



Comparative effects of Lime and Garlic extracts on the Bacterial Load and Nutritional Quality of processed Shrimps (*Penaeus notialis*) from Sombreiro River, River State

K. C. Nwaogwugwu^{1*}, B. J. O. Efiuvwevwere¹, O. K. Agwa¹
and N. U. Nwogwugwu²

¹Department of Microbiology, University of Port Harcourt, Nigeria.

²Department of Microbiology, Federal University of Technology, Owerri and World Bank African Center of Excellence for Oil Field Chemicals Research, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author KCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BJOE supervised and managed the analyses of the study. Authors NUN and OKA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Shrimps are highly valued worldwide. They deteriorate rapidly after harvest except preserved. This study was undertaken to determine the bacterial load and nutritional quality of shrimps subjected to 20% lime juice, 50% garlic extract and distilled water followed by smoking and storage at ambient (room) temperature for 28 days. Fresh shrimp samples from Sombreiro River in Degema Local Government Area of Rivers State were used for this study. The Shrimps were analyzed for total viable counts (TVCs), Coliform, Staphylococcal, Vibrio, Salmonella, and Shigella counts. The control samples had highest protein content of 21.2±0.018 immediately after treatment while after smoking, samples treated with lime and garlic had higher protein content; highest in samples treated with lime

*Corresponding author: E-mail: nwaogwugwu@gmail.com;

juice with protein content of 63.27 ± 1.20 , garlic-treated samples and control sample had protein content of 60.5 ± 2.49 and 51.27 ± 10.76 respectively at end of storage. Garlic-treated samples had highest ash content of 15.46 ± 0.11 , lime-treated samples and control had ash content of 13.29 ± 0.11 and 11.75 ± 0.11 at the end of storage period. There were slight increases in nutrient level with storage. Samples treated with lime juice had lowest bacterial count throughout the four weeks of storage. At day 0, samples treated with lime, garlic- treated samples and control had total viable count of 1.7×10^3 , 2.11×10^3 and 4.5×10^4 respectively, at the end of storage period. Lime-treated samples, garlic-treated samples and control had coliform count of 2.3×10^2 , 5.89×10^2 and 5.27×10^4 respectively at the end of storage period. Total Staphylococcal count for lime-treated samples, garlic-treated samples and control at the end storage period were 4.9×10^2 , 4.8×10^2 and 3.5×10^3 respectively. Samples treated with lime and garlic had no Vibrio, Salmonella and Shigella after smoking till end of storage. In this study 20% lime juice proved more effective against bacteria though with no significant difference ($p > 0.05$) and increased the nutritional value of smoked shrimps more than 50% garlic extract.

Keywords: *Penaeus notialis*; Nutritional quality; smoking.

1. INTRODUCTION

Shrimps are important seafoods globally and have high commercial value in export/international trade [1]. However, the nutritional qualities of shrimps deteriorate soon after harvest leading to great economic loss. Among seafoods, shrimps contribute about 20% by volume of the world seafood market. Shrimps were identified as a rich source of vitamin-B12, Selenium, w-3, highly unsaturated fatty acids (HUFA) and potent natural antioxidants. The nutritive values of edible marine organisms depend upon their biochemical composition, such as protein, amino acids, lipid, fatty acids, carbohydrate, vitamins and minerals. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body. Protein is essential for normal function, growth and maintenance of body tissues. Its content is considered to be an important tool for the evaluation of physiological standards. However, quantities of these constituents vary considerably within and between species, size, sexual condition, feeding, season and physical activity. Biological value of protein is obviously reflected upon its amino acid concentration. Amino acids are the building blocks of proteins and serve as body builders. Amino acids are important in osmoregulation and buffer capacity in the tissues of aquatic animals and some amino acids are involved in neurotransmission, it can be an important source of energy producing compounds. Lipid of shrimp contains mostly polyunsaturated fatty acids (EAs). These EAs available in shrimp provide health benefits for human e.g., eye (retina) and brain development and function. Among the body organic nutrients,

Carbohydrates are considered to be the first substances to be utilized for the synthesis of energy required for physiological activities. Carbohydrates serve as precursors for the synthesis of dispensable amino acids and certain nutrients, which are in free and bound state along with proteins as protein-bound sugars and glycogen [2]. The microbial load or the presence of bacterial pathogens in seafood is a good indication of the quality and the potential health risk they pose to consumers. These are considered to be the major organisms contributing to the rapid deterioration of shrimp quality [3].

Shrimps are sold and consumed in a variety of different forms, including fresh, frozen, breaded, cooked, dried and in paste form. In Rivers State, Shrimps are sold as smoked/dried or fresh delicacies. They are harvested mainly from rivers such as Gbile, Ololotoru, Andoni, Sombreiro. The fishermen usually harvest shrimps with locally crafted nets and after which they are taken to the traders for sale. They preserve these shrimps by freezing, although shortage of storage facilities could hamper storage of shrimps. Shrimps are also preserved by smoking, drying or boiling. Poor preservation approaches, lead to changes in the aesthetic values and make them unfit for consumption due to obvious microbial spoilage. Seafoods can be exposed to a wide range of hazards from the habitat, these hazards include, bacteria, viruses, parasites, natural toxins and chemical contaminants [4-5]. Contamination of habitats adversely affect the health of shrimps, as they become contaminated because they are filter-feeders, so they are able to concentrate any pathogenic spoilage microorganisms in water

and could infect consumers. Shrimps could become unfit for consumption following bacterial contamination due to hot climatic conditions (temperature over 30°C), lack of modern preservation infrastructure (for refrigeration and freezing), poor hygienic handling conditions as well as the long periods of time elapsed during transportation [6].

