

Microbial diversity and nutritional profile of processed and unprocessed *Tympanotonus fuscatus* (Periwinkle), *Crassostrea gasar* (Oyster) and *Rapana venosa* (Whelk)

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Abstract

A study of the microbiological quality and proximate composition of Shellfish purchased from different markets in Rivers State. The results obtained for raw and cooked shellfish are as follows: Total Viable Bacteria Count ranged from Log₁₀ cfu/g 3.39 -7.74, Total Salmonella Count ranged Log₁₀ cfu/g 1.5-6.47, Total Staphylococcus Count; Log₁₀ cfu/g 3.07 -5.58, Total Vibrio Count Log₁₀ cfu/g 0.2 – 4.3. Most samples analyzed did not meet ICMSF standards for raw and cooked samples. Twelve bacteria species were isolated from raw(unprocessed) and processed periwinkle, oyster and whelk; *Salmonella* sp, *Proteus* sp, *Escherichia coli*, *Micrococcus* sp, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Enterobacter* sp, *Klebsiella* sp, *Citrobacter* sp, *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp. Proximate analysis showed that cooking does minimal to insignificant damage to the nutrient content in the sea foods. Handling and consumption of seafood must be undertaken carefully and knowledgeably to avoid food borne infections.

Keywords: Shellfish; Microbial Quality; Food Borne Infectious; Proximate Composition

1. Introduction

Shell fish are essential animals in the estuaries because they act as buffer to filter out pollution and contaminants associated with water bodies. This is due to the fact that shell fish, especially oysters and clams are efficient filter feeders that help to remove excess nitrogen from waters by incorporating it into their shell and tissues as they grow [22].

In Nigeria, one good example of an estuary is the Bonny estuary, located on the coast of Rivers state, Nigerian, near Port Harcourt, which forms part of the Campo River Delta. Estuaries are also located in other southern states of Nigeria and in the coastal part of Lagos state.

Periwinkle, oyster and whelk are good examples of shell fish found in the bonny estuary. Periwinkle, oyster and whelk are very common seafood consumed in Nigeria, especially in the south. These shelled seafoods are a very cheap source of protein that can almost be afforded by every class. They are also very essential ingredients in many local dishes, especially those of coastal communities. Most marine communities in Nigeria also earn a reasonable amount of money annually from fishing and sales of these seafoods, which in turn has contributed to the economic development of the areas. In Nigeria average of 13.3kg of seafood is consumed per person each year and the riverine and coastal communities consume more [9].

Fresh seafood is highly perishable and this drastically reduces their shelf life. The spoilage of seafood could be as a result of microbial activity, autolysis or chemical oxidation, though microbial spoilage constitutes more spoilage than the

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others. Spoilage bacteria are commonly gram negative and produce off odours and flavours in seafood as a result of their metabolic activities [29]. The association of seafood with spoilage organisms and other microorganisms especially pathogenic organism is a worthwhile study because of the growing concerns about economic loss of shell fish due to spoilage organisms and the risk of food borne diseases of which seafood has been a major culprit. One of the major reasons for this is the continuous increase in pollution of water bodies by untreated human and animal waste and industrial wastes which continue to increase the population of spoilage and pathogenic organisms in our water bodies [24].

Periwinkles are univalve prosobranch brackish gastropod molluscs which belong to the super family Cerithioidea. This super family is characterized with gastropod that possess a turreted granular and spiny shell with a tapering end [3]. Periwinkles are small sea animals that are endemic in Nigeria and Africa and are found mainly in the tidal mudflats of estuarine ecosystems, as part of the fauna of the mangrove community. The super family Cerithioidea consists of two families Potamididae and Melanidae. The family potamididae is represented by two genera, *Tympanotonus fuscatus* and *Pychmellania aurita*. The genus *Tympanotonus* is a single species which has two varieties – *Tympanotonus fuscatus* var *fuscatus* (Linnaeus) and *Tympanotonus fuscatus* var *radula*. *Tympanotonus fuscatus* var *fuscatus* is distinguished from *Tympanotonus fuscatus* var *radula* by the absence of spiny tubercles on the shell [10,17].

Periwinkles are popularly called “Isam” in Rivers State and an essential ingredient in most local dishes especially those peculiar to the southern part of Nigeria. In Nigeria, periwinkles occur in lagoons, estuaries and mangrove swamps and very large quantities are harvested and consumed in Port Harcourt, Lagos, Bayelsa and Akwa Ibom states [34].

