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An Evaluation of the Bacteriological Quality of Some Molluscan Shellfish Preserved With Different Drying Methods

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Abstract

The bacteriological quality of *Thais califera* (whelk) *Tympanotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) preserved with different drying methods (sun, oven, multipurpose dryer and wood smoke) were investigated using standard microbiological methods. The total viable bacterial count in whelk, periwinkle and oyster ranged from 3.86×10^5 cfu/g - 7.56×10^6 cfu/g, 3.38×10^5 cfu/g – 7.02×10^6 cfu/g and 3.20×10^5 cfu/g – 8.08×10^6 cfu/g respectively. Total coliform range from 322 to ≥ 2400 MPN/100g. Faecal coliform ranged from 33 to ≥ 109 MPN/100g. *Staphylococcus* count ranged from 3.38×10^4 cfu/g – 1.99×10^4 cfu/g, 1.09×10^4 cfu/g - 1.58×10^4 cfu/g and 1.25×10^4 cfu/g - 2.51×10^4 cfu/g respectively. *Salmonella* count ranged from 3.04×10^3 cfu/g – 6.71×10^4 cfu/g, 2.9×10^3 cfu/g - 2.48×10^4 cfu/g and 3.25×10^4 cfu/g - 4.65×10^4 cfu/g respectively. While *Shigella* count ranged from 1.65×10^3 cfu/g -5.65×10^4 cfu/g, 1.79×10^3 cfu/g - 1.31×10^4 cfu/g and 0.95×10^3 cfu/g - 2.85×10^4 cfu/g respectively. Statistical analysis showed that drying significantly affected the bacteriological parameters at $p<0.05$ in all the shellfish studied. The bacteria isolated from *T. callifera*, *T. fuscatus* and *C. gasar* were *Micrococcus*, *Enterobacter*, *Escherichia coli*, *Staphylococcus*, *Arthrobacter*, *Citrobacter*, *Pseudomonas aeruginosa*, *Proteus*, *Salmonella*, *Shigella* and *Bacillus cereus*. The results obtained revealed that fresh shellfish have higher microbial load than the preserved samples. The order of reduction of bacteria load from the dried shellfish was oven dried > multipurpose dryer dried > smoked dried > sun dried samples. Preservation of molluscan shellfish by drying will increase the availability, durability, safety and wholesome supply of this protein rich food for the masses.

Key words: Shellfish, drying, preservation, bacteria, *Staphylococcus aureus*.

Introduction

Seafood is any sea animal or plant that is served as food and is eaten by humans. They include fish and shellfish including mollusks and crustaceans (Archibong *et al*., 2014). *Thias callifera* (Whelk) *Tympanotonus fuscatus* (Periwinkle) and *Crassostrea gasar* (Oyster) are among the molluscan shellfish of the world. They are the most dominant shellfish of the Niger Delta region of Nigeria (Deekae and

Idoniboye 1995). Molluscan shellfish is tasty and constitutes a major source of proteins, vitamins and minerals. Effective utilization of these protein rich aquatic resources is recommended to improve the protein intake of rural population who are usually affected by protein deficiency syndrome (Archibong *et al*., 2014).

Freshly shucked shellfish is soft and easily damaged; therefore rough handling may result in contamination of shellfish. Their short shelf life poses serious practical problems of their storage and distribution (Frazier and Westhoff, 2000). Whelk, periwinkle and Oysters will become unfit for human consumption within about one day after being shucked, unless it is subjected to some form of processing or preservation by drying. This will reduce or destroy the contaminating microbial load and in turn destroy intrinsic enzymatic activities in them. Traditional curing methods such as sun-drying and smoking are used in preserving shellfish that cannot be sold by fishermen (NIIR, 2003).

Molluscan shellfish is consumed raw (ingested whole), preserved or processed and form a much – cherished delicacy in some parts of the world including Nigeria. Consumption of raw or fresh shellfish has been known to result to the transmission of pathogenic organisms (BMJ, 1990). Survey on the microbiological quality of shellfish has shown shellfish to harbor pathogenic organisms. These pathogenic organisms have been implicated in outbreaks of food-borne disease in many parts of the world (FAO/WHO, 2003).

