



BIostatistical Evaluation of the Nutritional Qualities of Selected Molluscan Shellfish in the Port Harcourt Area of Rivers State, Nigeria

Beka Sylvia E^{1,3,5}, Beka Nathan E^{2,3,5} and Sokari T.G^{1,4}

¹Rivers State University of Science and Technology, Port Harcourt, Nigeria

²Igbinedion University Okada, Nigeria

³University of Hertfordshire, Hertfordshire, England

⁴University of Botswana, Gaborone, Botswana

⁵Inter-Enviroments Limited, Rumuodara, Port Harcourt, Nigeria

ABSTRACT

The nutritional and bacteriological qualities of popular street-vended molluscan shellfish were determined. The total viable bacterial plate counts were 4.06×10^7 cfu/g, 3.85×10^7 cfu/g and 1.21×10^8 cfu/g for *Thais callifera*, *Tympanotonus fuscatus* and *Arca senilis*, respectively. Species of *Vibrio*, *Staphylococcus*, *Shigella*, *Salmonella*, *Pseudomonas*, *Proteus*, *Mierococcus*, *Bacillus* and *Arthrobacter* as well as *Enterobacter aerogenes* and *Escherichia coli* were isolated from the street-vended seafood samples processed by artisans. No *Staphylococcus* spp. were isolated from all samples processed in the laboratory indicating that the presence of *Staphylococcus* in street-vended samples was as a result of poor food handling practices by street-vendors. Counts of more than 1100 coliforms per 100g were observed in both street-vended and laboratory processed samples indicating faecal contamination of the environment from which they were harvested and poor handling practices by the sea food vendors. It is clear from the foregoing, that the sea foods vended in Port Harcourt are of poor microbiological quality even though they are of high nutritional quality. Since these sea foods constitute a relatively inexpensive source of protein for the poor, it is proposed that processors and vendors of these sea foods be advised on the importance of food hygiene and microbial safety.

Keywords: *Bacteria, Nutrition quality, Mollusca, Port Harcourt, Pollution, Evaluation*

INTRODUCTION

A major problem reported to be associated with the consumption of sea foods is the prevalence of pathogenic bacteria due to coastal pollution by faecal wastes. Studies by Udotong and Sokari (1998) have shown high microbial counts in *Thais callifera* as compared with counts of water from which they were harvested. This is not surprising as molluscan shellfish are filter feeders. The presence of enteric bacteria like *Escherichia coli* and *Salmonella* species is a major cause for concern because of its public health implications in causing food borne diseases

It is important to note that sewage polluted waters usually contain sea foods that are potentially capable of transmitting various pathogenic microorganisms (Pelczar et al., 1986). A few bacteria species that are pathogenic to man may be found in natural aquatic environments. Typically, these organisms include the genera *Vibrio* and *Aeromonas*. Contamination of sea foods by the naturally occurring aquatic bacteria present a risk to public health if toxigenic strains multiply to high numbers during improper storage and handling (Lee et al., 1981).

Inadequate heat processing or preservation and storage after purchase of fresh street vended sea foods may also allow some pathogens particularly enteric viruses and bacteria to persist in them (Dupont, 1986; Sockette et al., 1985)).

BIOLOGICAL HAZARDS RELATED TO SEA FOODS:

Biological hazards related to sea foods include pathogenic bacteria (infectious or toxin producing), biogenic amines, viruses, parasites and aquatic biotoxins. However, the scope of this study deals specifically with pathogenic bacteria.

Pathogenic bacteria are defined as those bacteria that may cause illness in humans via food. Food-borne pathogenic bacteria are few among the many different types of seafood bacteria, which are causing no harm to humans (FAO/WHO, 2003).

Many bacteria contaminating food and water can cause acute gastroenteritis or inflammation of the stomach and intestinal lining.

