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A Comparative Analysis of the Microbial Load of Two Drying Methods for the Preservation of Clam (*Tegillarca granosa*)

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Author's contributions

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

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ABSTRACT

Tegillarca granosa (clam) was preserved by different drying methods. The molluscan shellfish sample was smoked dried and oven dried to predict the most effective method of drying based on the microbiological quality, proximate composition and sensory evaluation of the shellfish sample. Total viable bacterial counts of *Tegillarca granosa* ranged from 2.45±1.94 - 0.19±.28 x10^6 cfu/g, *Vibrio* counts ranged from 3.88±3.32 - 0.00±0.00×10^4 cfu/g, *Pseudomonas* count ranged from 3.65±3.25 - 0.00±0.00×10^3 cfu/g. *Salmonella* ranged from 3.46±2.70 – 0.00±0.00×10^5 cfu/g. *Shigella* ranged from 3.68±2.70 – 0.00±0.00 × 10^5 cfu/g, *Staphylococcus* counts ranged from 3.67±2.81 – 1.19±2.13 × 10^4 cfu/g. Coliform counts ranged from 5.74±2.63 - 0.00±0.00×10^5 cfu/g,. Fungal counts ranged from 4.13±2.75 - 0.03±0.07×10^4 cfu/g. The bacteria isolated were *Bacillus*, *Enterobacter*, *Escherichia coli*, *Klebsiella, Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus* and *Vbrio*. The fungi isolated were *Aspergillus flavus*, *Aspergillus niger, Penicillium* sp and *Saccharomyces cerevisiae*. Results obtained showed that clam contains unacceptable counts of bacteria and fungi, higher than the specified standard limits of 1.0×10^5 cfu/g for bacteria 1.0×10^2

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cfu/g for coliform. Proximate composition showed that smoked dried clam is nutritionally richer than oven dried clam. Protein content, carbohydrate content, fat content and moisture content were higher in smoked dried clam than oven dried clam while crude fiber and ash content were higher in oven dried clam. Sensory evaluation to determine the degree of liking showed that the smoke-dried clam was more preferred than the oven dried clam. The result of the microbial analysis revealed that fresh clam had higher microbial load than smoked dried and oven dried clam. Preservation by drying was effective in reducing the microbial load from the shellfish samples. The smoked drying method was more effective in reducing the microbial load of the shellfish samples than the oven dried samples.

Keywords: Clam; proximate composition; smoke drying; oven drying.

1. INTRODUCTION

Seafood could be sea animals, including fishes, crustaceans and mollusks or plants used as food Seafood is tasty, nutritionally balanced and a great significant source of proteins, fiber, vitamins and minerals. They are also low in carbohydrates and fats. Clam is used as a condiment in the Niger Delta region in Nigeria and other parts of the world with coastal areas. Studies by Umoh and Basir [1] showed that protein content and nutritional composition of shellfish are comparable to those of eggs. *Tegillacra granosa* (Clam) are found all over the world. They live in a variety of habitats including Arctic and Antarctic waters, coastal mud flats, the Deep Ocean and coral reefs. Most species are found in the ocean; however, some are found in fresh water. Most clams are found in shallow water in areas that are muddy or sandy.

Shellfish is consumed raw (ingested whole) in some parts of the world including Nigeria thereby leading to the transmission of pathogenic organisms. Thus, various bacteria that are aetiologic agent of shellfish related food infection such as salmonellosis, shigellosis, *Vibrio* and Hepatitis A virus had been isolated from shellfish. In addition, septicemia had been reported among consumers of raw oysters from the Gulf of Mexico [2,3].

Dried shellfish have unique flavor and are eaten as snacks or with fermented tubers without subjecting them to further cooking. Fresh seafood can spoil easily, thus rough handling may result in contamination of shellfish. Their short shelf life poses serious practical problems in their storage and distribution [4]. Seafood can be subjected to form of processing or preservation by drying to reduce or destroy contaminating microbial load which in turn destroy intrinsic enzymatic activities in them. Review on the microbiological quality of shellfish indicated that shellfish harbor pathogenic organisms. These pathogenic organisms have been implicated in outbreaks of food-borne disease in many parts of the world; since clams, are found in the bodies of water containing untreated human and industrial waste, there is the tendency that they may concentrate and accumulate high levels of pathogens and toxic contaminants which can pose a significant health hazard to consumers. This is not surprising as shellfish are filter feeders, improper storage and handling, inadequate heat processing or preservation and storage after purchase of fresh shellfish may allow some pathogens particularly enteric viruses, fungi and bacteria to persist in them.

