

Microbiological and Nutritional Quality of market and home smoke dried Shrimp

ABSTRACT

Bacteriological and nutritional quality of market and home smoke dried shrimp was carried out. Fresh and dried shrimps were bought from market. One set of the fresh shrimp was not rinsed before they were home smoke dried and used for analysis, another set was rinsed with clean water before they were home smoke dried and used for analysis and the market dried sample was used for analysis without any treatment. After the home smoke drying, the microbial load and types of microorganisms were determined using standard microbiological technique. The proximate compositions of the fresh shrimp, home smoke dried and market smoke dried shrimp were also determined. The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the market smoke dried were 4.23 ± 3.30 , 1.98 ± 3.15 , 0.97 ± 2.58 , 2.95 ± 2.96 , 1.22 ± 2.67 , 3.89 ± 3.22 and 1.88 ± 2.86 CFU/10g, respectively. The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the home smoke dried not rinsed were 4.18 ± 2.75 , 2.69 ± 3.43 , 0.4 ± 1.44 , 1.96 ± 2.8 , 1.47 ± 2.72 , 2.33 ± 3.08 and 2.85 ± 3.06 CFU/10g, respectively. The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the home smoke dried rinsed shrimps were 4.11 ± 2.48 , 1.09 ± 2.50 , 0.00 ± 0.00 , 0.00 ± 0.0 , 1.56 ± 2.62 , 3.35 ± 3.03 and 1.25 ± 2.17 CFU/10g, respectively. Eight bacteria genera which include *Bacillus*, *Klebsiella*, *Proteus spp*, *Providencia*, *Pseudomonas*, *Shigella*, *Staphylococcus* and *Vibrio* were identified. *Aspergillus niger*, *Penicillium* sp, *Rhizopus oligosporus* and *Saccharomyces cerevisiae* were the fungi isolates identified. Although the bacterial types could contain pathogenic strains, proper cooking could inhibit their presence in food. Smoke drying of the seafood could preserve the shellfish longer since the water activity of the seafood is reduced. Thus, smoke drying would be recommended for preservation since it does not reduce the nutritional quality of the shrimp and preservation of food has been a great challenge especially in developing countries where there is poor power supply. More so fresh seafood easily perishes especially when they are not well preserved.

Key words: *microbiological quality of shrimp, proximate, smoke drying.*

Introduction

Seafood could be sea animals, including fishes, crustaceans and mollusks or plants used as food. Seafood makes up a significant food component for a large part of the world population. Seafood is tasty, nutritionally balanced and a great significant source of proteins, fiber, vitamins and minerals. They are also low in carbohydrates and fats. Shrimps have been 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. 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 shellfish from the Gulf of Mexico [2].

In Nigeria seafood is a great and appealing food, especially a choice dish by Riverine dwellers in Niger Delta which they have been using in various cuisine. Archaeological findings have shown that humans have been making use of shellfish as a food item for hundreds of thousands of years. In the present, shellfish dishes are a feature of almost all the cuisine of the world. 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 [3]. 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 shrimps 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. Improper storage and handling, inadequate heat processing or preservation and storage after purchase of fresh shellfish may allow some pathogens particularly enteric viruses and bacteria to persist in them. Considering the massive consumption/demand and enormous nutritional and industrial importance of shellfish, the fishing industry cannot continue to remain neglected. Hence there is need to create awareness to the public on the health risk of consuming raw or inadequately cooked or preserved shellfish as this could be a channel of ingesting pathogenic microorganisms.

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 [4]. More so, there is dearth of information concerning the bacteriological and nutritional quality of market smoked dried and home smoked dried shrimps.

Materials and Method

Sampling collection

Fresh samples and market smoke samples of *Litopenaeus vannamei* (shrimp) were purchased from Creek Road Market in "Town Area" of Port Harcourt, Rivers State, Nigeria. Samples were placed in ice and transported to the laboratory.