The bacterial flora associated with fresh molluscan shellfish is principally a function of the environment in which the shellfish are harvested and of the handlers and not of the shellfish. More so, aquatic environments are extremely susceptible to microbial contamination. Shellfish-borne pathogenic bacteria may be conveniently dividend into 3 groups according to their ecology and origin, as those that are indigenous to the aquatic environment, the general environment and the animal/human reservoir (FDA, 2000; Farber and Peterkin, 2000). Food handlers may transmit pathogens, including enteric pathogens to food from a contaminated surface, from another food or hands contaminated with microorganisms from their gastrointestinal tract (BMJ, 1990).

Since whelk, periwinkle and oysters are found in bodies of water containing untreated human and Industrial waste, there is a tendency that they may concentrate and accumulate high levels of pathogens and toxic contaminants which can pose significant health hazards to consumers. This is not surprising as Mollusca shellfish are filter feeders. Improper storage and handling, inadequate heat processing or preservation and storage after purchase of fresh shellfish may also allow some pathogens particularly enteric viruses 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 (USFDA, 1998). A number of surveys have shown that consumer awareness about the quality of their food is increasing (FAO/WHO, 2003).

Considering the massive consumption/demand and enormous nutritional and industrial importance of Mollusca shellfish in the Niger Delta region, the fishing industry cannot continue to remain neglected. There is therefore the 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 microorganism.

This present study was undertaken to evaluate the effect of different drying methods on the bacteriological quality of *Thais callifera*, *Tymponotonus fuscatus* and *Crassostrea gasar*. The objectives of the study were: To isolate and enumerate bacteria from molluscan shellfish samples before and after drying with different drying methods; to characterize and identify the bacteria isolated from the shellfish samples; to subject the data obtained to statistical analysis and to identify a suitable drying method to preserve shellfish that will ensure low microbial load over a considerable period of time and reduction in the health risk in the consumption of shellfish.

Materials and Methods

Sampling location

The sampling location was Creek Road Market in "Town Area" of Port Harcourt, Nigeria. Freshly shucked *Thais callifera* (whelk) *Tympanotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) samples purchased were transported to the laboratory in a clean container within 2 hours of purchase for analysis.

Type of seafood samples used for analysis

Fresh seafood samples [*Thais callifera* (Whelk), *Tymponotonus fuscatus* (Perewinkle) and *Crassostrea gasar* (Oyster)] were purchased from seafood vendors in Creek Road Market in Port Harcourt. Samples were aseptically collected and transported to the laboratory immediately for processing and analysis. The fresh shellfish samples were subjected to different drying methods which included drying using dryer manufactured by (NSPRI); electric oven; sun drying; and wood smoke drying. Freshly shucked samples were used as control.

Methods of drying of shellfish samples

Sun Drying: The heat from the Sun was used in drying the shellfish samples during the day. Average temperature attained was 29^{o}C. The samples were turned after every 4 hours until drying was completed.

Oven drying: An oven dryer, electrically powered, was set to operate at 50^{o}C. The come –up time was one hour, 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 50^{o} C and 55^{o}C.

Multipurpose dryer drying: The dryer was built by Nigerian Stored Product and Research Institute (NSPRI). It has two parts: the stove and cabinet compartments. The stove portion is made of aluminum and the cabinet compartment mounted on it. The

cabinet portion is an enclosed wooden chamber containing layers of trays (wooden frames with wire mesh beneath). The enclosed cabinet has a small vent at the top and sides for smoke and moisture expulsion. The stove compartment was heated with a kerosene stove and the temperature was maintained between 50 and 55^{o}C.

Wood Smoke drying: 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 dried shellfish samples from the different drying methods were packaged in clean polythene bags, sealed and stored for 28 days.

Sample preparation and analysis

The dried shellfish samples which were stored for 28 days were aseptically milled into fine powder before being used for analysis. Bacteriological, analyses were conducted on the shellfish samples each parameter was determined in triplicate.