The method of preservation of shellfish for retail significantly influences the type and counts of pathogenic microorganisms that are isolated. These pathogens can be introduced into shellfish from the air during processing, unclean hands, unsanitary equipment, unsafe water, sewage and through cross contamination [3,5]. Thus, this study is aimed at providing the information especially on microbial load and proximate composition of smoked and oven dried clam.

2. MATERIALS AND METHODS

2.1 Source and Sample Collection

Fresh samples of *Tegillarca granosa* (clam) were purchased from the Creek Road Market in “Town Area” of Port Harcourt, Rivers State, Nigeria. Samples were transported to the laboratory in clean container for analysis.

2.2 Methods of Drying

2.2.1 Oven dry

An oven dryer, was set to operate at 180°C. The come –up time was 30 minutes, after which the metal trays loaded with the shellfish samples were introduced into it. To ensure uniformity in drying, the shellfish samples were turned hourly

with the positions of the trays being swapped-top trays brought lower and lower ones sent up. The temperature of the tray was maintained between 170°C and 180°C.

2.2.2 Smoked dry

Fire wood was used in drying the shellfish samples; this was done by putting the firewood under a metal drum and placing wire gauze on top. Temperature control was achieved by withdrawing or adding firewood.

2.3 Microbiological Analysis of Samples

Ten grams (10 g) of seafood samples were transferred into a blender and homogenized for 2 minutes with 90 ml of 8.5% normal saline to give a 10^{-1} dilution. The filtrate was used to carry out serial tenfold dilution by transferring 1 ml into 9 ml fresh diluents to give the required dilution. Aliquots (0.1 ml) of appropriate dilutions were transferred separately to plates of dried sterile Nutrient Agar in duplicates by spread plate method using a sterile bent glass spreader. Plates were incubated at 37°C for 24-48 hours and only plates having 30 to 300 colonies were counted, the average was taken and recorded as total heterotrophic counts of bacteria and expressed as colony forming unit per gram (cfu/g).

Similarly, Mannitol salt Agar was used for staphylococcal count, *Salmonella-Shigella* Agar for *Salmonella* and *Shigella* counts, Thiosulphate citrate bile salt sucrose agar for total *Vibrio* counts, Cetrimide Agar for total *Pseudomonas* counts, MacConkey agar for total coliform counts. Discrete colonies were subcultured onto nutrient agar plates and incubated at 37°C to obtain a pure culture. The colonies obtained were stored in MacCartney bottles containing nutrient agar slant and stored in the refrigerator as stock cultures for further biochemical tests such as gram stain, motility, catalase, oxidase, citrate, coagulase, indole, MR-VP and sugar fermentation tests. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the identification of Medical Bacteria and Bergey's Manual of Determinative Bacteriology.

In the case of moulds, 0.1 ml of appropriate dilutions of the sample was inoculated onto sabouraud's dextrose agar plates (SDA) in duplicate. Plates were incubated at 25°C for 3 to 5 days. Colonies formed were counted and the average counts for duplicated plates were recorded as total viable fungi in the samples. Discrete colonies were subcultured onto freshly prepared Sabouraud's dextrose agar (SDA) to obtain pure cultures. The sub cultured isolates in freshly prepared Sabouraud's dextrose agar plates were incubated at 25°C for 5 days to 7 days. The isolates that developed were further sub cultured onto agar slant and incubated at 28°C for 5 to 7 days, stored in refrigerator and used for further identification tests. Identification of fungi was done based on their macroscopic appearance on plates and by viewing them under the microscope [6].

2.4 Proximate Composition

The recommended methods of the Association of Official Analytical Chemists [7] were used for the proximate analysis of the fresh clam, smoked dried clam and oven dried clam and the moisture, crude fat, crude protein, ash content, carbohydrate and crude fiber were determined.

2.4.1 Moisture

This was determined using a thermostatically controlled forced air oven (Gallenkamp, England) operating at 105°C for 3 h. The difference in weight before and after drying was used to calculate the per cent moisture content.

Calculation: Moisture (%)

$$= \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight (g)}} \times 100 \quad (1)$$

2.4.2 Crude fat

This was done using the Soxhlet extraction apparatus; four (4) grams of samples were used. Petroleum ether (boiling point 40°C to 60°C), was used for extraction. The weight of fat divided by weight of sample was used to compute for the percent crude fat content.