Sample Preparation

Smoked Dryer

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. The smoke dried shellfish samples were packaged in clean polythene bags sealed and stored for 28 days and 3 months for further analysis.

Comment [DE1]: You can also specify what particular wood you used and why

Microbiological Analysis of Samples

Ten grams (10g) of fresh shrimp, market smoked dried shrimp and home smoked dried rinsed and unrinsed shrimp were blended for microbiological analysis. Samples were homogenized for 2 minutes with 90ml of sterile 8.5% normal saline to give a 10^{-1} dilution. The filtrate was used for 10-fold serial dilution by transferring 1ml of the diluent to test tubes containing 9ml sterile normal saline. Aliquots (0.1ml) of appropriate dilutions were transferred separately to plates of dried sterile Nutrient Agar for total heterotrophic bacteria counts, Mannitol salt Agar for staphylococcal counts, *Salmonella-Shigella* Agar for *Salmonella* /*Shigella* counts, Thiosulphate citrate bile salt sucrose agar for total vibrio counts, Cetrimide Agar for *Pseudomonas* counts, MacConkey agar used for total Coliform counts and Sabouraud Dextrose Agar for total fungal counts. Inoculation was done by spread plate method using a sterile bent glass spreader. Bacteria plates were incubated at 37°C for 24-48 hours while fungal plates were incubated at 22°C for 2-5 days. After incubation, plates were observed for proliferation of colonies. Colonies were counted; the average was taken and recorded and expressed as colony forming unit per 10gram (cfu/10g). Distinct colonies from different culture plates were selected and purified by inoculating on prepared nutrient agar plates.

Comment [DE2]: homogenized

The obtained pure isolates were stored in MacCartney bottles containing nutrient agar slant and sabouraud dextrose agar slants for bacteria and fungi, respectively, and stored in the refrigerator at 4°C. The stock was used for subsequent analysis including identification. Identification techniques for respective isolates was adopted from the methods and test described by Cheesbrough [5]. Bacterial isolates were identified with reference to Manual for the identification of Medical Bacteria [6].

Microscopic Examination of Fungi

Microscopic examination was done by wet mount method as described by [7]. In this method, a wet mount was prepared for the fungal isolates by placing a drop of water on a clean slide aseptically, fungal spores under test was teased into it using 2 (two) sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. Another method adopted was

the wet mount using lactophenol cotton blue [8]. Each prepared slide was gently covered with a cover slip to avoid air bubbles. The slides were observed under X10 and X40 objectives, and observation was recorded.

Proximate composition

The recommended methods of the Association of Official Analytical Chemists (AOAC) [9] were used for the proximate analysis of the shrimps and the moisture, crude fat, crude protein, ash content, carbohydrate and crude fiber were determined.

Moisture

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

Calculation: Moisture (%) = $\frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight (g)}} \times 100$ equation 1

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:

Extractable fat (%) = $\frac{\text{Weight (g) of flask with fat} - \text{Weight (g) of flask without fat}}{\text{Weight (g) of sample}} \times 100$ equation 2

Crude protein (%N × 6.25)

Was determined by Kjeldahl method. About 0.1g 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.

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

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.

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

Total carbohydrates

Total carbohydrate was obtained by using dried homogenized sample (0.1g) 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:

Total carbohydrates (as % glucose) = $25 \times \text{absorbance of dilute sample}$

Absorbance of dilute standard × weight of sample Equation 5

Determination of Crude Fiber

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

The fat free material in a 100ml beaker was added to 25ml of 1.25% sulphuric acid and was covered with watch glass. The content of the beaker was heated gently on a Gerhardt hot plate for 5mins (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.

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

Statistical Analysis

The data obtained from the microbiological analysis and proximate composition 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).