Bacteriological Analysis of Molluscan Shellfish

Enumeration of Total Aerobic Heterotrophic Bacteria

Ten grams (10g) of molluscan shellfish samples were transferred to a sterile blender and homogenized for 2minutes with 90ml of sterile 8.5% normal saline to give a 10^{-1} dilution. The filtrate was used to prepare serial tenfold dilution by transferring 1ml to fresh diluents. Aliquots (0.1ml) 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. The plates were incubated at 37^{o}C for 24 hours and only colonies of plates on which 30 to 300 colonies developed were counted, the average counts of triplicate plates were recorded as total heterotrophic counts of bacteria. Discrete colonies were subcultured on nutrient agar plates and incubated at 37^{o}C to obtain a pure culture. The colonies obtained were stored in MacCartney bottles containing nutrient agar slant and stored in refrigerator as stock cultures for further biochemical test.

Characterization and identification of bacterial isolates

Colonies were aseptically collected from the 24 hours culture and were inoculated on nutrient agar plates and incubated at 37^{o}C to obtained discrete colonies. The 24 hours cultures were further used for identification and characterization test. Cultural and morphological characteristics such as Gram stain and motility were performed. Gram staining reaction was used to identify microorganisms by their gram reaction (Gram positive or Gram negative) and morphology. It was performed as described by (Cappuccino, 2005). Further biochemical tests such as catalase, oxidase, indole production, citrate utilization, Methyle Red (MR), Voges Proskauer (VP), coagulase and carbohydrate fermentation test were also carried out. The isolates were identified following the method of Cowan and Steel (2003).

Estimation of Coliforms, Faecal Coliforms and E. coli

The conventional methods for testing for the presence of coliforms, feacal coliforms and *E. coli* which include a presumptive test (MPN), confirmed test (MPN) for feacal coliforms and *E. coli* and completed test for *E. coli* were carried out using methyl red broth, eosin methylene blue agar and gram stain. The MPN test was performed as described by Verma *et al*., (1999). Triple sugar iron (TSI) agar which contains lactose, sucrose glucose, ferrous ammonium sulphate and sodium thiosulphate was used for the identification of enteric organisms by their attack to utilize glucose, lactose or sucrose and to liberate sulphides from ammonium sulphate and sodium thiosulphate.

Isolation of Staphylococcus

Principle Mannitol salt agar is a selective medium prepared according to the recommendations of Chapman in 1945 for the isolation of presumptive pathogenic Staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. 10g of sample was blended in 90ml of 0.1% peptone water. Further 10-fold dilutions were prepared and 0.1ml of each dilution was pipetted onto surface of pre dried plates of thiosulphate citrate bile salt sucrose (TCBS) medium in duplicates. The plates were incubated at 37^{o}C for 24hours and only plates having 30 to 300 colonies were considered in determining plate counts. Colonies were counted again after 48hours incubation. To compute the plate count report as cfu/g the total number of colonies per plate was multiplied by the reciprocal of the dilution used.

Isolation of Salmonella and Shigella

Principal: Deoxycholate citrate agar is a selective differential medium for the isolation of Salmonella and shigella, which is inhibitory to coliform and proteus species. 10g of sample was blended in 90ml of 0.1% peptone water. Further 10-fold dilution was prepared and 0.1ml of each dilution was pipetted onto surface of pre dried plates of thiosulphate citrate bile salt sucrose (TCBS) medium in duplicates. The plates were incubated at 37^{o}C for 24hours and only plates having 30 to 300 colonies were considered in determining plate counts. Colonies were counted again after 48 hours incubation. To compute the plate count and report as cfu/g, the total number of colonies per plate was multiplied by the reciprocal of the dilution used.

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).

Results

The result of the mean count of total heterotrophic bacteria, faecal coliform, and thermo-tolerant coliform bacteria of the fresh and dried samples of *Thais callifera* (whelk), *Tymponotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) is presented in Table 1. The counts of the various groups of bacteria were highest in the fresh shellfish samples and lowest in the oven dried samples.

