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Full Length Research Article

BACTERIAL CONTAMINATION AND HEAVY METAL RESIDUES IN FROZEN SHELLFISH RETAILED WITHIN LAGOS METROPOLIS, NIGERIA

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ABSTRACT
Shellfish has become a worldwide delicacy amongst seafood lovers due to its essential nutrients which are beneficial to human health. An investigative study was carried out on eight different shellfish– White Shrimp (*Penaeus occidentalis*), Tiger Shrimp (*Penaeus monodon*), Pink Shrimp (*Farfantepenaeus notialis*), Guinean Mantis Shrimp (*Squilla aculeata calmani*), Royal Spiny Lobster (*Pallinurius regius*), Lagoon Crab (*Callinectes amnicola*), Smoothswim Crab (*Portunus validus*) and Periwinkle (*Tympanotonus fuscatus*) obtained from Better Life market- exploring their bacterial quality and heavy metal accumulation. The highest Total Bacterial Count ($2.71\pm0.03\times10^8$ CFU/g) was observed in *P. occidentalis* while the lowest count ($1.00\pm0.04\times10^8$ cfu/g) was observed in *T. fuscatus*. The Total Faecal Count of the shellfishes reduced under 44^0C temperatures except in *P. occidentalis* which increased from $1.40\pm0.05\times10^3$ to $1.70\pm0.01\times10^3$ CFU/g. Although, all identified bacteria (*Bacillus spp*, *Escherichia coli, Lactobacillus spp, Pseudomonas aeruginosa* and *Klebsiella spp*) were found in *C. amnicola* and *T. fuscatus* but the most contaminated shellfish was *Penaeus occidentalis*, with 38.5% *E. coli*, followed by *F. notialis* having 37.3% *P. aeruginosa*. The mean heavy metals concentration occurred in the following decreasing order: Iron> Zinc> Copper> Chromium> Manganese while Cadmium, Lead, Nickel and Arsenic were not detected in most of the samples. The study revealed that frozen shellfish are preserved under unhygienic environment most times and the fact that some levels of toxic metals were found in the samples is a cause for constant monitoring.

Keywords: Heavy metal, Bacterial, Shellfish, Frozen, Makoko, Nigeria

INTRODUCTION

Shellfish has become a worldwide delicacy amongst seafood lovers due to its essential nutrients which are beneficial to human health. The consumption and utilization of these natural resources for human consumption has improved rapidly over time. According to FAO (2012), fish (fin and shellfish) accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed. Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Abisoye, *et al.*, 2011). As fish constitute an important part of human diet, it is not surprising that the quality and safety aspects of this delicacy are of particular interest.

Safety of the fish products and their quality assurance is one of the main problems of the food industry today. The presence or absence of food borne pathogens in a fish product is a function of harvest environment, sanitary conditions, and practice associated with equipment and persons in the process environment (Huss, 1997 and FDA, 2011). Water-borne diseases (i.e., diarrhoea, gastrointestinal illness) caused by various bacteria, viruses and protozoa have been the causes of many outbreaks. However, the microbial load found in fish is believed to be a reflection of the general contamination of their habitat (Adebayo-Tayo *et al*., 2012). Consequently, the contamination of fish with pathogens and trace elements has been a major public health concern that requires continuous monitoring. The knowledge about the potential accumulation of heavy metals in fish and fishery products is very important for the health of the consumers. The safety level of the shellfish consumed in Lagos has not been documented. Hence, the aim of the study is to assess the bacterial quality and heavy metal residues of eight different shellfish– White Shrimp (*Penaeus occidentalis*), Tiger Shrimp (*Penaeus monodon*), Pink Shrimp (*Farfantepenaeus notialis*), Guinean Mantis Shrimp (*Squilla aculeata calmani*), Royal Spiny Lobster (*Pallinurius regius*), Lagoon Crab (*Callinectes amnicola*), Smoothswim Crab (*Portunus validus*) and Periwinkle (*Tympanotonus fuscatus*) retailed within Makoko Area of Lagos, Nigeria.

