



The Effects of Garlic Extract on the Proximate Composition and Microbial Load of Hot and Cold-Smoked *Clupea harangus* (Atlantic Herring Fish)

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Authors' contributions

This work was carried out in collaboration among all authors. Author STF carried out all laboratories analysis and performed the statistical analysis. Author BAA wrote the first draft of the manuscript, managed the literature searches and edited the manuscript. Author SOB conceived and designed the study. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to determine the effect of garlic extract on the proximate composition and microbial activity of cold and hot smoked *Clupea harangus* (Atlantic herring fish). In the study, fresh atlantic herring was thawed, eviscerated, weighed, washed properly and folded. Garlic extract was prepared at 100 ml, 75 ml, 50 ml and control with no garlic extract, each treatments were prepared in duplicates and in three batches. The folded atlantic herring was dipped into the garlic extract at the different concentration, the first batch of treatments was oven dried for 40°C for 6 hours (cold smoked), the second batch was oven dried for 65°C for 12 hours (cold smoked) and the third batch was oven dried for 75°C for 18 hours (hot smoked). After oven drying, it was allowed to cool for 20 minutes, samples for microbial analysis was immediately taken in a foil paper and stored in the

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fridge for a month while the remaining sample was immediately used for proximate analysis. At all hours of drying period, the moisture content decreases and protein content increases with increasing concentration of garlic extract, the lipid content was highest at 75 ml garlic extract and the ash content was highest at 50 ml garlic extract. Total bacteria count decreases with increasing concentration of garlic, increasing temperature and increasing hour of drying. No fungi growth was recorded at all hours of drying period.

Keywords: Proximate composition; microbial load; oven drying; garlic extract; *Clupea harangus*.

1. INTRODUCTION

An aquatic organism with adaptive physical features: mouth, operculum (gill cover) fins, eyes, lateral lines, scales, nostrils and barbell, among others, which enables it to live conveniently in water is called Fish. Major diets of people particularly in the developing countries contains fish which serves as a major source of food for humans, providing a high percentage of the protein, fats and fat-soluble vitamins intake. Also, a source of valuable medicinal, feeding and technical products is fish. It is a cheap source of animal protein compared to other source with little or no religious rejection [1]. The richest source of iodine in human diet are marine and shell fish [2]. Fish is a product that decays easily, in order to prolong its storage, it requires proper processing and storage [3]. In addition, nutritional loss can be caused by insufficient handling and processing methods. Over the years, in Nigeria and other parts of the world different kinds of methods such as: Chilling, freezing, salting, drying, canning and smoking have been used to prolong the storage period of fish [4]. These processing methods have their advantages and disadvantages which dictates their usage in particular parts of the world. Despite the success of fish smoking method to increase the storage period and nutritional properties, the high rate of post-harvest loss especially in the sub-Saharan African is still increasing. This makes it necessary to ensure methods that will prevent lipid oxidation and delay microbial activities [4]. The utilization of plants extracts as natural antioxidants has been employed due to global restriction on the use of synthetic substances, also plant extracts rich in antioxidant helps to preserve food [5]. In reference to food science and technology plants such as garlic and ginger does not only contribute to the aroma and taste of food but also contains different bioactive substances which are of great importance. These plants products may be used individually or combined together to prevent spoilage by deterring microbial proliferation, hence, they are called a bio preservative [6]. The ability of these plants products: garlic and ginger, to delay

spoilage is due to compounds such as phenolic compounds, tannins, essential oils, saponins and flavonoids [5]. In order to reduce fish post-harvest loss, and increase the nutritional quality of fish, this research critically examines the effects of garlic extract at different concentration levels and hour of drying period on the proximate composition and microbial load of *Clupea harangus*.