Calculation:

$$\text{Extractable fat (\%)} = \text{weight (g) of flask with fat} - \text{weight (g) of flask without fat} \times 100 \quad (2)$$

2.4.3 Crude protein (%N × 6.25)

Crude protein as determined by Kjeldahl method. About 0.1 g sample was weighed to the nearest mg each into 250 ml Pyrex conical flask containing the digestive catalyst. The product was digested with concentrated sulphuric acid, using copper sulphate as a catalyst, to convert

organic nitrogen to ammonium ions. Alkali was added and the liberated ammonia distilled into an excess of boric acid. The distillate was titrated with hydrochloric acid to determine the ammonia absorbed in the boric acid.

$$\text{Calculation: N (\%)} = \frac{\text{Titre value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times \text{sample weight}} \quad (3)$$

2.4.4 Ash content

Ash content was determined by incinerating 5.0 g of sample at 550°C overnight in a muffle furnace (Gallenkamp, England) and the weight before and after ashing used in calculating the per cent ash content.

$$\text{Calculation: Ash (\%)} = \frac{\text{Ash weight (g)} \times 100}{\text{Oven dry weight (g)}} \quad (4)$$

2.4.5 Total carbohydrates

Total carbohydrate was obtained by using dried homogenized sample (0.1 g) of each type of shellfish sample, weighing to the nearest mg into a flat bottom flask. The material was digested with perchloric acid. Hydrolysed starches together with soluble sugars were determined colorimetrically (Filter photo colorimeter, Electra system, model 321, Sn: 0208052) and expressed as glucose.

Calculation:

$$\text{Total carbohydrates (as \% glucose)} = \frac{25 \times \text{absorbance of dilute sample}}{\text{Absorbance of dilute standard} \times \text{weight of sample}} \quad (5)$$

2.5 Determination of Crude Fibre

Extract of 0.5 g of moisture free sample for 3 hours with petroleum ether using Soxhlet apparatus.

The fat free material in a 100 ml beaker was added to 25 ml of 1.25% sulphuric acid and was coved with watch glass. The content of the beaker was heated gently on a Gerhardt hot plate for 5 mins (Acid hydrolysis) and was filtered under vacuum through a Buchner funnel fitted with filter paper (Whatman No. 40) it was washed with boiling water until the washings is no longer acidic to litmus. The residue was washed back into the beaker with 1.25% NaOH and was covered with wash glass and the content was boiled for 5 minutes. The resulting insoluble material was transferred to a dried weighed ashless filter paper and was washed thoroughly with hot water until the washing is no longer alkaline to litmus. The filter paper and content were dried for 1 hour at 105°C, incinerate the filter paper and content to an ash for 1hr at 550°C using SXL muffle furnace. The ash was cooled using Desiccator and weighed. The weight of ash was subtracted from the increase weight on the paper due to the insoluble material and the difference reported as fibre.

$$\text{Crude fibre (\%)} = \frac{\text{weight of fibre}}{\text{weight of sample}} \times \frac{100}{1} \quad (6)$$

2.6 Sensory Evaluation

Smoked dried and oven dried clam samples were used for the sensory evaluation. Twelve panelists, who regularly eat seafood were randomly selected and trained for sensory evaluation. The degree of liking for color, texture, odour, taste and overall acceptability were determined using the 9 point hedonic scale.

2.7 Statistical Analysis

The data obtained from the microbiological analysis and proximate composition and sensory evaluation were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test significant differences ($p < 0.05$) among mean values obtained. Where significant differences existed, Duncan's least significance difference (LSD) test was applied to indicate where the differences occurred. The statistical packaged used was SPSS 17.0 (SPSS Inc. Chicago, IL, USA).

3. RESULTS

3.1 Microbiological Analysis

The result of the comparative analysis of the microbial load of two drying methods for *Tegillarca granosa* (clam) is illustrated in Table 1. The total heterotrophic bacteria, counts of *Vibrio,* counts of *Pseudomonas,* total fungi count, counts *Salmonella-Shigella,* staphylococcal counts and coliform of the smoked dried clam are 0.42±0.27 $\times 10^6$ cfu/g, 0.09±0.18 $\times 10^4$ cfu/g, 0.58±2.06 $\times 10^3$ cfu/g, 0.17±2.29 $\times 10^4$ cfu/g, 0.96±2.49 $\times 10^5$ cfu/g, 2.45±3.21 $\times 10^4$ cfu/g and 0.98±1.89 $\times 10^5$ cfu/g, respectively.