Table 1: Mean count of bacteria of the fresh and dried shellfish samples

Shellfish sample	Total het. bacteria count ($\times 10^4$cfu/g)	Faecal coliform (MPN Index/100g)	Thermo-tolerant coliform (MPN Index/100g)
Fresh Whelk	147.667	181.66	2400
Sundried	5.34	29	479.33
Oven dried	0.14	13	200.66
Multipurpose dried	1.81	13.33	209.66
Wood smoke dried	4.75	16	301.33
Freshly Periwinkle	886.5	79.66	1020
Sundried	8.058	18.33	207.33
Oven dried	2.663	3.66	104
Multipurpose dried	1.716	8.66	99
Wood smoke dried	2.009	14.66	140.66
Freshly Oyster	2244.0	109.66	2400
Sundried	24.39	13	322.66
Oven dried	4.91	5.66	170.33
Multipurpose dried	12.91	5.66	174.33
Wood smoke dried	49.4	8.33	230

The result of the mean count of *Staphylococcus*, *Salmonella* and *Shigella* of the fresh and dried samples of *Thais callifera* (whelk), *Tymponotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) is presented in Figure 1, Figure 2, and Figure 3 respectively.

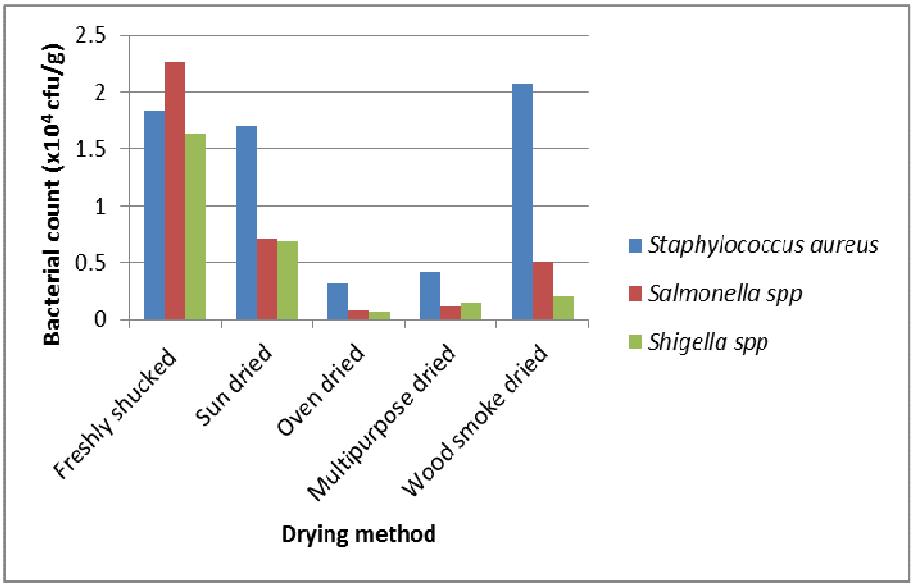

Fig. 1: Mean count of Staphylococcus, Salmonella and Shigella of whelk samples

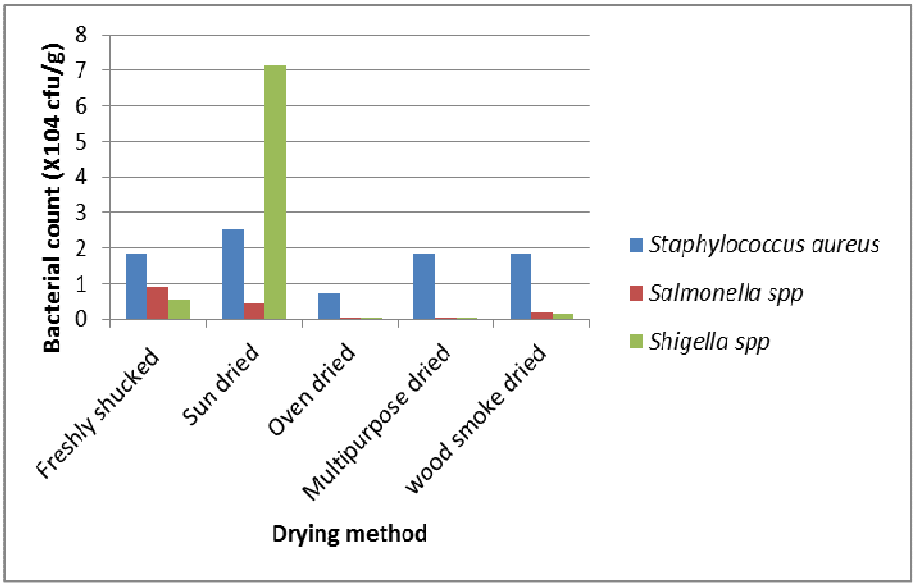

Fig. 2: Mean count of Staphylococcus, Salmonella and Shigella of periwinkle samples