The term Oyster is used to describe a distinct group of bivalve molluscs which live in marine or brackish habitat. Oyster is locally called “Mgbe” in Rivers State and are found in large quantities in riverine and coastal areas. Oysters are usually found in clusters attached to aerial roots and lower branches of mangrove trees [34].

Over the past two decades, oyster consumption has significantly increased all around the globe so that the oysters have become the second most commercially important marine organisms for aquaculture worldwide and imported oyster meat is consumed in many big hotels and restaurants in Nigeria and the world [35].

Whelk is the common name applied to various sea snails. Whelks are bivalve invertebrate gastropods that possess a spiral shell which usually occur in various shapes and sizes. Whelks can vary in size from under an inch in length (shell length) to more than 2 feet. The trumpet whelks are the largest in the world and can grow to over 2feet. Whelks possess a muscular foot which is used to move and hold their prey. The shell opening of whelks is covered by a hard operculum which the whelk uses for protection against predators. [19]. Whelks are popularly called “Ngolo” in Rivers State. Like periwinkles and oysters, whelk is also a cheap source of protein and an important ingredient in many southern dishes. Food borne diseases arising from consumption of periwinkle, oyster and whelk may be generally due to pollution of water bodies, poor hygiene of those who process them for commercial marketing, undercooking or the three combined, hence, the need for a comparative study of both raw and cooked samples. This study is both relevant and necessary as it evaluates the microbial population associated with periwinkle, oyster and whelk, especially pathogenic organisms which are the cause of food poisoning. This study was conducted to evaluate and ascertain the microbial and nutritional profile of processed and unprocessed periwinkle, oyster and whelk from different markets around Port Harcourt, Rivers State.

2. Material and methods

2.1. Study design

The research was carried out to determine the microbial and nutritional quality (profile) of both Raw and processed periwinkle (*Tympanotonous fuscatus*), oyster (*Crassostrea gasar*) and whelk (*Rapana venosa*) obtained from different vendors in different markets in Port Harcourt and sea food traders in Bonny and brought to Research laboratory in Department of Microbiology.

2.2. Sample Collection

A total of 120 samples. Were purchased and collected into sterile transparent bags, one for each and clearly labeled and transported (in ice packs for preservation in cases of long distances) to the research laboratory. 20 raw and 20 cooked samples of each of the sea food samples (periwinkle, oyster and whelk) was used for this research.

2.3. Preparation of the samples

2.3.1. Raw samples

Raw oyster and Whelk was shucked aseptically with a sterile stainless-steel instrument (Knife) which was inserted between the shell about 2cm from the hinge area the knife will be pushed into the shellfish the fluid and meat removed from the shell and placed in a sterile container as described by [1]. The raw periwinkle meat in their shell was cracked using a small sterile hammer on the improvised sterile anvil then the meat was extracted individually from the broken shell using a sterile forceps and transferred into a sterile container as described by [1] as modified by [30] The cooked shellfish samples were purchased from the market from different sellers

2.3.2. Microbial Analysis of Samples

Samples of the seafood (the fleshy edible part) were hygienically transferred to a sterile stomacher (Model BA 6021, Seward Medical, UK) according to the method described by [14]. This was later homogenized, using 225ml sterile 0.1% peptone water for a period of two minutes. Serially, tenfold dilution up to 10⁵ of the homogenates were made by transferring 1ml of fresh sterile dilutors to plates of surface-dried plate count Agar for total bacteria count and Salmonella-Shigella Agar for salmonella count, mannitol salt agar for Staphylococcus count. All plates were incubated at 37°C for 24-48hours. After 24hours, plates were removed from the incubator, inspected, colonies counted and records taken. Microbial counts of each petri plates were expressed in colony forming units per grams (CFU/g). Colonies were also observed for their morphological characteristics and all observations recorded

The different colonies of microorganisms found on each petri plates were picked individually using a sterile inoculating loop (which was frequently sterilized by red hot flaming) and sub cultured on dry formed nutrient agar plates (one for each) using the streak plate method. The plates were also incubated at 37 °C for 18 to 24hour biochemical identification of isolates were sterilized at 121°C 15 psi for 15 minutes. Enumeration of *Vibrio* spp. was done by using the medium Thiosulphate citrate bile salt agar (TCBS). Aliquot (0.1ml) each of the shellfish was pipette aseptically into TCBS plates in triplicates and spread with a sterile glass rod. The *vibrio* spp. was later enumerated using standard microbiological techniques [8].