Smoking process depends on many factors which include surface area, water content and fat content of shrimp, temperature, relative humidity and air velocity. It is important to achieve required quality by optimizing temperature, humidity and moisture content through optimizing the smoking process for each species of shrimp. The quality of mechanical smoking kiln is related to the final water activity (*aw*) and amount of smoke deposition on smoked product. Most bacteria do not grow and multiply at *aw* values below 0.95 [7]. Simultaneously with reducing moisture content, smoking process deposits smoke on body surface of shrimp. Smoke is the combination of chemical substances, which have anti-microbial effects and thus inhibit microbial growth. So, the cause of the longer storage life of smoked shrimp is not due to drying effect of smoke only but also the preservative effects of the chemical compounds deposited on shrimp from smoke.

Application of plant extracts as natural antioxidants has gained appreciable interest because of their low cost and minimal side-effects. One of the plants with antimicrobial characteristics is garlic (*Allium sativum*). It is also a medicinal plant which has been used worldwide for a long time as a food preservative. It has the ability to boost immune system and cure high blood pressure. It is effective against bacterial, fungal and viral infections [8]. It has the ability to stimulate the lymphatic system which expedites the removal of waste products from the body. It is also considered an effective antioxidant to protect cells against free radical damage. It can help to prevent some forms of cancer, heart disease, strokes and viral infections. In Europe and India, it was used to treat common colds, hay fever and asthma. Garlic is nicknamed as Russian penicillin for its widespread use as a topical and systemic antimicrobial agent [9]. It was used by Greek physicians, Hippocrates and Galen to treat intestinal and extra-intestinal diseases; ancient Japanese and Chinese used it to treat headache, flu and sore throat. In Africa, particularly in Nigeria, it is used to treat

abdominal discomfort, diarrhea, otitis media and respiratory tract infections [9]. It also imparts flavor on food and could be eaten when fresh, cooked, or powdered form. One of the active ingredients in garlic is allicin which has antimicrobial activity against a wide range of gram negative and gram positive bacteria.

Lime juice is another plant extract also used to treat some illnesses. It is sometimes used as an active ingredient in plants to cure several illnesses. It is also capable of extending shelf stability of food products [10]. Limes (*Citrus aurantifolia*) are small citrus fruit either be sugar and citric acid content than lemons and feature an acidic sour taste. The nutritional profile includes information on a full array of nutrients including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. While these flavonoids have been shown to stop cell division in many cancer cells, they are perhaps most interesting for their antibiotic effects. Lime juice was also found to have a strong protective effect against cholera, in [10]. Citric acid, the major organic acid in these juices, was found to be responsible for inhibiting the growth of *Vibrios* [10].

This study was undertaken to evaluate the comparative effect of 20% lime juice and 50% garlic extract on the bacterial load and nutritional quality of shrimps.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study area is Sombreiro River, located in Rivers State. It is one of the rivers that drains the western part of Rivers state. It provides nursery and breeding ground for a wide range of fish species [11]. It is located in three local government areas of River State: Ogba/Egbema/Ndoni and Degemasour or sweet, sour limes possess a greater between latitude 60 30' and 700E and longitude 40 12' and 60 17' N. It takes source of the River Niger which rises from northern boundary of Rivers state with Imo state as one of the series of Niger Delta river which drains into the Atlantic Ocean and is connected to other rivers via creeks in the coastal area of the Niger Delta [11]. It is contained within the tropical rainforest although

the lower reach is within the brackish mangrove zone. It is fringed by riverside forest. Many human activities such as transportation, logging or cutting of timber, cutting of mangrove, dredging, fishing and others occur in this area. These are potential sources of pollution or contamination to the environment. There are also refuse dumps and run-offs into the river from the riverine communities. Also waste from the communities may constitute source of pollution to the river. All these contribute to the contamination of seafoods in the area.

2.2 Preparation of the Food Preservatives

One thousand shrimp samples were purchased from traders (based on prior arrangement) and placed in ice packs (40C). These were taken to the laboratory and analyzed.

The lime (*Citrus aurantifolia*) was washed using sterile distilled water and cut with sterile knife and the juice was aseptically pressed into a sterile container and twenty percent (v/v) lime juice was prepared by squeezing 100ml of the lime juice into the measuring cylinder and adding 400ml of water to it, to make 20% of lime juice.

Garlic bulbs were cleaned, washed, cut with sterile knife and blended with electric blender and 200 ml of it squeezed into the measuring cylinder and 200 ml of distilled water was added to it to make fifty percent (50%) concentration of the extract Bandna, [12].

