Comparative Study on Bacterial Quality of Fresh and Frozen Shrimp (*Palaemonetes* spp.) Sold in Retail Markets in Port Harcourt, Rivers State, Nigeria.

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Abstract: Fresh and frozen shrimp sold in retail markets in Port Harcourt were examined for bacterial quality using standard plate count, total coliform count and pH value. A total of ten fresh and frozen shrimp samples were purchased and tenfold serial dilution were spread plated in duplicates on nutrient and MacConkey agar plates and incubated at 37^0C for 24 hours. Fresh samples had average pH of 7. 21±0.16 and gave total plate counts of 1.89 x10^5 to 3.1x10^7 cfu/g and total coliform counts of 2.72 x10^5 to 3.1 x 10^5 cfu/g. The frozen samples had average pH value of 8.55±0.28 and total plate counts of 8.00x10^6 to 9.9x10^6 cfu/g and total coliform counts of 8.50x10^4 to 1.00x10^5 cfu/g. All isolates were sub-cultured onto nutrient agar and identified based on standard biochemical tests. The following isolates were identified from fresh samples, *Proteus* sp., *Klebsiella* sp., *Serratia* sp., *Enterobacter* sp., *Staphylococcus* sp., and *Escherichia coli*, while the frozen samples had *Pseudomonas* sp., *Streptococcus* sp., *Alcaligenes* sp., *Bacillus* sp., and *Micrococcus* sp. Bacterial contaminants of shrimp is a reflection of the harvesting environment, the quality of the overlaying waters and post-process contamination through unhygienic handling, processing, packaging and storage as well as the filthy environment of the market place. The various bacterial isolates are potential pathogens and spoilage organisms. *Staphylococcus aureus* enterotoxin A (SEA) is extremely potent with as little as 100ng sufficient to cause intoxication. Hence shrimp marketed in Nigeria are mainly smoked or dried to make the product less hazardous. The International Commission on the Microbiological Specifications of Foods (ICMSF) recommended total bacterial count for shrimp as 1x10^7 cfu/g. The results revealed that both fresh and frozen shrimp sold in Port Harcourt retail markets did not meet the recommended ICMSF bacteriological standards for shrimp quality and so cannot be exported.
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1. Introduction

Shrimp (*Palaemonetes* spp.) are decapods, swimming crustaceans classified in the infra order Caridea. They can be found near the seafloor of most coasts including estuaries and in wells, rivers and lakes. Marine species are found at depths of up to 5, 000 metres (16,000 ft), (Fenner *et al*., 1989) and from the tropics to the Polar Regions. Shrimps are also farmed for human consumption. In 2003, the total global production of farmed shrimp reached more than 1.6 million tonnes, representing a value of nearly 9 billion U.S. dollars. (Gillett, 2008).Thailand remains the world's leading black tiger shrimp exporter with approximately 25% world market share with an average annual revenue of 2.32 billion U.S. dollars in 1998-2001. Also 75-80% is cultured while 20-25% is captured. Supermarkets set quality and sanitary standards which Thai suppliers must meet (Manarungsan *et al.*, 2005).Different countries have different requirements. Shrimps imported by Japan are inspected for presence of bleaching agents and residual antibiotics and allowable limits for food preservatives such as nitric acid, sodium sulphite and sodium hyposulfite. Limit for antibiotics chlortetracycline, oxtetracycline and tetracycline is 0.2 ppm.There should be zero *Bacillus sp.*in cooked shrimp. The European Union has banned use of chloramphenicol and nitrofurantoin as preservatives. In Canada, histamine, fecal coliforms and decomposed products were the top three causes of outbreaks of seafood illnesses. In Nigeria, shrimp is harvested from unknown tropical waters and studies by previous researchers reported high level of bacterial contamination with *Pseudomonas sp.* as the main spoilage bacteria (Aribisala, 1975).Importation standards for Nigeria are not known.

Shrimp have high levels of omega 3 fatty acids and low levels of mercury (Smith *et al.*, 2010). They are high in calcium, iodine and protein, essential minerals and vitamins but low in food energy (Jay, 1986a). Shrimp-based meal is also a significant source of cholesterol, from 122mg to 251mg per 100g of shrimp. Shrimp consumption, however, is considered healthy for the circulatory system because the lack of significant levels of saturated fat in shrimp means that the high cholesterol content in shrimp actually improves the ratio of low density lipid to high density lipid

cholesterol and lowers triglycerides (Elizabeth *et al.*, 1996).

