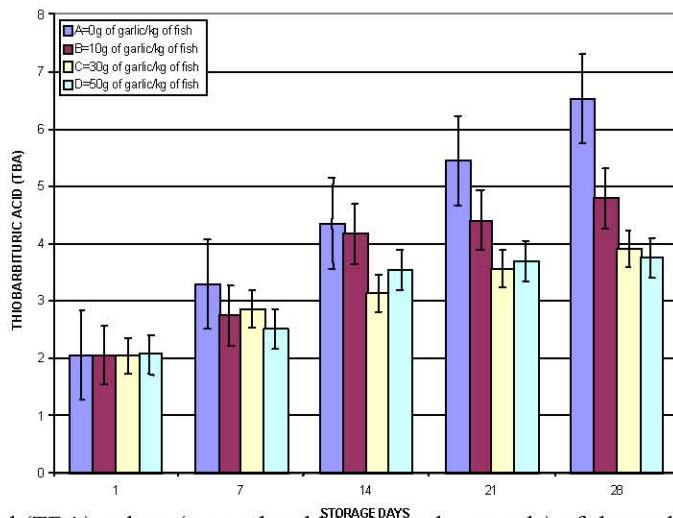


Fig. 1: Thiobarbituric acid (TBA) values (mg malonaldehyde per kg sample) of the garlic-treated *Clarias gariepinus* during the 28 days storage period

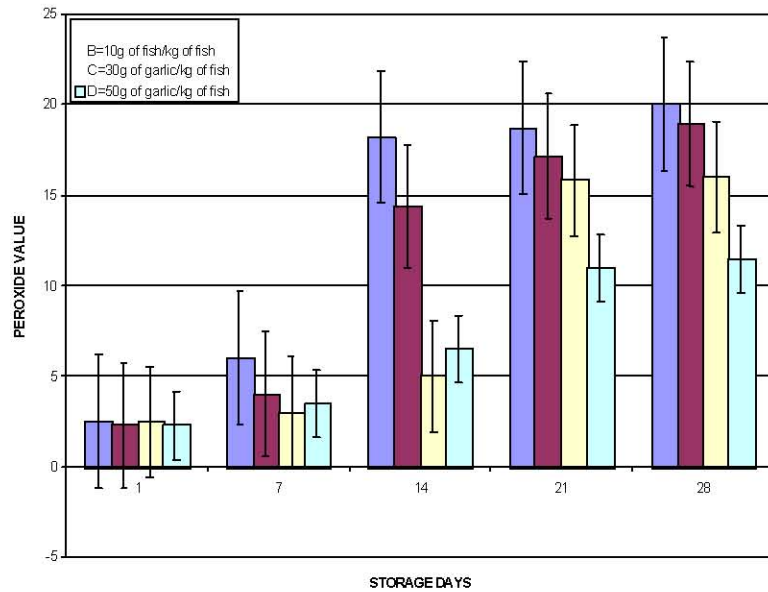


Fig. 2: Peroxide values (milliequivalent peroxide per kg of sample) of the garlic-treated *Clarias gariepinus* during the 28 days storage period

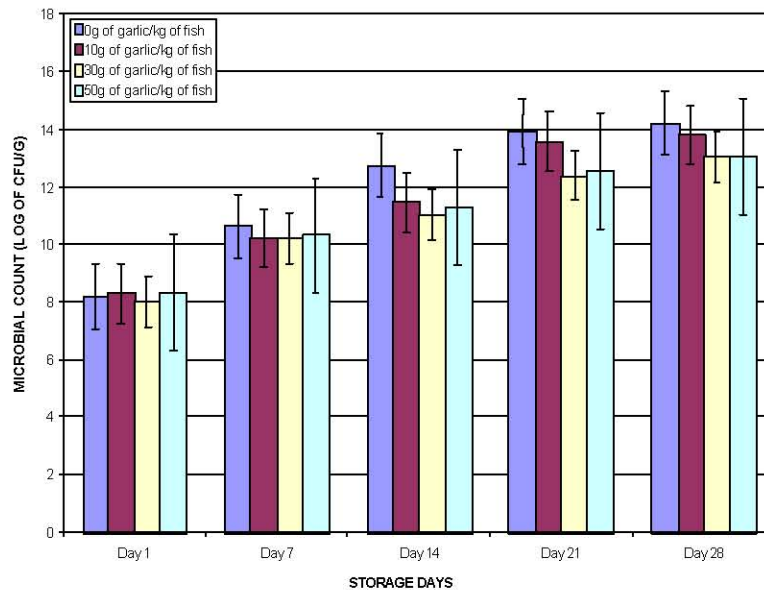


Fig. 3: Microbial growth (Log₁₀ CFU/g of fish sample) of the garlic-treated *Clarias gariepinus* during the 28 days storage period.

(0g of garlic/kg of fish) were higher than the garlic-treated samples. It was also observed that samples with higher concentration of garlic paste; 30g of garlic/kg and 50g of garlic/kg of fish had lower TBA and peroxide value when compare to the untreated samples. Table 1 showed that the highest value (4.32 ± 0.39 mg malonaldehyde per kg sample) of TBA was recorded in control that was not treated with garlic while the lowest value (3.09 ± 0.18 mg malonaldehyde per kg sample) was observed in the

sample treated with 50g of garlic/kg of fish. This difference was statistically significant ($p < 0.05$). The highest peroxide value (12.31 ± 2.06) occurred in the control (0g of garlic/kg of fish) while the lowest (6.91 ± 1.08) was found in the sample treated with 50g garlic/kg of fish. This difference was also significant ($p < 0.05$). The microbial count of the smoked catfish samples, *Clarias gariepinus* during the 28-day storage as shown