MATERIALS AND METHODS

Sample collection

Thirty frozen shellfish fillet samples each of White Shrimp, Tiger Shrimp, Pink Shrimp, Guinean Mantis Shrimp, Royal Spiny Lobster, Lagoon Crab, Smoothswim Crab and Periwinkle were randomly obtained from the Better Life market in Makoko Area of Lagos. After collection, they were immediately kept in an ice box and transferred to Food Hygiene Laboratory for bacteriological and chemical evaluations.

Laboratory analysis

Bacteriological analysis

Twenty five (25) grammes of examined shellfish fillet were transferred to a sterile polyethylene bag and 225 ml of 0.1% sterile buffered peptone H_2O in a blender at 2000 rpm for 1-2 minutes to provide a homogenous solution of 1/10 dilution. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9ml of sterile buffered peptone

water (0.1%) and mixed together to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to enumeration of Total Bacteria Count (as described by USDA, 1998) and Total Coliform (as described by AOAC, 1980) at varying temperatures (Room temperature, 37°C and 44°C). Culture media like nutrient agar was prepared according to manufacturers' specifications and distilled water used for serial dilution was sterilized in an autoclave at 121°C for 15minutes before use. Bacterial isolates were characterized using routine microbiological procedures as described by Olutiola *et al*. (1991) after which they were identified using Bergey's Manual of Determinative Bacteriology.

Determination of Heavy metals

The muscle tissues of the shellfish samples were oven dried to constant weight at 105 °C. The dried samples from each station were grinded into a fine powder with a pestle and mortar and placed in bottles and labeled well. Triplicate digestion was performed according to the procedure of Turkmen and Ciminli (2007). For background correction, six blanks was digested as pretest samples and each of the blanks analyzed for Hg, As, Mn, Fe, Cu, Zn, Cd, Pb, Ni and Cr by atomic absorption spectrophotometer after calibration.

Statistical Analysis

The data was analyzed using SPSS 2.0 package and Microsoft Excel 2013. All data are represented as mean of three replicates. The mean, range and standard deviation of each parameter were determined. Duncan multiple range test were conducted to derive the statistical significance and relationship of the different results.

RESULTS

The Total Bacterial Count (TBC) in frozen shellfish within Lagos metropolis is presented in Figure 1. It was taken at different temperatures at 37^{0}C, 44^{0}C and at room temperature. Results showed that the highest individual values of the shellfish samples were observed at room temperature. The highest TBC ($2.71\pm0.03\times10^8$ cfu/g) was observed in white shrimp (*Penaeus occidentalis*) while the lowest count ($1.00\pm0.04\times10^8$ cfu/g) was observed in periwinkle (*Tympanotonus fuscatus*). The TBC in 37^{0}C and 44^{0}C also increased slightly. Although, no significant differences were observed but highest bacterial counts were recorded in the shrimps as compared to lobster, crabs and periwinkle.

Table 1: Total bacterial count in frozen shellfish retailed within Lagos Metropolis

	SPECIES	Total Bacterial Count x 10^8 cfu/g		
		37^{0}C	44^{0}C	Room Temp.
Shrimps	*Penaeus occidentalis*	1.80±0.01	2.11±0.03	2.71±0.03
	Farfanpenaeus notialis	1.60±0.04	2.02±0.10	2.21±0.05
	Penaeus monodon	1.54±0.02	1.55±0.05	1.86±0.03
	Squilla aculeata calmani	1.70±0.03	2.04±0.03	2.17±0.02
Lobster	*Pallinurius regius*	1.50±0.03	1.45±0.02	1.64±0.02
Crabs	*Portunus validus*	1.40±0.01	1.51±0.01	1.71±0.03
	Callinectes amnicola	1.40±0.01	1.61±0.05	1.81±0.02
Periwinkle	*Tympanotonus fuscatus*	1.00±0.04	1.04±0.03	1.12±0.03

Values are not significantly different between samples ($p<0.05$)

The mean values of the Total Faecal Count (TFC) for the samples are shown in Table 2. The result revealed that the highest TFC ($1.80\pm0.03\times10^3$ cfu/g) was observed in Tiger shrimp (*Penaeus monodon)* while the lowest ($1.04\pm0.05\times10^3$ cfu/g) was observed in the periwinkle, *Tympanotonus fuscatus.* The TFC of the shellfishes reduced under 44^{0}C temperatures except *Penaeus occidentalis* which increased from $1.40\pm0.05\times10^3$ to $1.70\pm0.01\times10^3$ cfu/g. Values are not significantly different between samples ($p<0.05$).