There are reports that many coastal communities have a long history of disposing sewage onto tidal flats and mudflats, thereby contaminating the water and shellfish (Shiaris *et al.*, 1987). Foster *et al.,* (2003) reported that shellfish have low bacterial counts when freshly caught but during holding, however, the number of bacteria increases significantly, with some samples attaining total viable count values in the 10^7 cfu/g to 10^8 cfu/g range. The implication of these higher bacterial counts is the possibility of shellfish-borne diseases such as gastroenteritis caused by *E. coli, Salmonella* sp., *Vibrio vulnificus, V. parahaemolyticus, Bacillus cereus* and *Staphylococcus aureus* (Clemson University, 2012).Microbiological standards for shrimp set by Codex alimentarius commission (FAO/WHO,1982) on fish and fisheries products emphasised rejection of frozen shrimp with total aerobic plate count above 10^5 cfu/g, *Staphylococcus aureus* above10^2 cfu/g and the detection of *Salmonella*.

The objective of this study was to comparatively evaluate the bacteriological quality of fresh and frozen shrimp sold in retail markets in Port Harcourt to ascertain if they meet the standards of International Commission on Microbiological Specifications for Foods.

2. Materials and Methods

The samples used for this study were fresh, in-shell shrimp purchased from Creek Road market and frozen, peeled shrimp purchased from Superbod store in Port Harcourt. A total of ten samples each were purchased from the retailers, wrapped in sterile aluminium foil and transported to the laboratory for microbiological analysis within 2 hours.

2.1. Enumeration of bacterial contaminants

The shrimp samples were prepared by homogenizing 5g in 45ml sterile diluents (physiological saline) in a sterile Binatone blender for 2 minutes. The homogenized shrimp was then used for tenfold serial dilution and pH measurement. The spread plate technique was used and 0.1ml of the dilution required was plated in duplicate. Higher dilutions (10^{-4}, 10^{-5}) were plated for fresh samples while lower dilutions (10^{-2}, 10^{-3}) were used for frozen samples and both samples were plated on Nutrient agar and MacConkey agar for total bacteria and total coliforms respectively. Incubation was done for 24 hours at 37^{O} C before the total numbers of colonies were counted. Red colonies on MacConkey agar represented coliforms. Pure colonies were preserved on nutrient agar slants for identification. Bacterial load of samples was calculated as colony forming units per grams (cfu/g).

2.2. Identification of isolates

Standard procedures were used (Berger and Holt, 1994) and the colonial morphology, Gram reaction, motility test and biochemical tests (including indole production, methyl red, Voges–Proskauer, citrate utilization, catalase, oxidase tests) and carbohydrate fermentation tests were performed on isolates.

2.3. pH measurement

The digital pH Meter was used. It was switched on and allowed to stabilize for 15 minutes before sterilizing the electrode by rinsing with jet of 95% ethanol. Standardization was done with buffer 4 and 7. The electrode was rinsed again using distilled water before it was dipped into the 1:10 dilution of homogenate and the pH read.

2.4. Statistical analysis

The data generated in the study were subjected to statistical analysis to determine level of significance using the students''t' test. A value of $P<0.05$ was accepted as significant and $P>0.05$ was considered as not significant.

3. Results

Table 1 shows the average values for total aerobic plate count (APC), total coliform count and pH of 5 fresh shrimp samples. Total aerobic count ranged from 1.84 x 10^7 to 3.1 x 10^7 cfu/g with an average of 2.6 x 10^7 cfu/g or log count/g of 7.39 ± 0.14. Total coliform count ranged from 2.72 x 10^5 to 3.1 x 10^5 cfu/g with average of 3.0 x 10^5 cfu/g or log count/g of 5.48±0.03. Fresh shrimp had an average pH of 7.21±0.06, which falls within the expected value of 6.8 – 7.0 for fresh shrimp product (Jay, 1986a).

Table 1: Total aerobic count, total coliform count and pH values of fresh Shrimp at 37^0 C.

Samples(n)	pH values	APC (cfu/g)	Total coliform(cfu/g)
1	7.30	1.89 x 10^7	3.07 x 10^5
2	7.06	1.99 x 10^7	3.0 x 10^5
3	7.20	3.10 x 10^7	3.1 x 10^5
4	7.43	2.90 x 10^7	2.72 x 10^5
5	7.05	3.10 x 10^7	3.05 x 10^5

Table 2 shows the average values for total aerobic plate count, total coliform count and pH of 5 frozen shrimp samples. Total aerobic count ranged from 8.00 x 10^6 to 9.9 x 10^6 cfu/g with an average of 9.1 x 10^6 cfu/g or log count/g value of 6.96 ±0.04.