2. MATERIALS AND METHODS

2.1 Sources of Raw Material

The principal raw materials used in this research work are Atlantic herring fish (*Clupea harangus*) and garlic. The fish was purchased from a local cold store at Oja oba market in Akure town, Ondo state, Nigeria transported within 30 minutes in polythene bag with ice to the fisheries laboratory at Federal University of Technology Akure, Ondo state, Nigeria.

2.2 Sample Preparation

2.2.1 Preparation of garlic

The dry skin of the fresh garlic was peeled off before use. 600 g of garlic was weighed, washed properly with distilled water and blended with 400 ml of distilled water using a kitchen blender. The blended mixture was sieved and 700 ml of garlic extract was collected.

2.2.2 Preparation of fish

Each of the 12 raw frozen mackerel fish was allowed to thaw at room temperature. The fish were gutted (the gills was removed), eviscerated (the sperms, eggs and intestines was removed), washed properly and folded. The average weight of the gutted and eviscerated mackerel fish was 152.80 ± 3.42 kg.

2.2.3 Treatments preparation

Four treatments were prepared in three different batches, the folded fish was immersed into the treatments for two hours, an hour for each sides.

Sample treatment: Table I. Treatments and meanings

Treatment	Explanations
Control	Samples without garlic treatment
Treatment 1	50 ml of garlic extract and 100 ml of distilled water
Treatment 2	75 ml of garlic extract and 100 ml of distilled water
Treatment 3	100 ml of garlic extract and 100 ml of distilled water

2.2.4 Heating and cooling

The first batch of the treatment was oven dried for 6 hours at 40°C which represents the cold smoked fish, the second batch was oven dried for 12 hours at 65°C which represents the cold smoked fish and the third batch was oven dried for 18 hours at 75°C which represents the hot smoked fish. After drying, the samples were allowed to cool for 20 minutes.

2.2.5 Storage and analysis

After cooling, 3 g of fish sample was immediately collected from the treatments and wrapped in aluminum foil for microbial analysis, the collected samples for microbial analysis were stored in the freezer for 30 days. The remaining treatments was immediately analyzed for proximate composition.

2.3 Analytical Procedure

2.3.1 Proximate analysis determination

The proximate composition of the oven dried fish were performed according to the procedures of American Oil Chemists' Society (A.O.C.S) [7] and discussed briefly

2.3.1.1 Moisture content

Finely ground (2 g) of sample was weighed into a petri dish of known weight. It was dried in a hot air oven at 105°C for 4 hours; after the time has elapsed the sample was brought out and placed inside a desiccator for cooling. The moisture content was calculated as:

$$\text{Moisture \%} = [(W_1 - W_2) \times 100] \div W$$

W = Weight of sample

W₁ = weight of sample + weight of Petri dish.

W₂ = Weight of dried sample + weight of Petri dish.

2.3.1.2 Ash

5 g of sample was weighed and transferred in pre-weighed porcelain crucible. The weighed

sample was burned till smoke ceases. The crucible was then transferred to muffle furnace maintained at 550°C and incinerated until light grey ash was obtained. The crucible was then cooled in desiccator and weighed. The results were reported on dry weight basis.

$$\text{Ash \%} = [(W_1 - W_2) \times 100] \div W$$

W = Weight of sample

W₁ = weight of sample + weight of crucible.

W₂ = Weight of ash + weight of petri dish (after ashing)

2.3.1.3 Lipid

The dried samples were ground in a blender and 5 g of sample was weighed accurately and transferred to the thimble and defatted with petroleum ether in soxhlet apparatus for 6-8 hours at 80°C. The residue was procured and ether was removed by evaporation. The loss in weight of thimble was estimated as loss of lipids from sample and expressed as percent lipids in sample.

$$\text{Fat \%} = [\text{loss in weight of sample} \times 100] \div \text{weight of sample}$$

2.3.1.4 Protein

2 g of sample was weighed and put into the digestion tube. 20 ml of concentrated sulphuric acid (98%) and 2 tablets of digestion mixture as catalyst was added into the digestion tube. The digestion was carried out for 3-4 h (till the digested contents attained transparent colour). The digested material was then allowed to cool at room temperature and diluted to a final volume of 50 ml. The ammonia trapped in H₂SO₄ was liberated by adding 40% NaOH solution through distillation and collected in a flask containing 4% boric acid solution, possessing methyl indicator and titrated against standard 0.1 N H₂SO₄ solution.