Table 2: Total faecal coliform count in frozen shellfish retailed within Lagos Metropolis

	SPECIES	Total Faecal coliform Count x 10^3 cfu/g	
		37^{0}C	44^{0}C
Shrimps	*Penaeus occidentalis*	1.40±0.05	1.70±0.01
	Farfanpenaeus notialis	1.61±0.03	1.30±0.05
	Penaeus monodon	1.72±0.03	1.60±0.03
	Squilla aculeata calmani	1.41±0.02	1.30±0.01
Lobster	*Palinurius regius*	1.52±0.01	1.21±0.02
Crabs	*Portunus validus*	1.61±0.05	1.42±0.02
	Callinectes amnicola	1.34±0.05	1.10±0.03
Periwinkle	*Tympanotonus fuscatus*	1.10±0.02	1.04±0.05

Values are not significantly different between samples ($p<0.05$)

The percentage bacterial contamination of frozen shellfish retailed within Lagos metropolis is shown in Figure 1. *Penaeus occidentalis* was most contaminated with 38.5% *Escherichia coli*, followed by *Farfanpenaeus notialis* having 37.3% *Pseudomonas aeruginosa*. The least contaminated shrimps were *Penaeus monodon* (33% *Bacillus spp*, 33.5% *Klebsiella spp* and 33.5% *Lactobacillus spp*) and *Squilla aculeata calmani* (32.9% *Pseudomonas aeruginosa,* 34% *Escherichia coli* and 33.1% *Klebsiella spp*). *Pseudomonas aeruginosa* and *Escherichia coli* were not observed in *Penaeus monodon, Palinurius regius* and *Portunus validus* while all identified bacteria were found in *Callinectes amnicola* and *Tympanotonus fuscatus.*

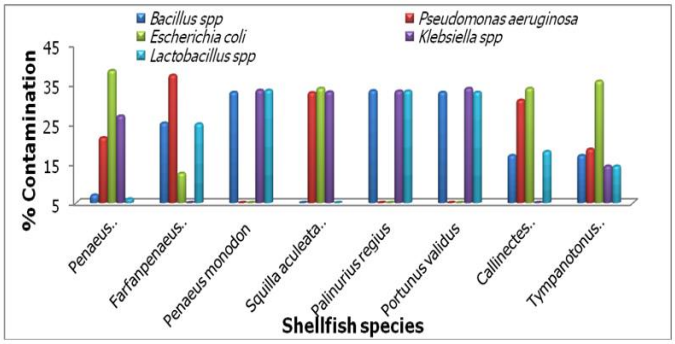

Figure 1: Bacterial contamination in frozen shellfish retailed within Lagos Metropolis

The metal concentration in frozen shellfish retailed within Lagos Metropolis is presented in Table 3. Except in *Penaeus occidentalis,* most of the highly toxic metals (Cadmium, Lead, Nickel and Arsenic) were not detected in the samples. The highest value of Manganese (0.04±1.5 mg/kg) was observed in *Callinectes amnicola*, while the lowest value (0.002±2.5 mg/kg) was recorded in *Penaeus monodon*. Iron occurred most in *Tympanotonus fuscatus* (0.756±1.3 mg/kg) while the least was observed in *Pallinurius regius* (0.08±0.5 mg/kg). Highest value of Copper (0.435±1.1 mg/kg) in *Callinectes amnicola* was

significantly differently (P<0.05) to the values obtained in other shellfishes. The highest value of Zinc (0.534±2.1 mg/kg) was detected in *Portunus validus* while the lowest value (0.085±2.5 mg/kg) was detected in *Squilla aculeata calmani*. The levels of Mercury were insignificant different (P<0.05) across samples. The highest value of Chromium (0.015±3.2 mg/kg) was recorded in *Tympanotonus fuscatus* while the lowest value (0.005±1.2 mg/kg) was recorded in *Penaeus occidentalis*.