2.4. Proximate Analysis

Proximate analysis was done as described by [20].

2.5. Statistical analysis

All analysis were done in duplicates for each of the samples, and data were reported for duplicate analyses of the same samples. All statistical analyses were carried out using analysis of variance (ANOVA). Significance of the differences was ascribed at the 0.05 level for ANOVA.

3. Results

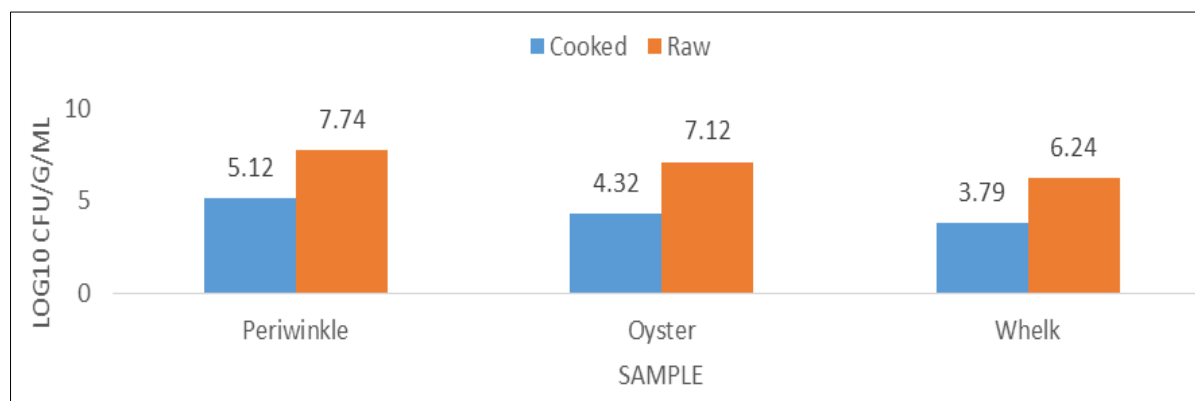
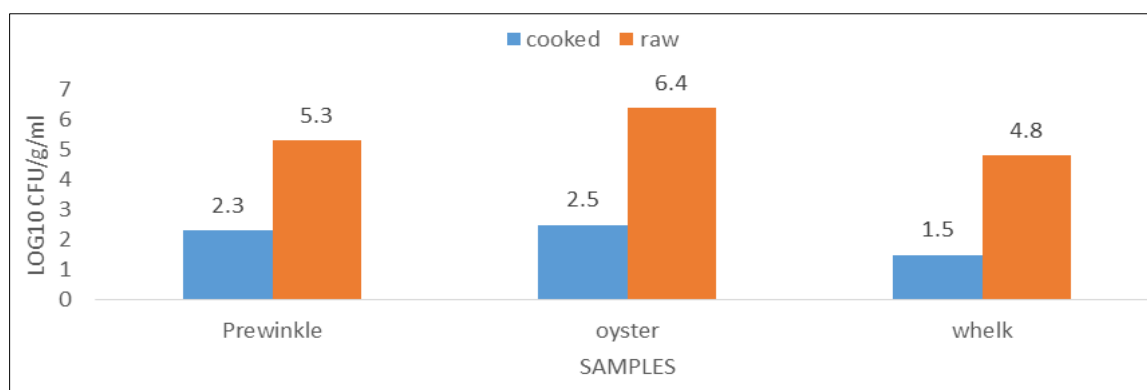
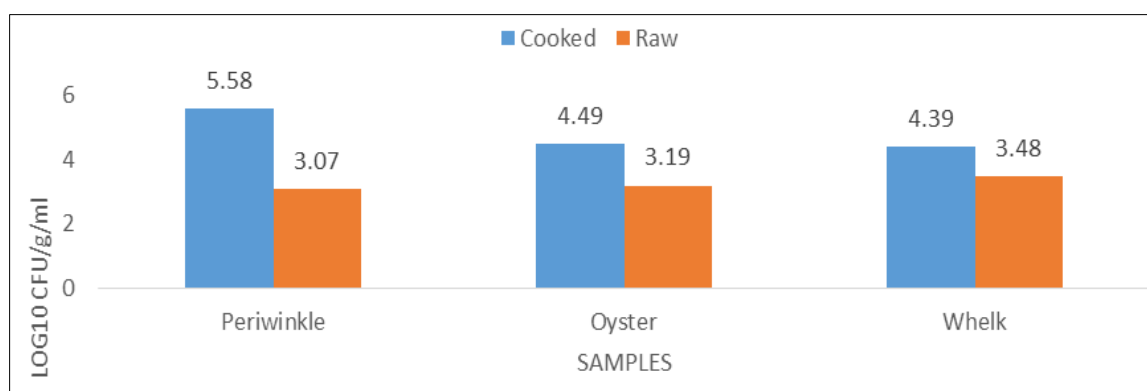