Calculation:

$$\% \text{ Total Nitrogen} = 14.01 \times (\text{sample titre} - \text{blank titre}) \times N / 10 \times \text{sample weight}$$

N = Normality of acid

i.e protein (crude) = % Nitrogen X
Conversion factor

Conversion factor = 6.25

2.3.2 Microbial evaluation

2.3.2.1 Determination of total viable count

The total viable count of each sample was determined by pour plate method using nutrient agar as the culture medium by Laboratory methods in food and dairy microbiology [8]. The test tubes were washed properly, 10 ml of distilled water was measured into the test tubes representing the stock and 9 ml in the other test tubes and covered at the top with a stopper made of cotton wool and aluminum foil. The Agar was prepared. The agar and the test tubes were then autoclaved for sterilization at 121°C for 15 minutes. The fish was grounded using a mortar and pestle. 1 g of the fish sample was weighed into the first test tube which is the stock and it was mixed thoroughly by shaking. Then 1 ml of the stock solution was transferred to the test tube and shaken, which was dilution 1 and it was mixed properly, 1 ml of dilution 1 was transferred into the next test tube which was dilution 2, serial dilution was continued until dilution 7. Thereafter, 0.1 ml was transferred from the stock test tube, dilution 5 and dilution 6 test tube into corresponding plate, then 15 ml of sterile nutrient agar medium was poured into the plates and mixed thoroughly by carefully rocking the plates and then allowed to gel. The plates were incubated upside down at 37°C for 24 hours after which colonies formed were counted and expressed as colony forming units per gram.

2.3.2.2 Mould count determination

The mould count for the fish was done according to the method of Laboratory methods in food and dairy microbiology [8]. 10 ml of distilled water was measured into the test tubes representing the stock and 9 ml in the other test tubes and covered at the top with a stopper made of cotton wool and aluminum foil. 65 g of potato dextrose agar was weighed out and dissolved in 1 litre of distilled water in a clean 500 ml flat bottom flasks and then sterilized in the autoclave at 15 psi for 20 minutes at 121°C. It was then allowed to cool a little bit in a desiccator and a tablet of antibiotics was added. The test tubes were then autoclaved for sterilization at 121°C for 15

minutes. The fish was grounded using a mortar and pestle. 1 g of the fish sample was weighed into the first test tube which is the stock and it was mixed thoroughly by shaking. Then 1 ml of the stock solution was transferred to the test tube and shaken, which was dilution 1 and it was mixed properly, 1 ml of dilution 1 was transferred into the next test tube and mixed properly which was dilution 2 and 1 ml of dilution 2 was transferred to the next test tube and mixed properly which was dilution 3. Thereafter, 0.1 ml was transferred from the stock test tube, dilution 2 and dilution 3 test tube into corresponding plate, then 15 ml of sterile potato dextrose agar medium was poured into the plates and mixed thoroughly by carefully rocking the plates and then allowed to gel. The plated dishes were incubated upside down for 48 hours at 30°C. The mould colonies on each plate were enumerated and calculated as colony forming units (CFU) per g of sample (cf u/g = No of colonies x Dilution factor). The moulds on the PDA were identified by their morphological features.