2.3 Shrimp Sample Preparation and Smoking

The shrimp samples were washed in sterile distilled water and using a surgical dissecting blade while, wearing hand gloves, the head and intestines were separated, the tail portions of it were used for bacterial analysis and proximate composition before and after smoking. After the removal of the head and abdomen of the shrimp samples some tail portions were dipped in 20% (v/v) lime juice for 5mins, some were dipped in the 50% garlic extract, allowed to drain for 15 minutes and then smoked for 3hours at 78°C [13]. Some portions of the tail were not dipped in preservative to serve as the control sample. The shrimp samples both the control and the preserved samples were smoked. They were placed on a platform of wire gauze which was supported by a framework of perforated metal drum. The bottom of the metal drum was filled with sand while firewood was used to generate

fire and allowed to heat up for 15 minutes the temperature was measured using mercury-in-glass thermometer.

The raw sample with or without preservative treatments were analyzed for bacterial load and proximate composition. The bacterial analysis was carried out for 28 days at 7day intervals (0,7,14,21,28 days) while the proximate analysis was carried out on days 0 and 28(beginning and the end of storage).

2.4 Bacterial Analysis

The method of analysis used was that of Efiuvwevwere et al. [1]. Serial dilutions were prepared by homogenizing 25g of each sample in 225ml of 0.1% peptone water. 10-fold dilutions were prepared and spread-plated (0.1ml aliquot) in triplicate on surface-dried plate count agar, MacConkey agar, Mannitol salt agar, Thiosulphate-citrate bile agar, Salmonella-Shigella agar and incubated at 370C for 18-24hr. The plates were examined for colonial growth and enumeration of total viable, total coliform, total staphylococcal, Salmonella and Shigella counts.

2.5 Identification of Bacterial Isolates

Typical representative colonies were randomly picked from plates showing 25-250 colonies, purified, characterized using motility, Gram reaction, spore stain, catalase, coagulase, urease, citrate utilization, indole production, Methyl-Red (MR), Voges-Proskauer and sugar fermentation tests (triple sugar iron agar, glucose, sucrose, lactose and mannitol) and subsequently identified based on colonial, cellular and biochemical characteristics [14,15].

2.6 Proximate Analysis

Proximate Analysis of samples were done on fresh samples before treatment (control), immediately after treatment and then after smoking at day zero and day 28. The proximate analysis was done using the method of Kjeldahl's [16] for moisture, crude protein, crude lipid, crude fibre, ash content and carbohydrate [17].

2.7 Statistical Analysis

The obtained data from the study were subjected to statistical analysis using Statistical Package for Social Science (SPSS) Version 21.0 IBM Corp, Armonk, NY: [18], Analysis of variance

(ANOVA) was used to determine the statistical difference at p-value < 0.05.

3. RESULTS

Fig.1 below presents the total viable count of shrimp samples. Before smoking, control samples had highest count of 4.2×10^7 while lime-treated samples had lowest count of 1.8×10^7 . After smoking till end of storage lime-treated samples had lowest count of 3.04×10^4 followed by garlic-treated samples (3.27×10^4).

Garlic-treated samples (GAT) had lowest Coliform count before smoking (4.71×10^5) as shown in Fig. 2, but after smoking till end of storage Lime-treated samples (LT) had lowest coliform count (2.3×10^2) followed by GAT (5.89×10^2) while control had highest count of (3.2×10^4). In Fig. 3, LT and GAT had lower count of staphylococcal spp before smoking (3.82×10^4), (4.06×10^4) and after smoking and end of storage (4.9×10^2) and storage (4.8×10^2) respectively while control had highest count of 3.5×10^3 at end of storage. In Figs. 4,5 and 6, LT had lowest count of vibrio, salmonella and shigella before smoking and after smoking, at end of storage, LT and GAT had no growth while control sample had count of 3.6×10^3 , 4.56×10^3 and 4.56×10^2 respectively.

Tables.1-3 presents proximate composition of fresh shrimp samples immediately after treatment with preservatives, after smoking and under storage. Control sample had highest

protein content of 21.21 ± 0.018 before smoking while after smoking and end of storage LT had highest protein content of 61.55 ± 4.65 and 63.27 ± 5.98 respectively. Garlic-treated sample had highest ash content of 1.415 ± 0.07 before smoking, after smoking (13.13 ± 1.16) and at end of storage (15.46 ± 0.11). LT had highest carbohydrate content of 0.485 ± 0.007 before smoking while after smoking GAT had highest carbohydrate content of 5.08 ± 1.02 and (5.39 ± 0.70) at the end of storage. Before smoking, LT and GAT had moisture content of 81.140 ± 0.014 and 77.765 ± 0.092 respectively while after smoking and at end of storage LT had lowest moisture content of 6.41 ± 0.01 and 4.71 ± 0.21 respectively. After smoking and at end of storage Control had highest moisture content of 15.17 ± 0.01 and 15.15 ± 0.01 respectively.