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$1.55 \pm 2.62 \times 10^5$ cfu/g, $3.59 \pm 3.19 \times 10^4$ cfu/g and $4.14 \pm 3.29 \times 10^5$ cfu/g, respectively. The highest total heterotrophic bacterial load was the same for the smoked dried and oven dried samples. The home smoked dried clam had the least counts of *Vibrio, Pseudomonas,* fungal, Salmonella- Shigella counts, staphylococcal counts and total coliform counts respectively. The lower microbial load in the smoked clam could be attributed to the processing technique which involved the use of smoke. Smoke contains over 400 substances (methanol, ethanol, alcohols, ethanoic acid, methanolic acid, furfuraldhyde phenols etc) some are antimicrobial in nature The combined effect of heat and antimicrobial substances in smoke may be responsible for the low microbial counts. In oven dried method it was only application of heat without antimicrobial substances. More so, the two drying methods showed reduction in the microbial load compared to the freshly shucked clam.

3.2 Proximate analysis

The proximate composition of the smoked dried and oven dried clam showed that the smoked dried clam has higher Moisture, fat, crude protein and carbohydrate content than the oven dried shrimps. While the oven dried smoked dried clam had higher ash content and crude fiber than the smoked dried clam. Despite the high moisture content in the smoked dried clam, the fresh clam had very high moisture content (Table 2).

The bacterial and fungal isolates isolated from the clam using the oven drying and smoke-drying methods are presented in Tables 3 and 4, respectively. *Aspergillus flavus Aspergillus niger,* and *Penicillium* sp, were the fungi isolated from the smoked dried clam while *Aspergillus flavus Aspergillus niger, Penicillium sp* and *Saccharomyces cerevisiae* were the fungi isolated from the oven dried samples. High humidity and warm environmental temperatures in the coastal areas of the Niger Delta predispose to growth of fungi [8].

However, there was no significant difference ($p<0.05$) between the moisture content of smoke-dried samples and oven dried samples but there was a significant difference ($p>0.05$) between the moisture content of freshly shucked clam, smoked dried clam and oven dried clam. There was a significant difference ($p>0.05$) between the ash content, fat content, crude fiber and crude protein of freshly shucked clam, smoked dried clam and oven dried clam. There was no significant difference ($p<0.05$) between the carbohydrate content of freshly shucked clam and oven dried clam but there was a significant difference ($p>0.05$) between the carbohydrate content of smoked dried and oven dried and freshly shucked clam.

3.3 Sensory Evaluation

The smoked dried samples were the most preferred in terms of taste than the oven dried samples. In smoked dried samples, the drying temperature was enough to cause maillard browning. However, smoke particles deposited on the smoked dried samples changed its colour compared to the oven dried samples. This may have influenced panelist's decision to choose the smoked dried samples over the oven dried samples in terms of colour.

The smoked dried samples were preferred in terms of odour than the oven dried samples. Smoke generally leads to enhanced flavor of foods. The presence of certain phenolic compounds such as guaiacol, 4-methylguaiacol, and syringol in smoke play an important role in characteristic flovour of smoked products [9].

4. DISCUSSION

The bacterial genera isolated from freshly shucked clam were *Bacillus, Enterobacter, Escherichia coli, Klebsiella, Pseudomonas, Salmonella, Shigella, Staphylococcus* and *Vibrio.* In smoked dried clam, only *Bacillus, Shigella, Staphylococcus* and *Vibrio* sp were isolated while *Bacillus, Escherichia coli, Klebsiella, Pseudomonas* and *Staphylococcus* sp (Table 3). The differences in the bacterial types could be attributed to the different processing methods. The microbial quality of the river, estuaries and seashores from which shellfish are harvested influence the microflora of shellfish samples [10]. In addition to the endogenous microflora of the shellfish, Molluscs are transported live to the point of sale or processing where the flesh can often be contacted by hand. Although contamination may occur at this stage, the significant public health problems associated with shellfish arise from the surrounding waters [11]. The initial microbial load on ready-to-eat foods is important; however, factors such as processing storage and display may influence the microbiological load of ready-to-eat foods at the point of sale [12,13]. Although drying reduces water activity and destroys bacteria through

Table 1. The microbial load of two drying methods for *Tegillarca granosa* (clam)

Clam	THBC $\times 10^6$	TVC $\times 10^4$	TPC $\times 10^3$	TFC $\times 10^4$	TSS $\times 10^5$	TSC $\times 10^4$	TCC $\times 10^5$
Freshly shucked clam	2.45 ± 1.94^{b}	3.88 ± 3.32^{b}	3.65 ± 3.25^{d}	4.13 ± 2.75^{b}	3.46 ± 2.70^{de}	3.67 ± 2.81^{b}	2.01 ± 1.05^{b}
Oven dried clam	0.42 ± 0.28^{a}	0.13 ± 0.61^{a}	0.90 ± 2.34^{abc}	0.75 ± 2.00^{a}	1.55 ± 2.62^{bc}	3.59 ± 3.19^{b}	4.14 ± 3.29^{c}
Smoked dried clam	0.42 ± 0.27^{a}	0.09 ± 0.18^{a}	0.58 ± 2.06^{ab}	0.17 ± 0.29^{a}	0.96 ± 2.49^{abc}	2.45 ± 3.21^{ab}	0.98 ± 1.89^{ab}