2.4 Experimental Design and Statistical Analysis

The treatments were arranged in a Completely Randomised Design (CRD). All analyses were conducted in duplicates. Data obtained were subjected to one-way analysis of variance (ANOVA) using the R software. Tukey HSD test was used to separate the means with significance level at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

The research shows that at all hours of drying (6 hours, 12 hours, and 18 hours) garlic extract has a great influence ($p < 0.05$) on the proximate composition of the fish samples. Across all hours drying period the moisture content of the fish samples decreases with increasing garlic extract concentration, the protein content of the fish samples increases with increasing concentration of garlic extract, the lipid content of the fish samples was highest at 75 ml of garlic extract, and the ash content of the fish samples was highest at 50 ml of garlic extract. This observation is in agreement with the findings of [9,10,11,12,13]. From Tables 1 and 2, it can also be deduced that at 6 hours and 12 hours of drying, the ash content at 50 ml of garlic extract was significantly different from other treatments. While from Table 3, the ash content at 50 ml of garlic extract was not significantly different from

other treatments. This indicates that, at 18 hours of drying, the garlic extract does not actively improve the ash content of the fish sample. The result of the proximate composition also confirms with Mohajira et al. [14], who observed that, the mixture of garlic extract and salt was more effective in reducing the moisture content, increasing the protein, lipid, and ash content of smoked catfish when compared to smoked catfish that were dipped in just salt concentration.

3.2 Microbial Evaluation

The microbial evaluation of garlic extract is in agreement with [14] and [15]. It exhibits antibacterial activity against a wide range of Gram-positive, Gram-negative and acid-fast bacteria which include *Bacillus*, *Clostridium*, *Enterococcus faecalis*, *Escherichia coli*, *Hafnia*, *Klebsiella*, *Lactobacillus acidophilus*, *Micrococcus*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella dysenteriae*, *S. flexneri*, *S. sonnei*, *Staphylococcus* and *Streptococcus* [15,16,17,18,19]. The fungi study agrees with [20]. *Aspergillus*, *Candida*, *Cryptococcus*, *Epidermophyton*, *Microsporum*, *Rhodotorula*, *Torulopsis*, *Trichophyton* and *Trichosporon* are all susceptible to garlic extracts. The lower growth rates during storage of oven dried sample also agree with the report of [21], that the drying

which takes place during smoking aids removal of water available for microbial growth, thereby retarding microbial growth while prolonging the product shelf life.

Garlic extract was significantly different across all treatments for all hours of drying period. Dilution 5 and 6 was used to evaluate bacterial count. Microbial evaluation indicates that bacteria count decreases with increasing concentration of garlic extract. This study agrees with [14,15,16,17,18]. These result of this research also correlates with Ibrahim et al [22] who discovered that garlic extract improved the shelf life and consumer acceptability of smoked *Clarias gariepinus*.

For all treatments at all hours of drying period, no fungi growth was observed. This indicates that garlic extract exhibit antifungi property and it is effective in preventive mould growth. This result agrees with the reports of [13,4,23,24,25]. This research also confirms with Omojowo et al. [26, 27] that garlic extract can be substituted for other synthetic antimicrobial agent like citric acid, potassium sorbate and sodium metabisulphate. In addition, the antifungi property agrees with Chuku [28], who reported that the fresh and dry samples of garlic recorded 100 percent inhibition on smoked catfish mycoflora.

Table 1. Proximate analysis of fish for 6 hours treatment

Concentration	Moisture content	Lipid content	Ash content	Protein content
Control	52.498±0.28 ^a	30.142±0.62 ^a	1.9600±0.10 ^a	6.4700±0.16 ^a
Treatment 1 (50 ml)	52.294±0.10 ^a	33.319±1.80 ^{ab}	2.7300±0.11 ^b	10.1600±0.62 ^{bcd}
Treatment 2 (75 ml)	50.101±0.35 ^{ab}	35.996±0.45 ^{bc}	1.3500±0.21 ^{cd}	10.0000±0.49 ^{cd}
Treatment 3 (100 ml)	49.317±1.33 ^b	33.623±1.24 ^{ac}	1.0100±0.13 ^d	11.2700±0.04 ^d

Mean values with different superscripts within the same column are significantly different (P < .05)