4. DISCUSSION

The bacteriological qualities of the shrimps varied due to the preservatives used as shown in Fig.1 to Fig.6. The preservatives used in this study help in the reduction of bacterial load of shrimp samples in this study, there is a significant difference ($p < 0.05$) in the microbial load of the different samples. The result of the total viable count detected in fresh shrimp samples (control) from Sombreiro River was high beyond the acceptable limit ($> 10^5$ cfu/g) recommended by International Commission on Microbiological Specifications for Food [19]. Lime was most effective in reducing the microbial load of shrimps immediately

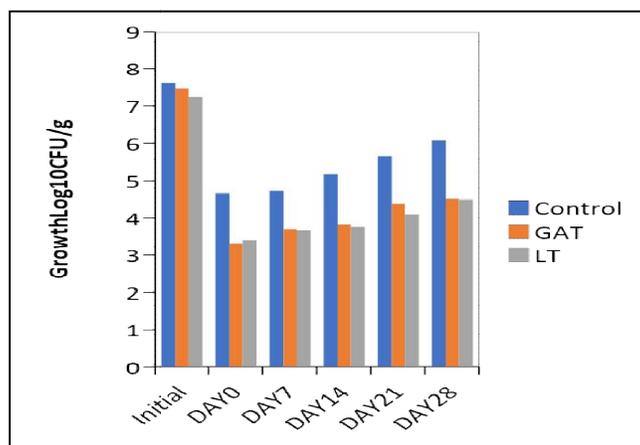


Fig. 1. Total viable count in Shrimp samples (Log10CFU/g)
 GAT=garlic- treated sample,
 LT=lime-treated sample

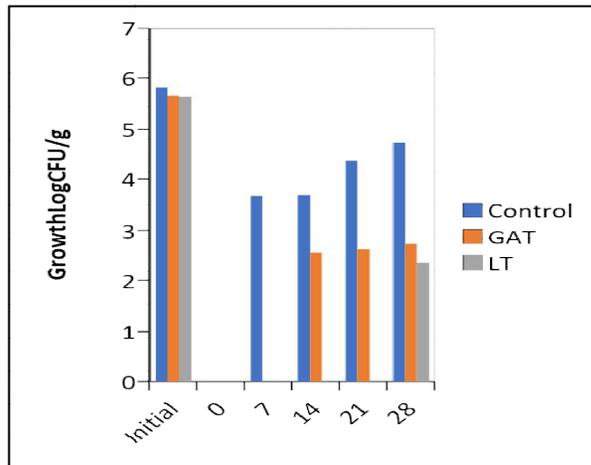


Fig. 2. Coliform count in Shrimp samples (Log10CFU/g)

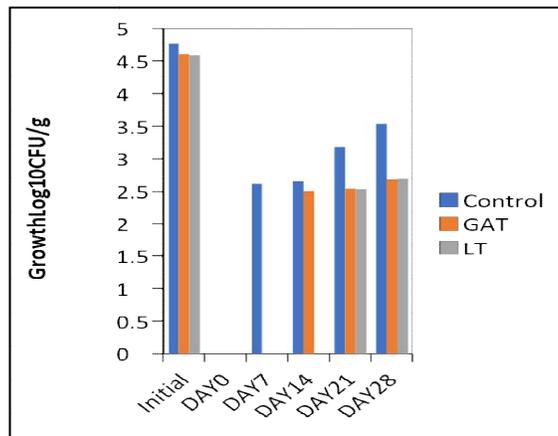


Fig. 3. Staphylococcal count in shrimp sample (Log10CFU/g)

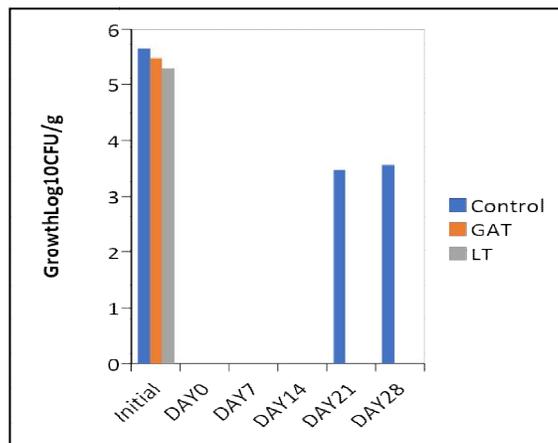


Fig. 4. Vibrio count in Shrimp samples (Log10CFU/g)

after preservative treatment (1.8×10^7) and after smoking at day 28 (3.04×10^4) followed by garlic treated sample (GAT) with a load of 3.27×10^4 while the control sample had the highest initial microbial load of 4.2×10^7 and highest load throughout the storage period 1.2×10^6 . Abu et al., [20], reported a total count ranging from 2.04×10^2 to 4.5×10^5 cfu/g in shrimp (*Penaeus monodon*). These disagree with the findings of this study, as the control sample had a total viable count of 4.2×10^7 .