Figure 1 Mean Log₁₀ Total Viable Count of Cooked (Processed) and Raw (Unprocessed) shellfish

Table 1 Percentage Occurrence of Bacteria Isolates in Periwinkle, Oyster and Whelk

Isolates	% in Periwinkle	% in Oyster	% in Whelk
<i>Salmonella sp</i>	12	11	26
<i>Proteus sp</i>	4	4	3
<i>Escherichia coli</i>	8	7	10
<i>Vibrio sp</i>	16	19	16
<i>Pseudomona sp</i>	8	8	6
<i>Bacillus sp</i>	16	15	13
<i>Klebsiella sp</i>	8	8	3
<i>Enterobacter sp</i>	4	8	10
<i>Citrobacter sp</i>	4	4	3
<i>Micrococcus</i>	12	8	3
<i>Staphylococcus</i>	8	8	7

**Figure 2** Mean Log₁₀ Total Salmonella Count of Cooked (Processed) and Raw (Unprocessed) shellfish**Figure 3** Mean Log₁₀ Total Staphylococcus count of Cooked (Processed) and Raw (Unprocessed) shellfish

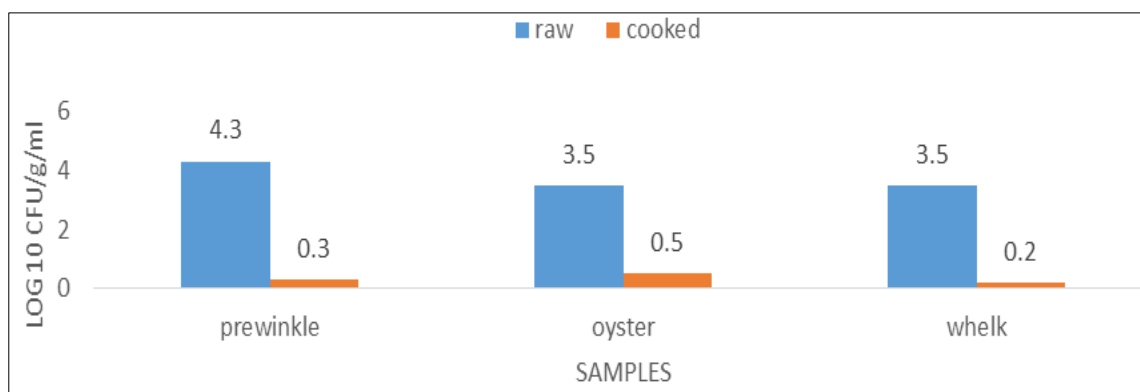


Figure 4 Mean Log₁₀ Total Vibrio count of Cooked (Processed) and Raw (Unprocessed) shellfish

Table 2 Proximate composition of the Shellfish samples

Composition (%)	Raw (unprocessed) Periwinkle	Cooked (processed) Periwinkle	Raw (unprocessed) Oyster	Cooked (processed) Oyster	Raw (unprocessed) Whelk	Cooked (processed) Whelk
Ash	13.74	10.82	5.03	11.28	12.73	15.73
Moisture content	17.62	12.36	40.14	21.17	52.93	57.24
Crude protein	32.53	48.05	18.66	28.74	17.02	12.83
Crude fibre	0.18	0.86	1.17	3.18	1.73	0.46
Crude lipid	7.3	5.94	3.73	0.52	8.57	9.02
Carbohydrate	28.63	21.97	31.27	35.11	7.33	4.72