Bacterial food-borne pathogens may be grouped into those that cause food intoxication and those that can result in food-borne bacterial infections. When food is the source of the pathogen, the condition is often called food poisoning. Alternatively, the pathogen may secrete an exotoxin that contaminates the food and is then ingested by the host. This is sometimes referred to as food intoxication because the pre-formed

toxin is ingested and the presence of living bacteria is not required

The number of viable bacterial cells necessary to cause disease called the minimum infective dose, (MID) varies considerably between bacterial species. Thus, the MID is known to be high i.e.> — 106 cells, for pathogenic *Vibrio* species (Twedt, 1989) and low for *some Salmonella typhi* and *Shigella* species .

Seafood-borne pathogenic bacteria may be conveniently divided 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.

Natural habitat of pathogen	Mode of action of disease		
	infection		Intoxication
High MID	low MID		
Aquatic environment	<i>Vibrio</i> spp., <i>Aeromonas</i> <i>Plesiomonas</i>		<i>Clostridium botulinm</i> type E (non-proteolytic)
General environment	<i>listeria monocytogenes</i>		<i>Clostridium botulinum</i> Type A Type B (proteolytic), <i>Clostridium perfringens</i> , <i>Bacillus cereus</i>
Aminal human reservoir	<i>Salmonella</i> , <i>Escherichia coli</i> (EPEC, ETEC)*	<i>Salmonella typhi</i> , <i>shigella</i> , <i>Escherichia coli</i> (EHEC)* Campylobacter	<i>Staphylococcus aureus</i>
Preventive measure	prevention of growth	good hygienic practice	prevention of growth

*MID, minimum infective dose; EPEC, *Enteropathogenic Escherichia coli*, ETEC, *Enterotoxigenic Escherichia coli*; EHEC, *Enterohaemorrhagic Escherichia coli*.
Source: FAO/ WHO, 2003

Table 1: Seafood-borne pathogenic bacteria and diseases

MATERIALS AND METHODS

Source of Samples:

Samples were collected from 6 popular sea food vendors at the Creek Road market and seafood harvesters at the waterside. The sea foods were all harvested from the Primroze Channel of Bonny River. Harvested samples included *Thais callifera*, *Tympanotonus fucatus* and *Arca senilis* (Fig. 1).

General sample preparation:

Some fresh samples (300g) of each type of seafood, processed in the laboratory were made homogenous prior to analysis using a procedure that protects labile nutrients. The samples were first dried in an air-circulation oven (BLT Laboratory thermal equipment SN: 7246017) set at 105°C to constant weight. After cooling, samples were ground to a fine powder in a sterile dry blender. The powder was used for further

analysis, as outlined in Fig.2.

Moisture (Air oven method):

A fresh sample of each type of seafood, processed in the laboratory, was dried to constant weight in an air-circulation oven (BLT Laboratory thermal equipment SN: 7246017) set at 105°C.

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

Ash content (Ashing):

One gram of dried homogenized sample of each type of seafood taken from stock made during general sample preparation was accurately weighed to the nearest mg into a porcelain crucible. Organic matter was burned of in a muffle furnace (Carbolite Sheffield LMF4) thermostatically controlled at 630°C for 3 hours. After ashing, the inorganic material remaining was cool and weighed.

Calculation: Ash (%) = $\frac{\text{Ash weight (g)} \times 100}{\text{Oven dry weight (g)}}$ -----(2)