Result and Discussion

The result of market and home smoke dried samples of *Litopenaeus vannamei* (shrimps) on the microbiological load is illustrated in Tables 1. The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the market smoke dried were 4.23±3.30, 1.98±3.15, 0.97±2.58, 2.95±2.96, 1.22±2.67, 3.89±3.22 and 1.88±2.86 CFU/10g, respectively.

The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the home smoke dried not rinsed are 4.18±2.75, 2.69±3.43, 0.4±1.44, 1.96±2.8, 1.47±2.72, 2.33±3.08 and 2.85±3.06 CFU/10g, respectively. The population of the aerobic bacteria, *Vibrio*, *Pseudomonas*, fungi, *Salmonella-Shigella*, staphylococcal and coliform of the home smoked dried rinsed shrimps are 4.11±2.48, 1.09±2.50, 0.00±0.00, 0.00±0.0, 1.56±2.62, 3.35±3.03 and 1.25±2.17 CFU/10g, respectively. The highest aerobic bacterial load was recorded in the market smoke dried shrimps, while the home smoke dried rinsed shrimps had the least aerobic bacterial load. The home smoke dried rinsed shrimps had the least counts of *Vibrio*, *Pseudomonas*, fungi and coliform, respectively, while the market dried shrimps had higher *Pseudomonas*, fungal and staphylococcal load. The home smoke dried not rinsed shrimps had higher *Vibrio* and coliform loads than the market dried and home smoke dried rinsed shrimps. The lower microbial load in the home smoke rinsed shrimps could be attributed to the processing technique which involved the use of clean water to rinse before drying. More so, the two drying methods showed reduction in the microbial load compared to the fresh shrimps.

The proximate analysis of the market smoke dried and home smoke dried shrimps showed that the market smoke dried shrimps has higher Moisture, Ash, fat, crude fiber and carbohydrate content than the home smoke dried shrimps. While the home smoke dried shrimps had higher crude protein than the market dried shrimps. Despite the high moisture content in the market dried shrimps, the fresh shrimps had very high moisture content. Also, in the nutritional content such as ash, fat and crude fiber of home and market smoked dried, there were no significant differences ($p < 0.05$) in proximate composition but there was a significant difference in crude fiber and carbohydrate content. (Table 2).

The bacterial and fungal isolates from shrimps using smoke dryer are presented in Tables 3 and 4, respectively. *Aspergillus niger*, *Penicillium* sp, *Rhizopus oligosporus* and *Saccharomyces cerevisiae* were the fungi isolated from the market smoked dried shrimps while *Aspergillus niger* and *Saccharomyces cerevisiae* were the fungi isolated from the home smoke dried samples. High humidity and warm environmental temperatures in the coastal areas of the Niger Delta predispose to growth of fungi [10].

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Table 1: Effect of smoke dryer on the microbial load of *Litopenaeus vannamei* (shrimp)

Shrimps	THB X10 ⁵	TCBS X10 ⁴	TPS X10 ⁴	TFC X10 ⁵	TSSC X10 ³	TSC X10 ⁴	TCC X10 ⁵
Fresh rinsed	7.13±3.02 ^c	4.17±2.64 ^b	3.06±3.07 ^b	4.02±2.8 ^d	4.65±1.82 ^b	3.49±1.48 ^{ab}	4.01±2.99 ^c
Market smoked dried	4.23±3.30 ^{ab}	1.98±3.15 ^a	0.97±2.58 ^a	2.95±2.96 ^{cd}	1.22±2.67 ^a	3.89±3.22 ^{ab}	1.88±2.86 ^{ab}
Home smoked dried not rinsed	4.18±2.75 ^{ab}	2.69±3.43 ^{ab}	0.4±1.44 ^a	1.96±2.8 ^{bc}	1.47±2.72 ^a	2.33±3.08 ^a	2.85±3.06 ^{bc}
Home smoked dried rinsed	4.11±2.48 ^{ab}	1.09±2.50 ^a	0.00±0.00 ^a	0.00±0.0 ^a	1.56±2.62 ^a	3.35±3.03 ^{ab}	1.25±2.17 ^{ab}