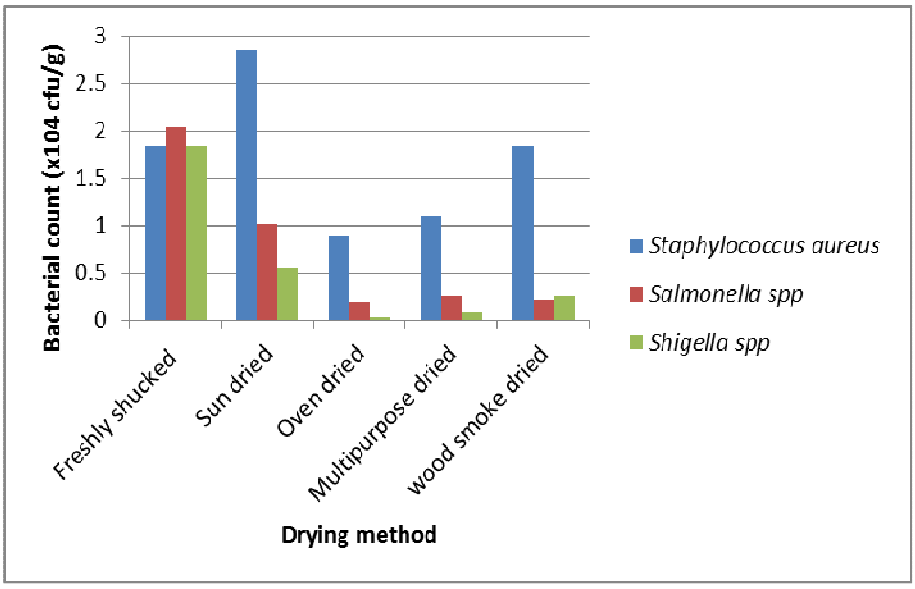

Fig. 3: Mean count of Staphylococcus, Salmonella and Shigella of oyster samples

The frequency (%) of isolation of bacteria from the shellfish samples is presented in Figure 4. *Staphylococcus* and *Bacillus* species had the highest frequency of isolation.