Table 3: Heavy metal residues in frozen shellfish retailed within Lagos Metropolis

Heavy Metals (mg/kg)	*Penaeus occidentalis*	*Penaeus notialis*	*Penaeus monodon*	*Squilla aculeata calmani*	*Pallinurius regius*	*Callinectes amnicola*	*Portunus validus*	*Tympanotonus fuscatus*
Manganese	0.003±1.5^a	0.007±0.6^a	0.002±2.5^a	0.004±0.5^a	0.006±0.6^a	0.04±1.5^b	0.005±0.1^a	0.008±0.5^a
Iron	0.142±0.5^a	0.203±2.5^a	0.149±1.8^a	0.082±1.2^b	0.080±0.5^b	0.292±2.3^a	0.198±0.5^a	0.756±1.3^c
Copper	0.018±2.1^a	0.029±1.7^a	0.011±0.5^a	0.025±1.3^a	0.142±1.5^b	0.435±1.1^c	0.037±1.2^a	0.057±2.1^a
Zinc	0.253±0.5^a	0.488±1.5^a	0.338±3.3^a	0.085±2.5^b	0.091±2.1^b	0.363±2.3^a	0.534±2.1^a	0.223±1.5^a
Mercury	0.004±0.2^a	0.004±2.4^a	0.004±2.6^a	0.004±0.6^a	0.003±1.5^a	0.004±1.2^a	0.003±0.5^a	0.005±1.1^a
Chromium	0.005±1.2^a	0.009±4.3^b	0.012±3.1^b	0.007±1.5^a	0.011±3.05^b	0.012±2.2^b	0.012±3.2^b	0.015±3.2^b
Cadmium	0.028±3.5	ND	ND	ND	ND	ND	ND	ND
Lead	0.012±0.5	ND	ND	ND	ND	ND	ND	ND
Nickel	0.028±1.4	ND	ND	ND	ND	ND	ND	ND
Arsenic	0.028±3.2	ND	ND	ND	ND	ND	ND	ND

Keys: Mean±Standard Error; ND= Not dectected; Values with different superscript across row are significantly different at (P < 0.05)

DISCUSSION

The bacterial data obtained in this study were similar across samples with non-significant differences (P>0.05) on the bacterial load of the eight different shellfish sampled in Makoko Market of Lagos. It shows that the quality standard of the products varies and could be as a result of handling techniques; as stated by the work of Hala *et al*., (2017) that the microbiological quality of frozen fish fillet depends upon various factors such as the nature of the raw material, its pre and post-harvest treatments and the sanitary condition of the processing factories, the rate and nature of freezing, the temperature and length of storage, the original numbers, growth during storage, thawing process and physical protection offered by the food.

The slight Total Bacterial Count (TBC) increase under 37^{0}C and 44^{0}C in this study agreed with Yeasmin *et al*., (2010) who reported an increase of bacterial load from 7.5×10 3 to 6.2×10 9 CFU/g. According to Jyh-Wei and Yin-Hung, (2000), TBC reflects the quality of food sanitation during manufacturing, shipping and storage, and also provides an index of food freshness and the bacterial load in frozen products is an indication of the initial bacterial load in the product before freezing, hygiene of processing plant, effectiveness of freezing, post process handling and time temperature abuse during storage and transportation.
The mean values of the Total Faecal Count (TFC) in this study reduced under 44^{0}C temperatures except in *Penaeus occidentalis,* though values are not significantly different (p<0.05). The increased TFC may be attributed to fillet quality, temperature fluctuations, time taken during the processing and time taken to transport shrimp. The presence of TFC is an indication of sewage contamination which may also occur during different processing steps such as transport and handling. Moreover, the contamination may also be caused by the water used for washing or icing (Boyd, 1990).

The study revealed the presence of *Bacillus spp, Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp* and *Lactobacillus* species associated with frozen shellfish in Makoko Area. The bacteria isolated are like those reported by Abolagba and Igbinevbo (2010). All the pathogens isolated are of food and public health implication and hence hazardous and injurious to human health if consumed without proper washing and cooking. The report is supported by the work of Ajayi (2011), who isolated bacteria that could cause ill health. The low presence of bacteria recorded in lobster, crabs and periwinkle could be as a result of the hard shell protection.