Figure 1: Tympanotonus fuscatus from the study Area

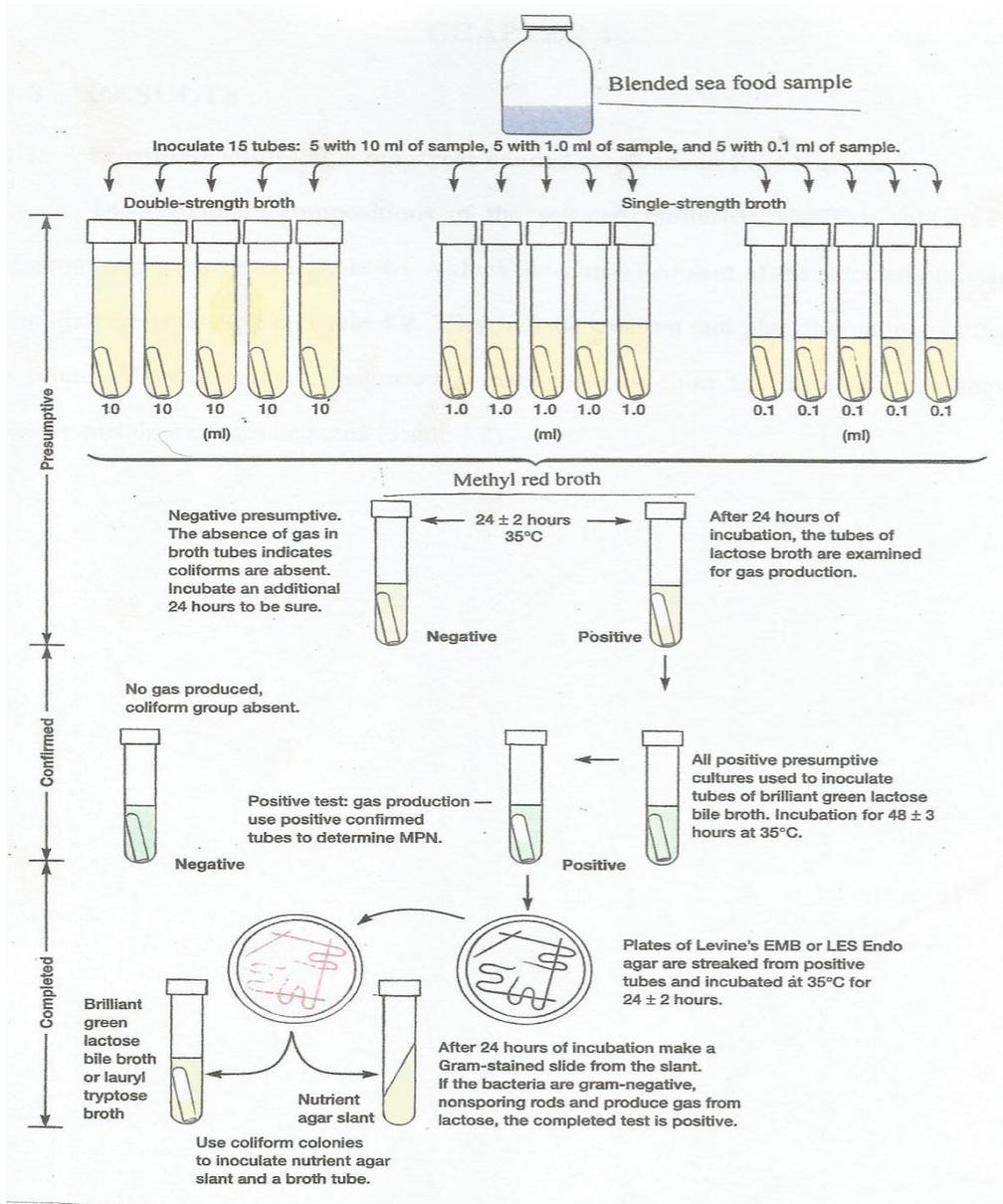


Figure 2: Multiple -Tube Fermentation test (Source: Prescott et al.,1996)

Total available carbohydrate (Manual Clegg Anthrone method):

Some dried homogenized sample (0.1 g) of each type of seafood taken from stock made during general sample preparation was accurately weighed 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 systems, model 321, Sn: 0208052) by the Anthrone method and expressed as glucose.

Calculation: Total available carbohydrate (as % glucose)

$$= \frac{25 \times \text{absorbance of dilute sample}}{\text{Absorbance of dilute standard} \times \text{weight of sample}} \text{-----(3)}$$

Absorbance of dilute standard x weight of sample

Extractable fat (Soxhlet method):

One gram of dried homogenized sample of each type of seafood taken from stock made during general sample preparation was accurately weighed to the nearest mg.

Each sample was transferred to an extraction thimble. The thimble was placed, in turn, in a soxhiet extractor. The fat was extracted with petroleum ether from the dried residue obtained during general sample preparation. The solvent was removed by evaporation and the residue of fat was weighed.