Table 1: Mean thiobarbituric acid (TBA) (mg malonaldehyde per kg sample), peroxide (milliequivalent peroxide per kg of sample) and microbial growth (Log_{10} CFU/g of fish sample) after 28 days storage period

Treatments	Tba	Peroxide	Microbial Counts
A	4.32±0.39 ^a	12.31±2.06 ^a	12.03±0.56 ^a
B	3.62±0.31 ^b	11.35±1.86 ^b	11.58±0.51 ^a
C	3.06±0.26 ^b	7.78±1.49 ^a	11.24±0.41 ^a
D	3.09±0.18 ^b	6.91±1.08 ^a	11.15±0.43 ^a

Values in the same column and with the same superscript values are not significantly different ($p>0.05$)

A = 0g of garlic/kg of fish; B = 10g of garlic/kg of fish; C = 30g of garlic/kg of fish; D = 50g of garlic/kg of fish

Table 2: Organoleptic analysis of garlic-treated *Clarias gariepinus* after 21-days storage period at 25 - 30°C

Treatment	Rancidity	Colour	Taste	Flavour General	Acceptance
A	3.84±0.36 ^a	3.80±0.38 ^a	3.84±0.35 ^a	3.68±0.39 ^a	3.88±0.45 ^a
B	4.40±0.23 ^a	4.36±0.27 ^a	4.28±0.21 ^a	4.28±0.24 ^a	4.24±0.28 ^a
C	4.48±0.36 ^a	4.60±0.14 ^a	4.60±0.18 ^a	4.52±0.19 ^a	4.44±0.19 ^a
D	4.80±0.28 ^a	4.58±0.13 ^a	4.16±0.18 ^a	4.60±0.14 ^a	4.16±0.09 ^a

Values in the same column and with the same superscript values are not significantly different ($p>0.05$)

A = 0g of garlic/kg of fish; B = 10g of garlic/kg of fish; C = 30g of garlic/kg of fish; D = 50g of garlic/kg of fish

in Figure 3 reveal a steady increase in microbial count with storage period in all cases. However, sample treated with fresh garlic paste, *Allium sativum* showed lower counts. Untreated sample (0g of garlic/kg of fish) had higher microbial load as from the 7th day of storage. Samples treated with 10g garlic/kg of fish equally accumulate high microbial loads after the 14th day. On the other hand, the samples treated with 30g garlic/kg of fish and 50g garlic/kg of fish showed lower microbial load throughout the 28 day storage period. Table 1 showed that in spite of the lower microbial loads recorded in samples treated with 30g garlic/kg of fish and 50g garlic/kg of fish, there was no significant difference ($p>0.05$) in the microbial loads among the treatments.

The result of the organoleptic analyses of the smoked catfish, *Clarias gariepinus* during the 28-days storage is shown in Table 2. It revealed that the control samples received lower panel scores than the garlic paste-treated samples with regards to rancidity, taste, colour, flavour and general acceptance. The taste panel rating showed that treated samples were rated better than untreated in all parameters studied. However, there was no significant difference ($p>0.05$) among the treatments in all the organoleptic parameters measured (Table 2).

DISCUSSION

The medicinal and anti-microbial properties of garlic have been studied extensively but its ability to retard lipid oxidation in fish has not been a subject of much studies. In the present study, the mean peroxide values in all samples (including the control) were below 25 meq of

active O_2/kg , which is considered as limit of acceptability in fatty foods [16]. The results of the present study showed that fresh garlic paste significantly reduced the thiobarbituric acid and peroxide contents of hot smoked *Clarias gariepinus*, which means that rancidity in the garlic-treated smoked *Clarias gariepinus* was significantly retarded when compared to the sample that was not treated with garlic. Similar results were obtained by Sallam *et al.* [9] in which garlic paste were effective in retarding the development of rancidity in chicken sausage. Sun *et al.* [17] attributed the anti-oxidative properties of fresh garlic to the presence of large amount of allicin which accounts for a lot of the biological activities of fresh garlic.

It was also observed that the treatment with the highest concentration of garlic paste had the lowest thiobarbituric and peroxide values and that with the lowest concentration of garlic had the highest thiobarbituric and peroxide contents. It can be inferred that the anti-oxidative ability of garlic depends on their concentration. Yang *et al.* [18] also presented a similar report but care must be taken when adding garlic to fish so that it does not become too much as to affect the palatability of the fish.

Fresh garlic was also effective in reducing microbial load in the stored fish (*Clarias gariepinus*), though, the difference in the microbial loads among the concentrations of fresh garlic studied was not significant ($p>0.05$). This could be attributed to high initial microbial load (8 Log_{10} CFU/g) of all the treatments, which must have been due to the storage temperature (25 - 30°C). This temperature range was chosen in order to simulate what happens in local communities where

there is no refrigerator to preserve perishable products. A lower storage temperature of 3 - 4°C may produce much lower microbial load as was reported by Sallam *et al.* [9].

Storage time had no effect on rancidity, colour, taste, flavour and general acceptance of garlic-treated *Clarias gariepinus* and the control that was not treated with garlic. This might be due to the duration of the study (28 days), although, Sallam *et al.* [9] obtained a similar result in their study in which they examined the effects of garlic in chicken sausage for 21 days. Samples treated with 30g garlic/kg fish and 50g garlic/kg of fish recorded the highest acceptability.

CONCLUSION

This study has shown that garlic (*Allium sativum*) has some anti-oxidative and anti-fungal effects which can retard oxidative rancidity, inhibit microbial growth, impact acceptable flavour and thus, extend the keeping quality of fish like *Clarias gariepinus*. However, the insignificance ($p > 0.05$) of the microbial growth and panel rating among the treatments suggest that further studies be carried out where the treated fish samples would be stored at lower temperature, say 4°C and the storage period of the experiment should be extended for a clearer result since there was a trend.

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