In case of total coliform count, significant reduction occurred after treatment, smoking and end of storage. Lime was most effective in

reducing the coliform load from 4.94×10^5 to 2.3×10^2 after treatment and after smoking till end of storage. The control sample had highest initial coliform count of 6.62×10^5 before smoking and the highest coliform at end of storage (5.27×10^4). This result disagrees with the work of Tairu et al. [21] who reported no growth in smoked fish treated with garlic and ginger after 4 weeks of storage reducing the coliform count from 9.0×10^6 to 0. This high load of coliform suggests contamination of shrimp habitats with sewage and contamination during handling. The result of staphylococcal count revealed count of 104 in all the samples. The control sample had the highest staphylococcal count

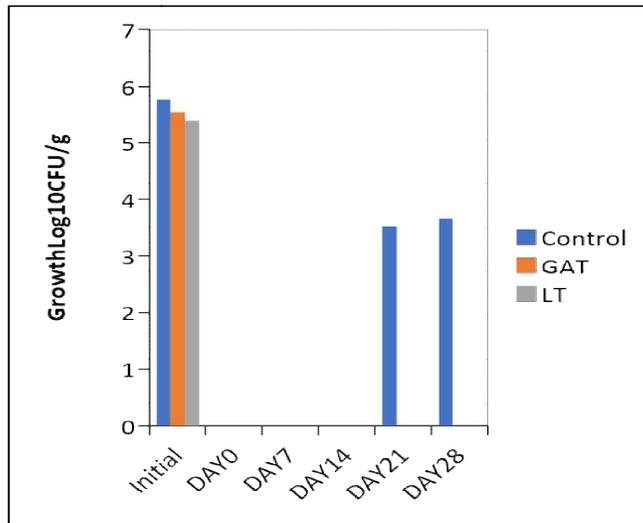


Fig.5. Salmonella count in Shrimp samples (Log10CFU/g)

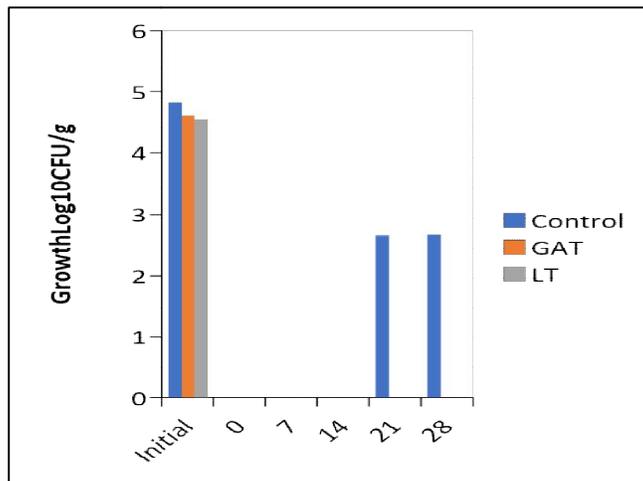


Fig.6. Shigella count in Shrimp samples (Log10CFU/g)

Table 1. Proximate composition of fresh shrimp samples

Treatment	Ash(%)	Crude.Lipid(%)	Crude Fibre(%)	Cho(%)	Moisuret(%)	Crude Protein (%)
Control	1.330 ± 0.014	0.605 ± 0.007	1.810 ± 0.014	0.178 ± 0.004	74.875 ± 0.007	21.223 ± 0.018
Garlic	1.415 ± 0.007	0.918 ± 0.011	2.673 ± 0.004	0.425 ± 0.007	77.765 ± 0.092	20.715 ± 0.021
Lime	0.545 ± 0.007	1.185 ± 0.007	0.570 ± 0.014	0.485 ± 0.007	81.140 ± 0.014	20.138 ± 0.004

Table 2. Proximate composition of smoked shrimp samples at day zero

Treatment	Ash (%)	Moist (%)	C.lipid(%)	C.fibre (%)	C.protein(%)	CHO (%)
Control	11.68±0.06	15.17±0.01	6.54±0.16	9.35±1.66	51.53±5.52	2.83±0.73 (%)
Garlic	13.13±1.16	6.78±0.31	7.51±0.65	11.69±1.97	61.1±0.42	5.08±1.02
Lime	12.46±0.16	6.41±0.01	8.44±0.30	12.29±1.52	61.55±4.65	4.39±0.37

Moist-moisture, C.lipid-crude lipid, C.fibre-crude fibre, C.protein-crude protein, CHO-carbohydrate

Table 3. Proximate composition of smoked shrimp samples at 28 day of storage

Treatment	Ash(%)	Moist(%)	C.lipid(%)	C. fibre(%)	C.protein(%)	CHO(%)
Control	11.75±0.11	15.15±0.01	6.51±0.16	9.31±1.17	51.7±10.76	2.85±0.69
Garlic	15.46±0.11	5.88±0.60	7.83±0.05	13.66±2.40	62.7±1.20	5.39±0.70
Lime	13.29±0.11	4.71±0.21	8.42±0.30	13.55±0.21	63.27±5.98	4.57±2.98

