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

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Abstract

Garlic powder was obtained through dry blending and sun-drying while garlic paste was produced by hand grating. Garlic oil was extracted from fresh garlic through hydro-distillation process. Ninety freshly killed *Clarias gariepinus* (250±20 g were procured and were gutted, washed, and spices with garlic paste, powder and oil. Fifteen fish samples were treated with garlic paste at three different concentrations (0, 10 and 30 g garlic paste/kg of fish). Each garlic paste concentration was applied to five fish. A similar process was used for the treatment of gutted fish samples in garlic powder (3 experimental units) and garlic oil (3 experimental units). The experimental setup is as follows: fifteen fish samples were spiced with garlic paste at 0 grams garlic paste/kg of fish (control), 10 grams garlic paste/kg of fish and 30 grams garlic paste/kg of fish. Another fifteen gutted fish samples were spiced with garlic powder at a concentration of 0 grams garlic powder/kg of fish (control), 5 grams garlic powder/kg of fish and 10 grams garlic powder/kg of fish. The last set of experimental unit had fifteen gutted fish samples treated with garlic oil at a concentration of 0 ml garlic oil/kg of fish (control), 1 ml garlic oil/kg of fish and 1.5 ml garlic oil/kg of fish. The treatments were replicated twice. The fish were smoked using a gas-powered smoking kiln at 85°C for 6 hours. Thiobarbituric acid (TBA), peroxide value (PV), microbiological and sensory analyses were performed to investigate quality changes and to determine the shelf stability of the products. The lowest TBA (23.02 ± 3.14 MDA/kg) and Peroxide (10.46 ± 2.31 mEq/kg) values were recorded in 1.0 ml garlic extract (oil) treated samples, while the highest TBA (31.27 ± 5.01 MDA/kg) and peroxide value (14.25 ± 3.41 mEq/kg) occurred in the control. However, TBA value, peroxide value and microbial count were not significantly different ($P>0.05$) among the treatments. Organoleptic assessment among treatments was significant ($p<0.05$). The study showed that garlic products could extend shelf life of *Clarias gariepinus*.

Keywords: Garlic, *Clarias gariepinus*, thiobarbituric acid, peroxide value, microbiological, organoleptic.

Introduction

The value of fish to man cannot be overemphasized. It is an excellent source of high quality protein (Kumolu - Johnson and Ndimele, 2001). Fish are a cheap source of protein. It has little or no religious or cultural rejection, which gives it an advantage over pork. Fish protein compares favourably with that of eggs, milk and meat in its amino acid composition (FAO, 2000), and in fact has higher

level of essential sulphur-containing amino acids such as cysteine, methionine and lysine, which are limiting in some legumes and most cereal-based diets (Evranus, 1993). Fish is low in saturated fatty acids. Oily fish in particular is an excellent source of long-chain omega-3 fatty acids.

However, fish is one of the most perishable of all foods and a medium for growth of micro-organisms. Igene (1983) reported at ambient temperature, spoilage is rapid; fish will spoil within 12-20 hours, depending on species and method of capture. Fish invariably becomes putrid within a few hours of capture unless they are subjected to some form of processing or preservation. Therefore, preservation techniques are needed to prevent fish spoilage and extend their shelf life. They are designed to inhibit the activity of spoilage bacteria and the metabolic changes that result in the loss of fish quality. Spoilage bacteria are the specific bacteria that produce the unpleasant odours and flavours associated with spoilt fish. To flourish, bacteria need the right temperature, sufficient water and oxygen, and surroundings that are not too acidic. Preservation techniques work by interrupting one or more of these needs (Ali *et al*., 2005).