Mean ± standard deviation values

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

Table 2. Proximate analysis of market dried and home smoke dried Shrimp

Shrimps	Moisture	Ash	fat	crude fibre	crude protein	CHO
Fresh	69.42±1.33 ^d	2.58±0.23 ^a	1.66±0.00 ^{ab}	3.86±0.18 ^a	17.81±0.78 ^a	4.18±0.08 ^a
Market smoked dried	15.92±0.23 ^c	10.34±0.72 ^b	0.96±0.05 ^a	11.13±1.22 ^b	51.07±0.00 ^b	10.59±0.6 ^b
Home smoked dried	9.50±0.00 ^b	8.95±0.07 ^b	0.73±0.11 ^a	9.48±0.60 ^b	67.34±0.00 ^d	4.02±0.79 ^a

Mean ± standard deviation values

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

CHO: carbohydrate

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

Isolate	Market smoke dried	Home smoke dried rinsed	Home smoke dried not rinsed
<i>Bacillus</i>	+	+	+
<i>Klebsiella</i>	+	-	-
<i>Proteus spp</i>	+	-	+
<i>Providencia</i>	+	-	-
<i>Pseudomonas</i>	+	-	+
<i>Shigella</i>	+	-	+
<i>Staphylococcus</i>	+	+	+
<i>Vibrio</i>	+	+	+

+ = present

- = absent

Table 4. Fungi isolated from *Litopenaeus vannamei* on different drying methods

Fungi isolate	Home smoke dried sample not rinsed	Market smoke dried sample
<i>Aspergillus niger</i>	+	+
<i>Penicillium sp</i>	-	+
<i>Rhizopus oligosporus</i>	-	+
<i>Saccharomyces cerevisiae</i>	+	+

+ = present

- = absent

The bacterial genera isolated from the market dried shrimps were *Bacillus*, *Klebsiella*, *Proteus*, *Providencia*, *Pseudomonas*, *Shigella*, *Staphylococcus* and *Vibrio*. In the home smoke dried rinsed shrimps, only *Bacillus*, *Staphylococcus* and *Vibrio* sp were isolated whereas the home smoked dried not rinsed were *Bacillus*, *Proteus*, *Pseudomonas*, *Shigella*, *Staphylococcus* and *Vibrio* sp (Table 3). The differences in the bacterial types could be attributed to the different processing method and handling which could introduce microbes and may lead to contamination of the shrimp. The microbial quality of the river, estuaries and seashores from which shellfish are harvested influence the microflora of shellfish samples [11]. In addition to the endogenous microflora of the shellfishes, crustaceans are transported 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 [12]. 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 [13, 14]. Although drying reduces water activity and destroys bacteria through the activity of heat, post processing contamination can occur especially during handling and transportation of processed foods to point of sale [15]. Processing of shellfish following proper food handling practices, especially the use of clean water for 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 shellfish cultured in contaminated waters [16]. 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 [17]. Studies have suggested that the presence of *Staphylococcus* and *Salmonella* species on ready-to-eat food may be as a result of improper handling, cross contamination and poor temperature control [18, 19]. In this current study, the smoke drying methods reduced the counts of *Staphylococcus* spp. but did not eliminate the contaminating microorganisms. *Vibrio* and *Shigella* were found to be present due to fecal contamination of the seafood from the water in which they were harvested.

Conclusion

The findings in this study have shown that smoke drying affected the microbial load inherent in shrimps and most of the microorganisms associated with shrimps in this study could be as a result of handling / processing techniques employed. More so, the use of smoke to dry shrimps affected the moisture content by causing a decline in moisture and aided longer preservation of shrimps. The home smoke dried shrimps showed higher protein and carbohydrate value than the market smoke dried sample. Thus, preservation of shrimp by rinsing in a clean water before smoke drying should be encouraged.

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