Moist-moisture, C.lipid-crude lipid, C.fibre-crude fibre, C.protein-crude protein, CHO-carbohydrate

before (5.71×10^4) and after smoking (3.5×10^3) at end of storage. After smoking all the samples had Staphylococcal count below the acceptable limit ($>10^4$) recommended by International Commission on Microbiological Specification for Food [19]. The presence of Staphylococcus species in shrimps in this study reveals contamination of shrimp through handling or from the environment. The result of vibrio count revealed count of 4.5×10^5 in control sample. Treatment with lime proved most effective in reducing vibrio count from 4.5×10^5 in control to 2.0×10^5 immediately after treatment. After smoking the vibrio count of all samples reduced to 0 while control had the highest vibrio load of 4.8×10^4 at the end of storage after smoking. The presence of vibrio species in shrimp is of public health concern as they may cause infection to the consumers as this count is beyond the acceptable limit (not to be detected in 25g of the sample) recommended by ICMSF [19]. The result of salmonella count revealed that lime juice treated sample had the lowest salmonella count after preservative treatment (2.54×10^5), after smoking salmonella count reduced to zero in all treated samples while control sample had the highest salmonella load (4.56×10^3) at end of storage. This result agrees with the work of Onyeagba et al. [22]. who reported that lime had higher antimicrobial effect than ginger and garlic against Salmonella species, Staphylococcal species and E. coli. Lime was most effective against Shigella, after treatment, lime treated sample had lowest Shigella count of 3.51×10^4 and zero after smoking till end of storage period, all treated samples had no growth of Shigella after smoking till end of storage but control sample had count of 4.72×10^2 at end of storage. The TVC of all treated samples were all below the acceptable limit (5×10^5 CFU/g) recommended by ICMSF [19] to the 4th week which signifies good quality. High levels of coliform bacteria were detected and Staphylococcal counts were above 104 in all samples after treatment but reduced in all treated samples after smoking till end of storage). The Staphylococcus and coliform counts were observed to be reduced significantly by the preservative treatments used. The control sample however, has TVC higher than 5×10^5 CFU/g in the fourth week and higher than the recommended limit in the 4th week. In addition, the coliform count for control already exceeded 104 even in the fourth week after smoking. This finding is of concern as a result of the associated public health implications.

The presence of E. coli, Bacillus sp, Staphylococcus sp, Klebsiella sp, Vibrio sp, Citrobacter sp, Salmonella sp and Shigella sp. was reported. Isolates like Vibrio sp, E.coli and Staphylococcus were the most frequent. These agree with the report of Narasimhan et al., [23] who isolated *Penaeus monodom* in shrimp culture ponds at east coast of thajavur district, Tamil Nadu, India, eleven different bacterial strains were isolated and identified as *Aureobacterium faciens*, *Aureobacterium erythreum*, *Bacillus subtilis*, *Escherichia coli*, *Vibrio cholerae*, *Enterobacter aerogens*, *Micrococcus luteus*, *Pseudomonas putida*, *Pseudomonas aeruginosa* and *Enterococcus pseudoavium*. Furthermore, this also agrees with the report of [24], whose detailed report suggest bacteria isolated from fresh shrimp were E. coli, *Citrobacter*, *Enterobacteria*, *Staphylococcus aureus* and *Bacillus cereus*.

The result of proximate composition revealed significant difference ($p < 0.05$) in ash content of control sample before smoking (1.33 ± 0.014) and after smoking (11.68 ± 0.06) at day zero and (11.68 ± 0.06) at day 28 of storage and other samples with preservatives as shown in table 1, 2 and 3. The ash content of treated samples such as GAT, and LT before and after smoking at zero day and day 28 of storage are (1.42 ± 0.07), (0.55 ± 0.07) and (13.19 ± 1.16), (12.46 ± 0.16) at zero day and (15.46 ± 0.11), and (13.29 ± 0.11) at day 28 respectively. The preservatives caused a significant increase in the ash content in the treated samples indicating that the minerals in shrimps are increased and made available by preservatives and smoking as the ash content of food is a measure of the quantity of mineral content of any food [25] and ash content changes with storage time. This result agrees with the findings of Ajeloja et al. [26] who reported increase in ash content of smoked fish treated with garlic and ginger. This result also can be compared to the work of Tairu et al. [21] who reported ash content of 20.49% and 18.8% in smoked fish treated with ginger and garlic respectively while control sample had ash content of 17.08%. The protein content of control sample in this work prior to smoking was 21.21%. There was a significant increase in the protein content of all samples after smoking but the lime-treated samples (LT) had highest percentage of protein after smoking (61.55 ± 0.06) and at end of storage (63.27 ± 1.99). This result agrees with the work of Tairu et al. [17] who reported protein content of 20.32% in untreated smoked tilapia fish, increased protein content of