In Nigeria, only a negligible proportion of the fish caught in inland waters is market fresh. A greater portion is preserved by smoking and sundrying (Ikeme and Bhandary, 2001). Fish smoking is the process where volatiles from thermal combustion of the wood or charcoal penetrate fish flesh (Ligia, 2002). Smoking is the major means of preservation in Nigeria, as the absence of advanced preservation technologies such as fermentation canning and modified atmosphere packaging of fish, poses a worrying concern. Hence, there is need to improve the traditional fish smoking methods in order to prolong smoked fish shelf life. One strategy that can help to achieve this is the use of antioxidants.
The use of synthetic antioxidants has been very effective in controlling rancidity in foods, but consumers require more natural products with lower chemical treatment because it may alter the nutritional and sensory properties. Spices are edible plant materials that possess anti-oxidative, antiseptic and bacterio-static properties. Garlic is chosen because is used as ingredients in some Nigerian cooked foods and is readily available. Historically, garlic has been used for centuries worldwide by various societies to combat infections. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic (Ranjan *et al.,* 2012). Garlic exhibit a broad antibiotic activity against both gram negative and gram positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Rlebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Helicobacter*, *Pylori* (Durairaj *et al.,* 2009) and on diarrheagenic organisms (Egbobor *et al.,* 2007). The raw juice of garlic was effective against many common pathogenic bacteria, strains that have become resistant to antibiotics and also prevents toxin production by some pathogenic strains (Bongiorno *et al.,* 2008). Allicin, the active ingredient of garlic acts by partial inhibition of DNA and protein synthesis and also total inhibition of RNA synthesis as a primary target (Alli *et al*., 2011). Similar to ampicillin, garlic inhibit cell wall synthesis by inhibiting transpeptidation enzyme involved in cross linking of polysaccharide chain of bacterial cell wall and also activate cyclic enzyme (Alli *et al*., 2011).

The aim of this study is to determine the antioxidative and antimicrobial effects of different forms of garlic (paste, powder and oil) on the shelf life of hot-smoked *Clarias gariepinus*

Materials and Methods

Fish Samples and Processing

Clarias gariepinus procured from a reputable farm in Lagos, Nigeria with an average weight of 250±20 g were transported live in large plastic containers full of water to the Post-Harvest Research Laboratory, Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos State, Nigeria.

The fishes were degutted and washed thoroughly with clean water. The fish samples were divided into seven batches of ten pieces each. Fresh garlic (*Allium sativum),* were bought from Alaba market, Ojo, Lagos, Nigeria and the outer coat was scrapped off and cleaned properly. The garlic was divided into three parts: a part was grounded properly into fine paste, another was

grounded into powder form and oil was extracted from the third part through the hydro-distillation process.

The fish samples were spiced with garlic paste at 0 grams garlic paste/kg of fish (control), 10 grams garlic paste/kg of fish and 30 grams garlic paste/kg of fish. Garlic powder was applied at a concentration of 0 grams garlic powder/kg of fish (control), 5 grams garlic powder/kg of fish and 10 grams garlic powder/kg of fish, while the garlic oil was applied at a concentration of 0 ml garlic oil/kg of fish (control), 1 ml garlic oil/kg of fish and 1.5 ml garlic oil/kg of fish. Ten fish (5 fish per replicate) from each treatment were smoked. The fish were smoked using a gas powered smoking kiln at 85^{0}C for 6 hours (Ikeme *et al*., 2001).

After smoking, each treatment was arranged in different basket, stored at room temperature for 8 weeks. The fish samples were subjected to chemical, microbiological analyses and organoleptic assessments during the 8 weeks of storage.

Peroxide Value Analysis

The oxidative stability of the samples was measured using titrimetric determination of the amount of peroxide and hydro-peroxide group (the initial products of lipid oxidation) according to Association of Official Analytic Chemist (AOAC, 1995). 10 grams of fish samples was added to 1 gram powdered potassium iodide and 20 cm^3 solvent mixture (2 volume of glacial acetic acid+ 1 volume of chloroform) placed in boiling water for 30 seconds. The content was then poured into a flask containing 20 cm^3 of potassium iodide solution (5%) and titrated against 0.002N sodium thio-sulphate using starch as indicator. Peroxide was calculated and expressed as milli-equivalent peroxide per kilogram of sample.

Peroxide value (Meq/kg) = (S-B) (N) / Unit of sample

Where B= titration of blank; S= titration of sample;
N= normality of sodium thio-sulphate.

Thio Barbituric Acid Analysis

The oxidative stability of the samples was measured according to AOAC (1995). 10 grams of the fish sample was macerated in 10 cm^3 of water for 2 minutes and washed into distillation flask with 47.5 cm^3 of water. 2.5 cm^3 of 4m hydrochloric acid (HCl) was added to bring the pH to 1.5. The flask was heated with an electric mantle until 50 cm^3 of distillate is collected after 10 minutes. 5 cm^3 of the distillate was pipette into a glass stoppered tube and filtrated with TBA reagent (0.2883 gram/100 cm of 90% glacial acetic acid). The test tube was cooled in water for 10 minutes and the absorbance measured against the blank at 538 nm using UV-VIS Spectro-photometer (model UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg sample.