Table 2. Proximate analysis of fish at 12 hours treatment

Concentration	Moisture content	Lipid content	Ash content	Protein content
Control	34.565±0.18 ^a	37.130±1.21 ^a	4.577±0.11 ^a	11.3000±0.08 ^a
Treatment 1 (50 ml)	34.122±0.68 ^a	44.307±0.049 ^{bc}	5.263±0.08 ^b	14.8900±0.11 ^b
Treatment 2 (75 ml)	31.168±0.45 ^{bc}	46.463±0.34 ^c	3.567±0.08 ^{cd}	17.6800±0.06 ^c
Treatment 3 (100 ml)	30.385±0.50 ^c	40.610±0.10 ^d	3.603±0.05 ^d	21.5400±0.06 ^d

Mean values with different superscripts within the same column are significantly different (P < .05).

Table 3. Proximate analysis of fish at 18 hours of drying period

Concentration	Moisture content	Lipid content	Ash content	Protein content
Control	6.658±0.03 ^a	34.268±0.41 ^a	9.277±0.04 ^a	19.9600±0.12 ^a
Treatment 1 (50 ml)	6.454±0.04 ^a	43.444±0.08 ^b	9.963±0.04 ^a	23.1200±0.04 ^b
Treatment 2 (75 ml)	4.261±0.07 ^b	45.121±0.03 ^c	9.267±0.02 ^a	27.4100±0.03 ^c
Treatment 3 (100 ml)	3.477±0.09 ^c	37.748±0.10 ^d	8.303±0.57 ^a	31.1700±0.08 ^d

Mean values with different superscripts within the same row are significantly different (P < .05)

Table 4. Bacteria count for 6 hours treatment

Treatment	Stock	Dilution 5	Dilution 6
Control	Very numerous to count	15.5×10^8	7.8×10^9
50 ml	Very numerous to count	0.5×10^8	No bacteria growth was observed
75 ml	Very numerous to count	No bacteria growth was observed	No bacteria growth was observed
100 ml	Very numerous to count	No bacteria growth was observed	No bacteria growth was observed

Table 5. Bacteria count for 12 hours treatment

Treatment	Stock	Dilution 5	Dilution 6
Control	Very numerous to count	7.5×10^8	5.0×10^9
50 ml	Very numerous to count	No bacteria growth was observed	No bacteria growth was observed
75 ml	250	No bacteria growth was observed	No bacteria growth was observed
100 ml	No bacteria growth was observed	No bacteria growth was observed	No bacteria growth was observed

Table 6. Bacteria count for 18 hours treatment

Treatment	Stock	Dilution 5	Dilution 6
Control	Very numerous to count	4.5×10^8	3.0×10^9
50 ml	No bacteria growth was observed	No bacteria growth was observed	No bacteria growth was observed
75 ml	No bacteria growth was observed	No bacteria growth was observed	No bacteria growth was observed
100 ml	No bacteria growth was observed	No bacteria growth was observed	No bacteria growth was observed

Table 7. Fungi count for 6 hours treatment

Treatment	Stock	Dilution 2	Dilution 3
Control	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
100 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
75 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
50 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed

Table 8. Fungi count for 12 hours treatment

Treatment	Stock	Dilution 2	Dilution 3
Control	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
100 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
75 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
50 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed

Table 9. Fungi count for 18 hours treatment

Treatment	Stock	Dilution 2	Dilution 3
Control	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
100 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
75 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed
50 ml	No fungi growth was observed	No fungi growth was observed	No fungi growth was observed

4. CONCLUSION

This research study clearly demonstrates that garlic (*Allium sativum*) extract have a great potential to increase the protein, lipid, ash content and to decrease the moisture content of *clupea harangus* (Atlantic herring). Also, garlic extract exhibit antimicrobial properties that can hinder the growth of bacteria and fungi, thereby prolonging the shelf life of *clupea harangus*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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