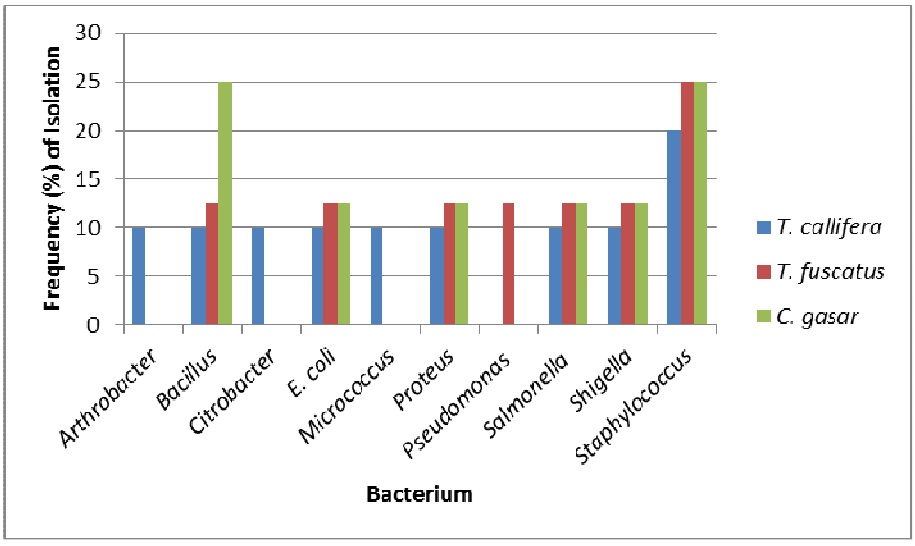

Fig. 4: Frequency (%) of isolation of bacteria from shellfish sample

Discussion

An evaluation of the microbiological properties of freshly shucked and dried *Thais callifera* (whelk), *Tymponotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) samples has revealed the mean count of total heterotrophic bacteria, faecal coliform, and thermo-tolerant coliform bacteria of the fresh and dried samples of *Thais callifera* (whelk), *Tymponotonus fuscatus* (periwinkle) and *Crassostrea gasar* (oyster) as presented in Table 1. The counts of the various groups of bacteria were highest in the fresh shellfish samples and lowest in the oven dried samples.

Of the shellfish samples dried with the various drying methods, the oven-dried samples had the lowest bacteria load while the sun dried samples had the highest bacteria load. The order of reduction of bacteria load from the dried shellfish samples was oven dried > multipurpose dryer dried > smoked dried > sun dried samples. Statistical analysis showed that drying significantly affected the bacteriological parameters at $p<0.05$ in all the shellfish studied.

The present study has also revealed that these shellfish contained various bacteria including *Enterobacter* spp, *Escherichia coli*, *Micrococcus* spp, *Bacillus* spp, *Pseudomonas* spp, *Salmonella* spp, *Shigella* spp, *Staphylococcus* spp, *Arthrobacter*, *Citrobacter and Proteus.* Most of these bacteria have also been reported by previous workers (Rhodes and Kator, 1988). The microbial quality of the river, estuaries and seashores from which shellfish are harvested influence the microflora of shellfish samples (Adams and Moss, 2005). 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 (Adams and Moss, 2005). The initial microbial load on ready-to-eat foods is important; however factors such as processing, storage and display for sale may influence the microbiological load of ready-to-eat foods at the point of sale (Beuchat and Ryu, 2004). Although drying reduces water activity and destroys bacteria through the agency of heat, post processing contamination can occur especially during handling and transportation of processed foods to point of sale. The process of shucking with bare hands in an uncontrolled and filthy environment, with little or no regard for proper food handling practices could result in further contamination of the shellfish with bacteria from the hands, nails, nasal passage and mouth of the handlers during processing and poor handling practice during retailing and purchase results in further contamination.

The detection of faecal coliform in the shellfish samples is attributed to faecal contamination of the environment by residents of the coastal areas of the Niger Delta from where these shellfish were harvested. Some household and public toilet facilities and pier latrines built directly over the river banks resulting in constant deposition of faecal matter in the river. These waters contaminated with sewage pose the risk that enteric organisms from infected individuals may be present and concentrated by the filter feeding activities of shellfish. 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 (FAO/WHO, 2003). Most strains *of Escherichia coli* are harmless commensals; however some strains are pathogenic and can cause diarrhea 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 (Brayan, 1973) as practiced by the molluscan shellfish vendors.

Staphylococcus species was isolated from freshly shucked and dried samples of *Thais callifera*, Tympanotonus *fuscatus* and *Crassostrea gasar*. 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 (Christiansen and King, 1991). Street shellfish vendors use their bare hands during processing and constantly dip their fingers into basins containing fresh and dry seafood during haggling for price. Food handlers with hand infection or with cold or with sore throat may transfer enterotoxigenic strains of *Staphylococcus* to food. When given optimum conditions, it grows, generate toxins and cause staphylococcal intoxication. Growth to levels above 10^6cfu/g is required for toxin formation and since *Staphylococcus aureus* is a mesophilic organism, some degree of temperature abuse precedes intoxication (FAO/WHO, 2003). Drying samples of the shellfish contaminated with *Staphylococcus* spp. using different drying methods reduced the plate count values of *Staphylococcus* spp. but did not eliminate the contaminating microorganisms. Isolation of *Bacillus* spp, feacal coliforms and *Shigella* spp indicated that the seafood was contaminated from where they were harvested. The display of the dried whelk, periwinkle and oyster meat without any form of packaging could also be attributed to contamination. Being frequently displayed and uncovered, the shellfish meat will become liable to contamination to bacterial origin. Some strains of *Bacillus* (e.g. *B. cereus* and *Staphylococcus aureus* are known enterotoxin producers (Bryant, 2007). The inherent danger in the association of *B. cereus* and *S. aureus* with or without their metabolic products in various foods, without further heat treatment is the possible outbreak of serious food-borne illness. Keeping processed seafood for retail free of contamination with *Staphylococcus* spp. is best ensured by observing proper food handling practices involving minimal contact with human skin. There was a significant difference ($p<0.05$) between the values of freshly shucked samples, sun dried samples, oven dried samples, multipurpose dryer sample and market smoked dried in *Thais callifera* samples while there was no significant difference in samples of *Tymponotonus fuscatus* and *Crassostrea gasar*.