Calculation: Extractable fat (%)

$$= \frac{\text{Weight (g) of flask with fat} - \text{Weight (g) of flask without fat}}{\text{Weight (g) of flask without fat}} \times 100 \text{-----(4)}$$

Total nitrogen and crude protein (Macro Kjeldahl method):

During general sample preparation was accurately weighed to the nearest mg each into a 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.

$$\text{Calculation: N (\%)} = \frac{\text{Titre value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times \text{sample weight}} \text{-----(5)}$$

1000 x 20 x sample weight

$$\text{Crude protein} = \text{N\%} \times 6.25 \text{-----(6)}$$

Bacteriological analysis:

Bacteriological analysis of the four treatments of each of the sea foods was carried out as described in APHA (1970) . Some sample (log) was blended in 90ml of 8.5% normal saline to give a 10⁻¹ dilution. Further 10 fold dilutions were prepared and total viable counts determined in duplicates followed by spread plating on Dextrocholate citrate agar for isolation of *Salmonella* and *Shigella* spp.; Mannitol salt agar for isolation of

Staphylococcus spp.

Thiosulphate citrate bile salt sucrose agar for isolation of *Vibrio* spp. and the multiple tube fermentation test for the presence faecal coliforms. Isolation, characterization and identification of representative discrete colonies were carried for qualitative determination using colonial, morphological and biochemical characteristics. Morphological features of bacterial isolates included Gram reaction and colonial morphology, while the biochemical tests carried out were: indole, methyl red, Voges-Proskauer, citrate, coagulase, oxidase and triple sugar fermentation test using lactose, sucrose, glucose for identification of bacterial isolates.

Determination of pH:

The pH of the sea foods was determined by homogenizing 10g of the meat in 20ml distilled water and then measured using CD 640 Digital pH meter.

Statistical analysis:

The data obtained were subjected to analysis of variance (ANOVA) and mean separation (LSD) for determination of mean differences at P 0.01.

PRESENTATION OF RESULTS:

The proximate compositions of the selected molluscan shellfish sold Port Harcourt are presented in Table 2. Values for mineral content of the selected molluscan shellfish are also discussed. They include calcium and phosphorus in significant amounts. Values for iron, magnesium, manganese, sodium and zinc are also shown (Table 2).

Parameter	value(%)		
	<i>Thais callifera</i>	<i>Tympanotonus fuscatus</i>	<i>Arca senilis</i>
Ash	9.07	9.46	8.09
Carbohydrate	23.50	11.11	12.50
Lipid	1.73	1.53	3.36
Crude protein	50.31	62.13	74.37

*Values are means of two replications

Table 2: Gross chemical composition of some street- vended seafoods in Port Harcourt

Bacteriological analysis and Composition:

Counts of Salmonellae:

Isolation of *Salmonella* species and *Shigella* species counts were 1.07×10^4 cfu/g, 2.00×10^2 cfu/g and 3.50×10^4 cfu/g in street-vended samples of *Thais callifera*, *Tympanotonus fuscatus* and *Arca senilis* respectively, and *Shigella* plate counts were 1.07×10^4 cfu/g, 2.00×10^2 cfu/g and 3.50×10^4 cfu/g in the same order.

Coliform counts:

Total coliform counts and faecal coliform counts were lower in samples of *Tympanotonus fuscatus* than in samples of *Thais callifera* and *Arca senilis*. Completed tests for the presence of faecal coliforms and *Escherichia coli* are presented in Tables 3 and 4.

Sample code	Holding temperature(°C)	<i>Thais callifera</i>		<i>Tympanotonus fuscatus</i>		<i>Arca senilis</i>	
		pH of Sample	MPN/100g	pH of sample	MPN/100g	pH of sample	MPN/100g
Lab1*	30.0	8.72	>1100	8.78	240	6.46	1100
Str1*	30.0	8.48	>1100	8.77	150	6.44	>1100
Str2	30.0	8.60	>1100	8.72	120	6.68	>1100
Str3	28.5	8.62	>1100	8.72	>1100	6.68	>1100
Lab2	28.5	8.69	93	8.65	>1100	6.74	1100
Str4	28.5	8.48	>1100	8.77	>1100	6.44	>1100
Str5	28.5	8.62	>1100	8.73	150	6.68	>1100
Str6	28.5	8.62	>1100	8.73	>1100	6.68	>1100
Lab	29.0	8.69	>1100	8.72	1100	6.74	1100
Str	29.0	8.63	>1100	8.65	>1100	6.69	>1100
Rin*	29.0	7.55	>1100	8.72	>1100	6.74	>1100
Pre*	29.0	7.54	>1100	8.62	4	6.70	20