The study also showed that the mean heavy metals concentration occurred in the following decreasing order: Iron> Zinc> Copper> Chromium> Manganese while Cadmium, Lead, Nickel and Arsenic were not detected in most of the samples. This is similar to the report of Jumbo *et al*. (2015) on the heavy metal concentrations of some fin and shell fish from Ogoniland, southern Nigeria.

Conclusion

The result obtained indicates that most times, frozen shellfish are preserved under unhygienic environment. This lack of proper sanitary in retailed frozen shellfish handling technique is of public health significance. Therefore, to minimize the risk of infectious disease, it is advisable to follow better hygienic and culinary processes prior consumption. The results of this finding also present a valuable baseline data on the heavy metals in the various shellfish species market within Lagos State, Nigeria. The fact that some levels of toxic metals were found in the samples is a cause for constant monitoring of the sources.

REFERENCES

Abisoye, B. F., Ojo, S. K. S., Adeyemi, R. S. and Olajuyigbe, O. O. (2011). Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. *Prime Journal of Microbiological Research*, **1**(2): 23-26.

Abolagba, O. J. and Igbinevbo, E. E. (2010). Microbial load of smoked fish (*Clarias sp*.) marketed in Metropolis, Nigeria.

Research Journal of Fisheries and Hydrobiology, **5**: 99-104.

Adebayo-Tayo, B. C, Odu, N. N, Anyamele, L. M, Igwiloh, N. J and Okonta, I. O. (2012). Microbial quality of fresh fish sold in Uyo Metropolis. *Journal of Nature and Science,* **10** (3): 71-77.

Ajayi, A. O. (2011). Bacteriological study of catfish, *Clarias gariepinus* from fish pond source in Akungba-Akoko Community, Nigeria. *British Microbiology Research Journal*, **2** (1): 1 – 9.

Association of Official Analytical Chemists (AOAC) (1980). Official methods of analysis (Vol. 534). W. Horwitz (Ed.). Arlington, VA, Washington DC: AOAC.

FAO (2012). The state of world review of fisheries and aquaculture. Retrieved from http://www.fao.org/docrep/016/i2727e/i2727e01.pdf..

Food and Drug Administration, FDA (2011). Fish and fishery products hazards and controls guidance. Food and Drug Administration, U.S.A. 5pp.

Hala, A. M., Gehan I.E. and Shereen A. Y. (2017). Assessment of the bacterial quality and toxic heavy metal residues of frozen fish fillet in Kaferelsheikh Markets. *Alexandria Journal of Veterinary Sciences*, **54**(1):108-116.

Huss, H.H. (1997). Control of indigenous pathogenic bacterial in seafood. *Food Control,* **8**: 91-98

Jumbo, A., Wegwu, M.O., Belonwu, D.C. and Okerenta, B.M. (2015). Assessment of heavy metal Concentrations of Selected Fin and Shell Fish from Ogoniland. *Journal of Environment and Earth Science*, **5**:15-19.

Jyh-Wei, S. and Yin-Hung, H. (2000). Investigation for contamination of parasite and aerobic bacteria in frozen tilapia fillets in Taiwan. *Journal of Food Drug Analysis*, **8** (1): 51-56.

Olutiola, P.O., Famurewa, O. and Sontang, H.G. (1991). An Introduction to General Microbiology-A practical Approach. Ca. Heidelberg verlagsanstaltund Dreuckerei GMbh., Heidelberg, Germany. Pp 520.

Turkmen, M. and Ciminli, C. (2007). Determination of metals in fish and mussel species by Inductively Coupled Plasma-atomic emission spectrometry. *Food Chemistry*, **103**: 670 – 675.

USDA/FSIS; United States Department of Agriculture Food Safety and Inspection Service (1998): Microbiology Laboratory Guidebook, 3 rd Ed., Washington, DC: USDA–FSIS.

Yeasmin, T., Reza, M. S., Shikha, F. H., Khan, M. N. A., and Kamal, M. (2010). Quality changes in formalin treated rohu fish (*Labeorohita, Hamilton*) during ice storage condition. *Asian Journal of Agricultural Science*, **2**(4): 158-163.