Isolation of *Salmonella* species and *Shigella* species from the shellfish samples canbe attributed to possible chronic carriers, from feaces to other persons by the oral-feacal route, which may be water-borne, food borne or by contact with hands and other formites. Classically the vehicle of spread from this source is water. Therefore eating raw shellfish as well as undercooked or improperly cooked shellfish that may have fed in contaminated water can cause illness to the unsuspecting consumer. Drying contaminated shellfish samples reduced bacterial load. There is no significant difference ($p < 0.05$) for the different drying methods. However there was a significant difference ($p > 0.05$) between the freshly shucked samples and the dried samples

Shigella usually associated with amoebic and bacillary dysentery, may survive up to six months in water and may also survive for long period of time in shellfish. The infectious dose of *Shigella* is low, approximately 10 to 100 cells (FAO/ WHO, 2003), therefore its presence in food must be avoided. However, the methods of drying used in this present study were effective in reducing microbial load of the shellfish.

The order of reduction of bacteria load from the dried shellfish samples was oven dried > multipurpose dryer dried > smoked dried > sun dried samples.

The putrid odour of the sun dried sample, due to proteolysis, must have encouraged the proliferation and growth of bacteria which in turn resulted in the observed high load of bacteria in the sundried samples. Another factor that helps with drying food is humidity. Since drying involves extracting the moisture from the food items and expelling it into the surrounding air, low humidity will help with the drying process. If the humidity is high, drying will be slower simply because the surrounding air would also be laden with moisture.

The high humidity levels in the Niger Delta make sun drying difficult. Sun drying is a slow and time-consuming process since the unpredictable and uncontrollable weather is the drying agent. Moreover, it is this unpredictability that also makes sun drying a risky process. For instance, in the Niger Delta, sudden rains can ruin the entire process of drying. Not only that, having the ideal mix of temperature, humidity and air flow is often difficult to achieve and this prompts one to look for other methods of drying food.

The use of multipurpose dryer in drying these shellfish samples gave a better dried product compared to sun dried samples. It is effective and has low operating cost, no electricity energy required, low maintenance and does not require expert operation. The resulting product can be packaged and exported for sale as to earn foreign exchange for the country.

Conclusion

Microbiological evaluation of molluscan shellfish preserved with different drying methods showed that drying actually reduced the amount of microorganisms harbored by fresh shellfish samples. Preservation of food extends its shelf life while ensuring its safety and quality. From the results obtained the oven drying method had the least microbial load and is thus regarded as the best method of preservation.

The microbial content of the shellfish samples are related to the microbial quality of the water bodies from which seafood is harvested and other industrial and human activities taking place in the water. Therefore attention should be paid to their safety through proper harvesting, processing, handling procedures, storage, packaging and good hygienic practices.

Recommendations

Since the water bodies in which shellfish grow and feed are also used for dumping sewage and industrial wastes, wastewaters should be properly treated to remove pathogenic organisms before discharging into the water bodies. Considering the massive consumption and demand of edible shellfish, and its commercial and indus-

trial importance, preservation of molluscan shellfish by drying will reduce the microbial content; increase the availability of shellfish, durability, safety and wholesome supply of this protein rich food for the masses. The government should make efforts to make electricity available to the citizenry so that the preservation of food with the appropriate drying method will be feasible. The government should also establish organizations to monitor and control the preservation and quality packaging of wholesome quality shellfish which can be exported for sale as to earn foreign exchange for the country.

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