Total coliform count ranged from 8.50 x 10^4 to 1.00 x 10^5 cfu/g with an average of 9.0 x 10^4 cfu/g or log count/g value of 4.67±0.04. Frozen shrimp had an average pH value of 8.55±0.28, which implies alkalinity and formation of spoilage amines such as putrescine, cadaverine, trimethylamine, ammonia, which are indicative of putrefaction (Jay, 1986b).

Table 2: Total aerobic count, total Coliform Count and pH values of Frozen Shrimp at 37^0 C.

Samples(n)	pH values	APC (cfu/g)	Total coliform(cfu/g)
1	8.45	9.90 x 10^6	1.00 x 10^5
2	8.50	9.05 x 10^6	8.50 x 10^4
3	8.43	8.00 x 10^6	8.70 x 10^4
4	9.04	9.40 x 10^6	9.00 x 10^4
5	8.35	9.20 x 10^6	8.95 x 10^4

Statistically, the difference of means (0.428) is less than the critical ratio (6.62) indicating significant difference ($p<0.05$) between the two products. Frozen samples have lower count. The fresh samples have pHs close to neutrality (normal, 6.8-7.0) while the frozen shrimp's pH are basic or alkaline, indicating that it was stored under fluctuating temperatures due to power failure and have undergone cycles of freezing and thawing(Reay and Shewan,1991).

Table 3 shows that fresh shrimp contained mainly enteric bacteria in the family Enterobacteriaceae, indicative of possible contamination from human sources through unhygienic practices while frozen shrimp had more of psychrophilic microorganisms such as *Pseudomonas, Alcaligenes, Aeromonas* genera.

Table 3: Frequency of Bacterial Isolates from Fresh and Frozen Shrimp at 37^0 C.

Fresh Shrimp	Frequency/5 samples	Frozen shrimp	Frequency/ 5 samples
Proteus	2	*Pseudomonas*	4
Klebsiella	3	*Streptococcus*	4
Serratia	2	*Alcaligenes*	4
Enterobacter	2	*Bacillus*	4
Citrobacter	2	*Micrococcus*	2
Staphylococcus	4	*Aeromonas*	3
Escherichia coli	4	*Proteus*	2

4. Discussion

The International Commission on Microbiological Specifications of Foods (ICMSF, 1986) recommended microbiological limits for frozen raw crustaceans as 10^7 cfu/g for total bacterial count, 500 cfu/g for *E. coli*, 0 count for *Salmonella*, 10^3 cfu/g for *Vibrio parahaemoliticus* and 10^4 cfu/g for *Staphylococcus aureus*, while total aerobic plate count for cooked peeled tail-on shrimp is 10^5 cfu/g. Most shrimp harvested by trawlers are washed and frozen immediately before processing in various forms. Some have the tail removed, some are peeled and boiled, and some are breaded, with or without the intestines and standards exist for these various forms and are different for various countries accepting imported shrimp. Standards for some countries are very stringent. In France, frozen shrimp should have no coliform bacteria and in Spain, no *Vibrio cholerae* (Manarungsan et al., 2005).The high count of 9.4 x 10^4 cfu/g coliforms in the frozen shrimps in this study would imply that they are not acceptable for local consumption or export even though the average total aerobic plate count of 9.1 x 10^6 cfu/g was slightly below the limit of 10^7 cfu/g.

The high level of contamination of fresh shrimp, 2.6 x 10^7 cfu/g for average total bacterial load supports the existence of unhygienic environment of Nigerian markets and the lack of monitoring of food standards by the National Agency for Foods and Drugs Administration and Control (Ibe, 2008). The occurrence of enteric organisms in the shrimp sample is an indication of the pollution of the harvesting and overlaying waters with untreated faecal waste and sewage (Elliott and Michener, 2003). The high level of various isolates from shrimps may play major roles in causing seafood-borne diseases and illnesses and this is why samples need to meet standards (FAO/WHO,1982). Most illnesses from naturally contaminated sea foods are associated with eating under-cooked or raw shellfish particularly molluscs (Jay, 1986a). *Staphylococcus aureus* enterotoxin A (SEA) is extremely potent with as little as 100ng sufficient to cause intoxication (Evenson *et al*., 1988). Other seafood problems are due to recontamination time and temperature abuse. In Nigeria shrimp is preserved by smoking, a process which imparts flavour to the product and reduces the bacterial load. Although frozen shrimp had lower counts than the fresh, both did not meet the recommended ICMSF bacterial quality standards for shrimp and so cannot be exported.