4. Discussion

Shellfish especially periwinkle, oyster and whelk play an essential role in the diet of consumers as a cheap source of protein for a vast section of the world's population. Although a cheap source of protein, seafood has been implicated a good number of times as vehicles for food borne disease as they, especially in their uncooked state are almost always associated with pathogenic microorganisms that are responsible for food borne infections [24]. In this research. The bacterial contaminants of raw periwinkle, cooked periwinkle, raw oyster, cooked oyster, raw whelk and cooked whelk were determined. The Mean Total Viable Bacteria Count for raw shellfish ranged from Log₁₀ cfu/g 6.34 – 7.74 While the cooked shellfish ranged from.log₁₀ 4.32- ,5.74

The microbiological analysis of the different shellfish showed high microbial load, however, the count was significantly higher in Raw samples when compared to the Boiled samples($p < 0.05$)., this work corroborates with previously reported work by [11] that periwinkles from Ishiet and Oron had high microbial load.[28], where the total viable bacteria count on raw periwinkles showed such high values.[15] also reported similar values in her work on periwinkles and oysters and [14], reported similar high bacteria contamination of whelk samples The statistical analysis on the data also showed that their microbial load was above the acceptable limit stipulated by the International Commission on Microbiological Specifications for Food [16] which state that the maximum microbial count in shell fishes should not exceed the acceptable limit of 1×10^5 cfu/g for the safety of the consumers. The result of the Boiled shellfish samples showed that boiling of shellfish will help to drastically reduce the microbial load on shell fishes.

Mean Total salmonella count for raw and cooked shellfish ranged from Log₁₀ cfu/g 1.5 – 2.54 While the raw shellfish ranged from.log₁₀ 4.48-6.74. cfu/g. The raw samples were significantly higher than the cooked samples. ($p < 0.05$). *Salmonella spp* is one of the most important food-borne pathogens and are indicators of sewage contamination and it is found to be associated with a number of non-human hosts such as reptiles [5]. This bacteria species has been reported to survive and persist in the aquatic environment and has been detected in seafood in different creeks of Niger Delta [30] and causes new born meningitis and infantile diarrhea [30]. Total salmonella counts for samples analyzed in this

research exceeded ICMSF standards and Microbiological Guidelines for Food, [26] of 1.0×10^2 for processed samples and 1.0×10^4 for raw samples,

Total staphylococcus count for cooked shellfish ranged from $\log_{10} 4.39$ - 5.58 cfu/g. While the raw shellfish ranged from $\log_{10} 3.07$ - 3.48 cfu/g. it was observed that the cooked samples had high staphylococcus count as compared to the raw samples. Processed samples, especially those of periwinkle and oyster showed higher counts compared to the raw ones probably because processed samples sold in the market are undercooked and are mostly used as cooking ingredient for further cooking process. This undercooking makes it very possible for organisms found in raw samples to be detected in the cooked samples. Also, poor hand washing of seafood processors who remove the samples from their shells and sell (with bare hands) to consumers increase the microbial population. Processed samples sold in markets are mostly kept open in jars and allowed to interact with surrounding air which contains droplets and microorganisms. Furthermore, these processed samples are washed and sometimes soaked in water which may be unclean and contain microorganisms, after which, these undercooked samples are finally left for long periods of selling in the market (morning to evening) which gives room for the growth and multiplication of organisms already contained in the samples. Some seafood vendors also admitted that processed seafood samples which were not sold were refrigerated and or soaked with warm water for re-selling the next day. This is especially true for oyster which are very seasonal and whose samples are processed and preserved for very long periods (weeks to months).

Total vibrio count for raw shellfish ranged from $\log_{10} 3.5$ - 4.3 cfu/g while cooked shellfish ranged from $\log_{10} 0.2$ - 0.5 cfu/g. The presence of *Vibrio* spp. in samples of raw shellfish suggests that food borne illness could arise if this seafood is consumed in the uncooked state. This may be due to human activities, such as bathing, defecation by the local populace which are common phenomenon in the area. Conversely, [38] reported that increased level of human activities could bring about the elevation of organic matter resulting in high microbial load in the water, therefore high microbial population in an aquatic environment, is an indication of the input of microorganisms from domestic and industrial sources consequent of human activities. Very close values were recorded by [33,28 11], for total counts for *Salmonella*, *Staphylococcus* and *Vibrio* Sp. [15] also reported similar values in her work on periwinkles and oysters and [14], reported slightly higher values for total *Salmonella* and *Vibrio* counts for whelk. Microbiological Guidelines for Food [26] and ICMSF standard [16] of not more than 5.0×10^5 for raw shellfish and not more than 1.0×10^5 for processed shell fish. According to ICMSF standards and Microbiological Guidelines for Food, [26], values obtained are unsatisfactory. Similarly, values for total salmonella counts for samples analyzed in this research exceeded ICMSF standards and Microbiological Guidelines for Food, [26] of 1.0×10^2 for processed samples and 1.0×10^4 for raw samples. Most samples analyzed did not meet ICMSF standards and Microbiological Guidelines for Food, [26] for *Staphylococcus* count of not more than 1.0×10^4 for both raw and processed samples and for vibrio count of less than 20 in 25g of processed samples and 1×10^3 in raw (unprocessed) samples. According to ICMSF standards and Microbiological Guidelines for Food, [26], all samples analyzed are considered unfit for consumption as counts obtained are beyond satisfactory limits. Bacteria isolated from processed and unprocessed periwinkle, oyster and whelk samples are *Salmonella* sp, *Proteus* sp, *Escherichia coli*, *Micrococcus* sp, *Vibrio* sp, *Enterobacter* sp, *Klebsiella* sp, *Citrobacter* sp, *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp. *Vibrio* species identified are *Vibrio Cholerae* and *Vibrio Parahaemolyticus*.