Microbial Limit Test For Smoked Samples

The total coliform count was determined according to the method of Fawole and Oso (1995).

Sample preparation

5 grams of fish from each treatments were weighed on analytical balance previously disinfected with 10% absolute alcohol, they were then transferred aseptically into 45 ml of distilled water to form 1/10 dilution and 1/100 dilution was also made. One ml sterile syringe was used to dispense the samples into a sterile Petri-dish aseptically and 20 ml of SDA in water bath was poured into the same Petri dish to mix the SDA with 1 ml of the samples. They were thoroughly mixed and allowed to settle on the bench. The Petri dish was incubated at room temperature for 5 days. The number of colony forming unit was read on each plate and recorded against each dilution.

Organoleptic Assessment

Subjective evaluation of the product quality was carried out in the accordance with Poste *et al.* (1991) by a panel of 5. Coded samples accompanied by questionnaires were presented to the panelists. Quality attributes studied include; Appearance, Taste, Texture, Smell, Colour and General Acceptance. The hedonic scale used was from 10 (data were transformed into scale as follows A (excellent) 10, B (good) 8, C (fair), 6, D (unsatisfactory) 4, E (unacceptable) 2. Scoring was done on a weekly basis for eight weeks.

Statistical Analysis

Analysis of variance (ANOVA) was applied to the treatment value obtained using SPSS V. 17.0 for Windows. Statistical significance was set at $p < 0.05$. Fisher's Least Significant Difference was used to separate differences in treatment means.

Results

Table 1 below shows that the highest value (31.27±5.01 mg/ kg) of TBA was recorded in the control, while the lowest value (23.02±3.14 mg/ kg) was observed in the sample treated with 1 ml of garlic oil/kg of fish. The highest peroxide value (14.25±3.41 mEq/kg) was also recorded in the control, while the lowest value (10.46±2.31 mEq/kg) was observed in the sample treated with 1 ml of garlic oil/kg of fish.

Organoleptic Analysis

The result of the organoleptic assessments of the smoked *Clarias gariepinus* during the 8 week storage period is shown in Table 2. The report shows that the sample treated with ginger oil/ kg of fish received the highest panel score. There was significant difference ($p>0.05$) among the treatments in all the organoleptic parameters treated (Table 2).

Discussion

The mean peroxide value in all the samples was below the considered limit for fatty foods (25 mEq/kg) (Evranuz, 1993). The peroxide value decreased with an increase in the concentration of garlic. It was observed that at the fourth week, the peroxide values of all the concentrations and the control was not yet rancid, this means that it is still acceptable, but by the fifth week the peroxide value of the control had gone up this explains the indication of rancidity. But between the fifth and the sixth week there was an increase in the values especially for the control and by the eight week there was no more increase in the values. This explains that by the sixth week rancidity has totally set in and the fish is no more acceptable. It was observed that the samples treated with 1ml of

Table 1: Mean Thiobarbituric acid TBA, peroxide and microbial growth (Log10 CFU/G of fish sample) for 8-weeks storage period

Treatment	Tba (mg/kg)	Peroxide (meq/kg)	Microbial count (cfu)
CONTROL	31.27±5.01^{a}	14.25±3.41^{a}	2.82±0.19^{a}
Garlic powder 5g/kg	26.66±2.74^{a}	11.51±2.28^{a}	3.03±0.16^{a}
Garlic powder 10g/kg	27.49±3.51^{a}	12.06±2.62^{a}	3.08±0.18^{a}
Garlic oil 1ml/kg	23.02±3.14^{a}	10.46±2.31^{a}	2.93±0.22^{a}
Garlic oil 1.5ml/kg	23.32±2.93^{a}	10.55±2.25^{a}	3.03±0.19^{a}
Garlic paste 10g/kg	25.19±3.44^{a}	11.44±2.56^{a}	3.22±0.21^{a}
Garlic paste 30g/kg	26.04±3.09^{a}	11.31±2.33^{a}	2.97±0.18^{a}

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

Table 2: Organoleptic analysis of garlic treated *Clarias gariepinus* after 8-weeks storage period at 25-30^{0}C