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References

1. Aribisala,O.A.(1975): The quantitative and qualitative bacteriology of shrimp and the role of bacteria in shrimp spoilage. *Annual report*. Ministry of Agriculture and Rural Development Discussion, Nigeria.
2. Bergey, D.H. and J.G. Holt (1994). *Bergey's Manual of Determinative Bacteriology. (9th ed.)* The Williams and Wilkins Company Baltimore.
3. Clemson University Cooperative Extension (2012).Food borne illnesses related to seafood. *www.clemson.edu/extension/hgric/food/food_safety/illnesses/hgic.*
4. Elizabeth, R., E. De Oliveira, E. Cynthia, J. Jaon, C. Lisa, M. Frank, and L. Jan (1996). Effects of shrimp consumption on plasma lipoproteins. *American Journal of Clinical Nutrition,* **64(5)**: 712-717.
5. Evenson, M.I., M. W. Hind, R.S. Bernstein and M.S. Bergdoll (1988). Estimation of human dose of Staphylococcal enterotoxin A from a large outbreak of Staphylococcal food poisoning involving chocolate milk. *Int. J. Food Microbial.* **7**:311-317.
6. Elliott, R.P., and H.D. Michener (2003). Review of microbiology of frozen foods. In conference on frozen food quality. USDA. *Agricultural Research Service. Pp.* 74-21.
7. FAO/WHO Codex alimentarius commission (1982).Report of the 15th session of the codex committee on fish and fishery products. Section 234.
8. Fenner, A.J., R. Chace, and P.H. Donard (1989). Caridea shrimp. *In* Robert, H. M., D. Abbott and E. C. Haderlie (*ed*.). *Shrimp Intertidal Invertebrates of California*. Stanford University Press.*Pp.* 567-576.
9. Foster, E.M. (2003). Interpretation of Analylical result for bacterial standards enforcement. *Association of Food and Drug.Pp.* 543-548.
10. Gillett, R. (2008). Global study of shrimp fisheries. Rome, Italy: Food and Agriculture Organization. *Fisheries Technical* paper. *Pp.* 475-521.
11. Ibe, S.N. (2008), Microbiological Standards for Foods: Are they relevant in Nigeria? University of Port Harcourt Inaugural Lecture Series, No.60. University of Port Harcourt Press. *Pp.* 48.
12. Jay. J.M. (1986b). Spoilage of Fresh and Processed Meats, Poultry and Seafood. *Pp.* 205-238. *In Modern Food Microbiology* (3rd ed.) Van Nostand Reinhold Co., New York.
13. Jay, J.M. (1986a).Intrinsic and Extrinsic Parameters of Foods that Affect Microbial Growth. *Pp*. 33-60.*In* Modern Food Microbiology (3rd ed.). *Pp.* 33-60. Van Nostrand Reinhold Co., New York.
14. International Commission on Microbiological Specifications of Foods (ICMSF, 1986), Microorganisms in foods.vol. 2. Sampling for microbiological Analysis: Principles and Specific Applications. (2nd. ed.). University of Toronto Press, Buffalo, NY.
15. Manarungsan, S., J. Naewbanii, and T. Revangijakrabhet. (2005) Shrimp, Fresh Asparagus and Frozen Green Soybeans in Thailand. *In* Agriculture and Rural Development Discussion. *Paper16*. The World Bank. Washington DC.Pp. 3-25.
16. Reay, G.A. and J.M. Shewan (1991). The spoilage of shrimp and its preservation by freezing. *Adv. Food Res*., **2(5)**:343-398.
17. Shiaris, M.P., A.C. Rex, G.W. Pettibone, K. Keay, M.A. Rex, J. Eberosle, and E. Gallagher (1987). Distribution of bacterial indicators and *Vibrio parahaemolyticus* in sewage polluted intertidal sediments. *Applied and Environmental Microbiology*, **53**:1756-1761.
18. Smith, K.L., and J.L. Guentzel (2010). Mercury concentrations and omega-3 Fatty acids in fish and shrimp: Preferential consumption for maximum Health benefit. *Marine Pollution Bulletin*, **60(9)**: 1615-1618.

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