64.75% in ginger treated smoked fish and 63.52% in garlic treated smoked fish. However, the other samples show some corresponding higher value of protein more than the control. The increase in protein in smoked samples could be attributed to aggregation of proteins after removal of water molecules present between proteins Ali et al., [27]. A significant difference ($p < 0.05$) occurred in moisture content in all samples before and after smoking. The moisture content of control, GAT and LT before smoking are (74.87 ± 0.27), (77.7 ± 0.02), (81.14 ± 0.14), after smoking at zero day (15.17 ± 0.01), (6.78 ± 0.32), (4.65 ± 0.10), and (6.41 ± 0.01) and, (15.15 ± 0.01), (5.88 ± 0.60), and (4.71 ± 0.21), 28 day of storage respectively. The reduction in moisture content in all samples after smoking could be attributed to effect of heating process and preservatives which reduced the water present in shrimps. This low moisture content indicates that smoked treated shrimps have the tendency to be very stable. as food with moisture content higher than 13% is susceptible to decomposition by microorganisms, Akuamuo et al., [28]. There was a significant increase in crude lipid, and crude fibre in all samples after smoking and during storage. This present study reported a difference in crude lipids before and after smoking in control (0.605 ± 0.07), garlic (0.918 ± 0.011), and lime treated sample (1.185 ± 0.007), suggesting that there was a significant variance at p -value < 0.05 as seen in Table 1, 2 and 3. After smoking lime treated sample had highest lipid content of (8.42 ± 0.30). Control sample had the lowest crude lipid of 0.6 ± 0.01 before smoking and after smoking (6.54 ± 0.16) at zero day and at end of storage lipid (6.51 ± 0.16), however there was significant difference in crude lipids with increase in storage periods. The increase in crude lipid in all samples in this study could be as a result of metabolized glycogen of the cell wall of shrimps. Available carbohydrate for garlic ($0.42 \pm 0.01\%$) had no significant difference just as the lime (0.48 ± 0.14) had no significant effect on the shrimp nutritional attributes when compared to the control. After smoking carbohydrate percentage increased in all the samples, GAT (5.08 ± 1.02), LT (4.39 ± 0.37) and Control (2.83 ± 0.03) at zero day and increased with storage as seen in Table 2 and 3. There was a significant increase and difference ($p < 0.05$) between crude fibres of fresh shrimp and the smoked and treated samples, before smoking garlic treated samples had highest crude fibre of 2.67 ± 0.22 while control sample had crude fibre of 1.8 ± 0.27 , after

smoking, the lime treated sample had highest crude fibre of 12.29 ± 1.52 but at end of storage garlic treated sample had the highest crude fibre of (13.66 ± 2.40) followed by lime treated sample (13.55 ± 0.21). This increase in crude fibre in all samples after smoking suggests that smoked shrimps preserved shrimps offer a better dietary advantage of helping to reduce constipation. Akintola et al. [29]

5. CONCLUSION

This study has revealed that the samples treated with Lime and garlic before and after smoking showed significant reduction in bacterial load and maintained a low level throughout the 4th weeks of storage. Lime can be used as a first choice preservative in smoked shrimps without adversely affecting quality in terms of bacterial and nutritional quality and garlic may be used in the absence of lime. The use of 20% lime as a choice antimicrobial agent is hereby recommended since it has been found to keep smoked shrimps in a very good condition for four weeks, reducing the coliform to 2.3×10^2 CFU/g, Vibrio count to zero, Shigella count to zero and Salmonella count to zero CFU/g at the end of four weeks storage. This will ensure prolonged shelf life and safety of shrimps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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REFERENCES

1. Efiuvwevwere BJO, Ogugbue CJ, Emoghen G, Ngbara-ue AK. Application of Hazard Analysis Critical Control Points (HACCP) concept using Sodium metabisulphite and Hypochlorite to enhance microbial safety of shrimps, American Journal of Microbiological Research. 2020;8(1):24-33.
2. Ahkam M, El-Gendy, Fatten El-Feky, Neveen H, Mahmoud, Ghada SA.