- ❖ Lab, laboratory processed sample; Str, street vended sample; Rin, rinsed street- vended sample; Pre, precooked street vended sample; culture media were EMB agar and nutrient agar

Table 3: Faecal Coliform counts in samples of street- vended seafoods in Port Harcourt

Sample code		<i>Thais callifera</i>		<i>Tympanotonus fauscatus</i>		<i>Arca senilis</i>	
	Green metallic Sheen on EMB Medium	Gram reaction & cell morphology	Green metallic sheen on EMB medium	Green reaction & cell morphology	Green metallic sheen on EMB medium	Gram reaction & cell morphology	
lab1*	+++	-, rods*	+	-, rods	++	-, rods	
Str1*	++	-, rods	++	-, rods	+++	-, rods	
Str2	+++*	-, rods	+++	-, rods	+	-, rods	
Str3	++	-, rods	+	-, rods	--	--	
Lab2	+++	-, rods	+	-, rods	++	-, rods	
Str4	--*	--	+	-, rods	+	-, rods	
Str5	+	-, rods	+++	-, rods	+++	-, rods	
Str6	+	-, rods	+	-, rods	+++	-, rods	
Lab	+++*	-, rods*	+	-, rods	+	-, rods	
Str	++	-, rods	+	-, rods	--	--	
Rin*	--	--	+	-, rods	++	-, rods	
Pre*	++	-, rods	--	--	--	--	

*-, rods, gram negative rods; +, ++, +++, indicate density of metallic sheen colour of colonies-- , colonies exhibiting green metallic sheen not seen; lab, laboratory processed sample; Str, street vended sample; Rin, rinsed street-vended sample; pre, precooked street- vended samples.

Table 4: Completed test for the presence of faecal coliforms in samples of street-vended sea foods in Port Harcourt

DISCUSSION

An evaluation of the bacteriological quality of the selected street-vended sea foods showed that these seafoods contained various bacteria including *Vibrio* spp., *Enterobacter* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp. and *Shigella* spp. and *Staphylococcus* spp.

The microbial quality of the river, estuaries and seashores from which seafoods are harvested influence the microflora of seafood samples (Pelczar et al., 1986). The isolation of faecal coliforms, *Shigella* spp. and *Bacillus* spp. from samples processed in the laboratory would indicate that the seafoods were contaminated from where they were harvested.

Faecal pollution of the environment may be because residents of this area deposit untreated faecal and household wastes in the river and along the river banks. Some household and public toilet facilities are pier latrines built directly over the river banks resulting in constant deposit of faecal matter in the river.

The use of this same water for processing and retail of samples will in turn contribute to further contamination of fresh processed samples for retail. Processing of seafood following proper food handling practices, especially the use of clean water for shucking, rinsing and retail may reduce numbers of coliform

bacteria in samples, though that reduction may not be substantial in seafood that have been harvested from polluted river estuaries as strains of *Escherichia coli* accumulate in the gut of molluscan shellfish cultured in contaminated waters (FAO/WHO, 2003). This was observed in faecal coliform counts of laboratory processed samples and rinsed street-vended samples. Values ranged from as low as 20 coliforms per 100g to more 1100 coliforms per 100

E. coli strains can multiply and generate *enterotoxins* when contaminated foods are kept at room temperature for several hours (Bryan, 1973) as practiced by the street vendors.

Therefore, consumption of highly contaminated raw seafood and improperly cooked seafood could result in some form of gastroenteritis and diarrhea disease.