Treatments	**Taste**	**Texture**	**Color**	**Smell**	**Appearance**	**General Acceptance**
Control	6.600±1.61^{b}	7.040±1.60^{b}	6.200±1.50^{b}	6.280±1.50^{b}	7.040±1.50^{b}	6.960±1.56^{b}
Garlic oil (1ml/kg)	7.840±1.63^{a}	7.960±1.56^{ac}	7.360±1.50^{a}	7.120±1.49^{ab}	8.040±1.47^{ac}	8.200±1.49^{c}
Garlic oil (1.5ml/kg)	7.840±1.65^{a}	7.920±1.60^{a}	7.760±1.58^{a}	7.680±1.53^{a}	8.180±1.45^{c}	8.220±1.63^{c}
Garlic paste (10g/kg)	7.983±1.29^{a}	7.197±1.09^{ab}	7.012±1.45^{b}	7.658±1.19^{a}	7.872±1.06^{a}	7.974±1.28^{a}
Garlic paste (30g/kg)	7.965±1.14^{a}	7.360±1.03^{a}	7.966±1.22^{a}	7.602±1.41^{a}	7.521±1.23^{a}	7.844 ± 1.39^{a}
Garlic powder (5g/kg)	7.221±1.31^{a}	7.486±1.09^{a}	7.288±1.33^{a}	7.683±1.29^{a}	7.599±1.18^{a}	7.601±1.05^{a}
Garlic powder (10g/kg)	7.622±1.35^{a}	7.429±1.49^{a}	7.609±1.11^{a}	7.408±1.42^{a}	7.307±1.56^{a}	7.703±1.19^{a}

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

garlic oil/kg of fish had the best oxidative stability (10.46±2.31 mEq/kg) as shown in Table 1. The samples treated with garlic oil had relatively lower peroxide value compared to the ones treated with paste (11.31±2.33) and powder (11.51±2.28). This means that 1ml of garlic oil worked better as a preservative although it was not significant.

The TBA value also decreased with increase in the concentration of garlic. It was observed in the fifth week that the fish in the control has started deteriorating and this reflects the high TBA value. Between the sixth and eight week, it reached its peak indicating that spoilage had fully set in by the sixth week. The lowest value (23.02±3.14 mg/ kg) was recorded in the samples treated with 1 ml of garlic oil/kg of fish, followed by 10g/kg of garlic paste (25.19±3.44 mg/kg) and 5g/kg of garlic powder (26.66±2.74 mg/kg). It can be deduced from the results above that garlic oil should be preferred to garlic paste and garlic powder in retarding rancidity in hot smoked catfish. These results are in agreement with the studies of Kumolu-Johnson and Ndimele (2011) where it was observed that there was a reduction in microbial proliferation and lipid oxidation in the samples treated with garlic paste. Similar results were reported in Ikeme and Bhandary (2001) and Salam *et al.,* (2004) where ginger and garlic paste were effective in retarding the development of oxidative rancidity in mackerel, *Scomber scombrus* and in chicken sausage respectively, and the effectiveness of the spice was directly related to their concentration.

Similar findings were reported by Çakli *et al.,* (2005), Yanar *et al.* (2006), and İzci (2010). The increase in the TBA value during fish storage has been demonstrated in previous studies; Tokur *et al.,* (2006) for fish fingers made from *Cyprinus carpio,* İzci (2010) for fish fingers made from *Carassius gibelio,* Patır *et al.,* (2009) for fish fingers produced from shrimp, Yanar and Fenercioglu (1999) for fish balls made from carp, Boran and Köse (2007) for fish balls made from *Merlangius merlangus*. These results also indicate that garlic which is a 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).

The weekly microbial load measured in $\log_{10}$ $Cfug^{-1}$ fish, revealed that treated samples had lower microbial growth than the untreated samples (control) in the first week. However in subsequent weeks, the control had relatively lower values. The highest microbial load (3.22±0.21 $\log_{10}$ $Cfug^{-1}$) was recorded in the sample with 10g garlic paste/kg of fish. This could be due to the fact that the sample was in paste, which may be suitable for microbial growth. The results of organoleptic evaluation for the smoked catfish samples were presented in Table 2. There was significant difference ($P < 0.05$) in the tested parameters. The panel rated all the fish samples high during the first six weeks, but by the seventh week, the control samples received a lower panel score relative to the treated samples, especially in appearance and smell. The samples treated with garlic oil had the highest panel score as shown in Table 2. This implies that garlic does not only retard oxidative rancidity and microbial proliferation, but also increases the sensory qualities of hot smoked catfish.

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