- Elsbakhy. Evaluation of Nutritional Quality of Green Tiger Prawn, *Penaeus Semisulcatus* from Land Fisheries (Alexandria) and Market (India). *Egyptian Journal of Hospital Medicine*. 2018;70(6): 924-934.
3. Ramesh, B.K, Govinda, R.V, Krishna, N.M, Geetha S, Kakara RR. Assessment of Bacteriological Quality in selected commercially important shrimps of Visakhapatnam, East of India. *International Journal of Microbiology and Biotechnology*. 2020;2(2):102-10
 4. Ehigiator FAR, Akise OG, Eyong MM. Bacteria and fungi load of raw processed shrimp from different meat shops in Benin metropolis. *Journal of Agriculture, Food and Environment*. 2014; 10(3):1-7.
 5. Dutta CS, Nath S, Chowdhury AK, Panigrahi, Sengupts C. Study of HACCP and microbial quality of seafood during processing in plants around Kolkata *Asian Journal of Animal Sciences*. 2016; 11(2):140-147.
 6. Sabine Ninelle Nga Ombede, Pierre Marie Kaktcham, Malang Seydi, François Zambou Ngoufack. Changes in sensory, physicochemical, and microbiological properties of fresh captured tropical pink shrimps (*Penaeus duorarum notialis*) inoculated with *Lactobacillus plantarum* Lp6SH, *Lactobacillus rhamnosus* Yoba, and their cell-free culture supernatants during storage at 40C; 2018. DOI: 10.1111/jfs.12579
 7. Tapia MS, Alzamora SM, Chirife J. Effects of Water Activity (aw) on Microbial Stability: As a Hurdle in Food Preservation; 2008. DOI: 10.1002/9780470376454.ch10
 8. Fufa KB. Anti-bacterial and Anti-fungal properties of Garlic extract (*Allium sativum*): A Review. *Microbiological Research Journal International*; 2019. DOI:https://doi.org/10.9734/mrji/2019/v28i330
 9. Gebreselema Gebreyohannes, Mebrahtu Gebreyohannes. Medicinal values of garlic. *International Journal of Medicine and Medical Sciences*. 2013;5(9):1-15.
 10. Bina LJ, Tista PJ, Anjana S, Kayo DY. Study of Antimicrobial Activity of Lime Juice against *Vibrio cholera*. *Environmental Laboratory of Nepal Academy of Science and Technology*; 2010. DOI: 10.3126/sw.v8i8.384
 11. Wokoma OAF, Umesi NN. Benthic macrofauna species composition and abundance in the Lower sombreiro river, niger delta, Nigeria *European Journal of Biology and Medical Science Research*. 2017;5(1):17-23.
 12. Bandna C. Antibacterial effect of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) against *Staphylococcus aureus*, *Salmonella typhii*, *Escherichia coli* and *Bacillus cereus*. *Journal of Microbiology, Biotechnology and Food Science*; 2013.
 13. Guinares RC, Agbon MCEB, Campeon ASB, Belinario MFMT. Effect of garlic *Allium Sativum* extract on lipid oxidation, microbiological, and organoleptic qualities in Hot – smoked sardinella longrceps during frozen storage. *International Journal of Fisheries and Aquatic Studies*. 2014;1(6) 87-94.
 14. APHA. Compendium of methods for the microbiological examination of foods 4th ed. Washington. DC. American Public Health Association; 2001.
 15. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, UK. 2005;23-35.
 16. Association of Analytical Chemists. Official Methods of Analysis (AOAC) 18th Ed., AOAC international, suite 500, 481 north frederick avenue, Gaithersburg, Maryland, USA. 2005;20877-2417.
 17. Amadi BA, Agomuo EN, Ibegbulem CO. *Research Methods in Biochemistry*. Supreme Publishers, Owerri. 2004;55-93.
 18. IBM SPSS Statistics for Windows, version 21.0.2012. Armonk, NY: IBM Corp
 19. The International Specifications for Foods. *Microorganisms in Foods, Sampling for Microbiological Analysis: Principles and Specific Applications* (2nd Ed.) Blackwell Science, Oxford. 1986;310
 20. Abu HY, Md Kawser A, Sabina Y, Nazmul A, Md Mujibur, Md Monirul I. Prevalence of Microbial Load in Shrimp, *Penaeus Monodon* and Prawn, *Macrobrachium rosenbergii* from Bangeledesh. *World Journal of Agricultural Sciences*. 2008;4(5):852-8.
 21. Tairu HM, Adedokun MA, Adelodun AB, Badmus BD. Microbial Examination of some selected natural preservatives on the shelflife and safety of smoked Tilapia fish (*Oreochromis niloticus*). *Journal of Microbiology and Biotechnology*; 2017.

22. Onyeagba RA, Ugbogu OO, Okeke CU, Iroakasi O. Studies on the Antimicrobial Effects of Garlic, Ginger and Lime. African Journal of Biotechnology. 2004;3(10):552-554
23. Narasimhan N, Maharajothi P, Sukumaran M, Ravichelvan R, Ravichandran R. Biochemical Studies on Protein content in different tissues of selected shrimps (*Penaeus monodon* and *Penaeus vannamei*). International Journal of Pure and Applied Zoology. 2013;1(1):92 -96.
24. Makinde OO. Microbiological and heavy metals analysis of some seafoods from Buguma and Ekerekana Creeks, Niger Delta. Ph.D Thesis submitted to University of Port Harcourt. Port Harcourt, Rivers State.; 2014.
25. Omotosho OE, Oboh G, Iweala EEJ. Comparative effects of local coagulants on the nutritive value, *in vitro* multi enzyme protein digestibility and sensory properties of Waracheese. Int. J. Dairy Sci. 2011;6: 58-65.
26. Ayeloja AA, George EOA, Akinyemi AA, Atanda AA. Effects of spices on the chemical properties of hot-smoked catfish (*Clarias gariepinus*). Proceedings of 28th annual conference. Nov. 25- 30; 2013.
27. Ali Aberoumand. Effects of the traditional fish processing method on the proximate composition and Ph of fish, Black pomfret (*P Parastromateus niger*. Potravinarstvo Slovak Journal of Food Sciences; 2020. DOI: 10.5219/1294
28. Akuamoah F, George TO, Nii Korley K. Nutritional and shelf life studies of dry smoked and gamma irradiated shrimps (*Penaeus notialis*) from three different water sources in Ghana(4)1. Journal of Cogent Food and Agriculture. 2018;4(1). DOI: ORCID.
29. Akintola LS. Effects of smoking and sun drying on proximate, fatty and amino acids, compositions of southern pink shrimp (*Penaeus notialis*) Journal of Food Science and Technology. 2015;52(5): 2646-2656.

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