Keeping processed seafood for retail free of contamination with *Staphylococcus* spp. is best ensured by processing with clean hands and avoiding contact with human skin. At the 1% significance level, the differences occurring between the four treatments presented in isolation of *Salmonella* spp. and *Shigella* spp. from street-vended samples of *Thais callifera*, *Tympanotonus fuscatus* and *Arca senilis* using Desoxycholate citrate agar are discussed.

Mean plate count values of 1.07×10^7 cfu/g, 2.00×10^2 cfu/g and 3.50×10^7 cfu/g, respectively for *Salmonella* spp. and 1.26×10^7 cfu/g, 3.02×10^7 cfu/g and 1.339×10^7 cfu/g, respectively for *Shigella* spp. were observed. *Salmonella* spp. were detected in twenty three percent (23%) of samples of street-vended seafood studied in the laboratory. *Salmonella* spp. were not detected in all the samples processed in the Laboratory.

The infectious dose of *Salmonella* is high, typically around 10^6 cells; however, growth to such levels were not detected in the street-vended seafoods. Open marine waters are usually free from *Salmonella*, but estuaries and coastal waters contaminated with human or animal excreta may harbour *Salmonella*.

The sources of infection are usually chronic carriers; from faeces to other persons by the oral-faecal route, which may be water-borne, food-borne or by contact with hands and other forms. Classically, the vehicle of spread from these sources is water.

Therefore, eating raw seafood as well as undone or improperly cooked sea food that may have fed in contaminated river estuaries or seashores, or that have been processed using contaminated water, can cause illness to the consumer. Rinsing contaminated street vended seafood using sterile distilled water reduced bacterial load. Load was further reduced by precooking at 80°C for 3 minutes. However, at the 1% significant level, the differences occurring between the four treatments were non-significant.

The difference between the mean values of street-vended seafoods and street-vended seafoods rinsed with potable water was non-significant.

The pH of the seafoods is optimum for microbial growth. Most bacteria have pH minimums ranging from 4.0 to 5.0. The mean holding temperature of the seafoods by the street vendors is 29°C and pathogenic bacteria grow well in seafood stored at ambient temperature, with most bacteria having optimum temperature ranging from 25°C to 37°C, and up to 47°C for *Clostridium botulinum*. A very low generation time at high temperatures (e.g. 12-18 minutes at 30°C) allows the organism to proliferate rapidly. *Vibrio* spp. are capable of proliferation in live shellfish especially during storage. However, subsequent cooling to 3°C reduces numbers, and the extent of decrease depends on food matrix, salinity and other factors (FAO, 2003). The mere presence (in low numbers) of pathogens from the aquatic and general environments, and from the animal/human reservoir in raw seafood is of limited safety concern. This is because growth of these pathogens is only possible at elevated temperatures (>5°C) and at this condition, spoilage will proceed very rapidly and the seafood will probably be rejected due to off-odours and off-flavours long before either toxic or infective organisms reach high numbers, and in borderline cases, cooking will destroy the pathogen (FAO/WHO, 2003).

CONCLUSIONS AND RECOMMENDATIONS

Thais callifera, *Tympanotonus fuscatus* and *Arca senilis* are common seafoods used in local delicacies in Port Harcourt and other parts of the Niger Delta region. This work has shown that these street-vended seafoods are of high nutritional quality, but are however of poor bacteriological quality. Hygienic food handling involving the use of clean potable water and minimal contact with bare hands as well as mild heat processing of the seafoods was observed to have improved the overall bacteriological quality of the seafoods. It is noted that one of the core principles of prevention of consumption of seafoods contaminated with microorganisms which may be pathogenic to consumers or that may cause food poisoning, is prevention of contamination by food handlers at wholesale and retail levels. However, the barriers of old practices and perhaps the most important of all, the yet unfulfilled need for continuing education of food handlers and street vendors at all levels of processing and retail make implementation of the acknowledged principles difficult. Consumers can protect themselves from potential problems that could arise from contamination, by refrigerating street-vended shucked shellfish as soon as possible after purchase and consuming them, adequately cooked, within two to three days.

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