Means with same alphabet across the columns shows no difference ($p \geq 0.05$)

Key: THBC = Total Heterotrophic Bacterial Counts, TVC=Total Vibrio Counts, TPC= Total Pseudomonas Counts, TFC=Total Fungi Counts, TSSC= Total Salmonella- Shigella Count, TSC= Total Staphylococcus Counts, TCC=Total Coliform

Table 2. Proximate composition of *Tegillarca granosa* (clam)

Parameter	Freshly shucked clam	Oven dried clam	Smoked dried clam
Moisture content	74.68±2.35[b]	5.25±0.24[a]	7.32±0.39[a]
Ash content	1.72±0.04[a]	19.81±0.0[c]	8.43±0.36[d]
Crude fat	1.92±0.12[b]	0.83±0.09[a]	4.48±0.70[c]
Crude fibre	3.27±0.33[a]	17.47±0.56[c]	5.31±0.00[b]
Crude protein	14.96±0.79[a]	54.45±0.00[b]	56.08±1.23[bc]
Carbohydrate content	3.09±0.56[a]	2.17±0.11[a]	18.39±2.6[b]

Means with same alphabet across the columns shows no difference (p≥0.05)

Table 3. Distribution of bacterial isolates in the different drying methods

Isolate	Freshly shucked clam	Smoked dried clam	Oven dried clam
Bacillus	+	+	+
Enterobacter	+	-	-
Escherichia coli	+	-	+
Klebsiella	+	-	+
Pseudomonas	+	-	+
Salmonella	+	-	-
Shigella	+	+	+
Staphylococcus	+	+	+
Vibrio	+	+	-

+ = present
- = absent

Table 4. Fungi isolated from *Tegillarca granosa* (clam)

Fungi isolate	Smoked dried clam	Oven dried clam
Aspergillus flavus	+	+
Aspergillus niger	+	+
Penicillium sp	+	+
Saccharomyces cerevisae	+	-

+ = present
- = absent

the activity of heat, post processing contamination can occur especially during handling and transportation of processed foods to point of sale [14]. Processing of shellfish following proper food handling practices, especially the use of clean water for shucking, rinsing and retailing may reduce numbers of coliform bacteria in samples, though that reduction may not be substantial in shellfish that have been harvested from polluted rivers and estuaries as strains of *Escherichia coli* accumulate in the gut of molluscan shellfish cultured in contaminated waters [15]. Most strains *of Escherichia coli* are harmless commensals; however, some strains are pathogenic and can cause diarrheal disease. The infectious dose of *E. coli* is quite low, so as much as possible their mere presence must be avoided. *E. coli* strains can multiply and generate enterotoxins when contaminated foods are kept at room temperature for several hours [16]. Studies have suggested that the presence of *Staphylococcus* species on ready-to-eat food may be as a result of improper handling, cross contamination and poor temperature control [17,18]. In this current study, the drying methods reduced the counts of *Staphylococcus* spp. but did not eliminate the contaminating microorganisms.

The relatively low protein content of the oven dried clam may be attributed. Putrefaction leads to the production of ammonia and pyruvate [19]. The ammonia released and the conversion of amino acids to pyruvate would lead to loss of nitrogen, which is the element quantitatively determined in the Kjeldahl method of protein determination. Thus, a reduction in the nitrogen content invariably would lead to a reduction in the protein content of the sample. This may account for the relatively lower protein content obtained for the sun-dried samples. The relative

low carbohydrates content observed in the home smoked dried could be attributed to browning. Previous studies have reported that browning which is as a result of Maillard reaction could cause low values of carbohydrate since the reducing sugars that participate in the Maillard reaction could be transformed into various compounds and therefore lost in the process [20,21].

5. CONCLUSION

The findings in this study have shown that drying was able to reduce the microbial loads inherent in clam and most of the microorganisms associated with clam in this study could be as a result of the different handling / processing techniques. Bacteria of aerial origin and fungi were also isolated from clam. The results obtained from this study also showed that smoke dried clam had relatively low microbial load than oven dried clam. Proximate composition showed that smoked dried clam is nutritionally richer than oven clam. More so smoked dried and oven dried clam had higher percentage values in proximate composition than fresh clam. Drying of clam affected the moisture content which caused a decline in moisture and aided longer preservation of clam.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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