Salmonella species are typically found in animal and human intestine and are shed through faeces. Humans serve as reservoir to the organism; *Salmonella* species cause diseases called salmonellosis and humans become infected by consuming contaminated food and water [12]. The detection of salmonella in raw samples could be due to pollution of water bodies with faeces from human and animals, whereas, contamination of processed samples could be linked to poor hand washing of those who process these foods and generally poor hygiene. *Proteus* sp are mostly found in the intestine of humans as part of the intestinal flora. The presence of *Escherichia coli* suggests a faecal contamination of samples and/ or water bodies as *E. coli* is associated with the gastrointestinal tract. *E. coli* is the causative agent of diarrhea, dysentery, bladder infection, septicemia and meningitis [23]. *Vibrio cholerae* cause cholera (*Vibrio cholera*) and is characterized by severe diarrhea, of which several outbreaks have been linked to sea foods. *Micrococcus* sp usually found freely in the environment. Contamination of seafood by *Staphylococcus* is mainly due to unhygienic food handling. *Staphylococcus* sp cause infections ranging in severity from minor skin infection, food poisoning to life threatening infections [18]. Because *Staphylococcus aureus* is a normal flora on the skin, small numbers of *Staphylococcus aureus* are expected in products handled by humans, limits must be maintained as toxins can be produced when in large numbers.

Although, raw and processed samples of periwinkles, oysters and whelks are highly loaded with pathogenic organisms, they are also highly nutritious, especially rich in protein, their need as a cheap source of protein becomes significant. The result obtained for proximate analysis of raw periwinkle (*T. fuscatus*) agrees perfectly with the study carried out by [13], although values for protein and carbohydrate in the study conducted by [5] showed higher values. The differences in nutrient content of processed *T. fuscatus* depends on the extent of cooking. [6] showed relatively higher

values for moisture content and lower protein and lipid content of raw *C. gasar*, however, ash content was within range. [21] obtained slightly higher value for moisture content, but protein, carbohydrates and lipid were significantly lower than those obtained in this study. [37] confirmed from study that differences in the proximate composition of *C. gasar* is size dependent; the larger the size, the higher the carbohydrate, lipid and protein content. [36] showed much higher values for the nutrient content of unprocessed whelk samples. Cooking has almost no effect on the lipid but increases the protein content of cooked *Rapana venosa* [25, 27,].

Proximate analysis showed that boiling does minimal to insignificant damage to the nutrient contained in the shellfish analyzed. This means that seafood can be reasonably cooked without the erroneous assumption that a short but reasonable amount of cooking will totally destroy all nutrients. This is because, cooking is one reliable way in the destruction of microorganisms contained in food and undercooking on the other hand is one major cause of food poisoning associated with seafood since if the seafood is cooked properly, it will drastically reduce the microbial population and destroy pathogenic microorganisms in food. Therefore, extra care must be taken in the final cooking or processing of these seafood to ensure their safety for consumption. A minimum of 160F for 15mins is required to destroy most pathogenic organisms, while the nutrients would not experience significant damage. However, the higher the temperature the lower the time required, [2]. Pre-processed seafood samples should not be eaten without further cooking with the assumption that they are cooked as they become contaminated again as they get cold and are manipulated with human hands, and may contain even higher numbers of microorganisms than the raw ones.

Recommendation

Consumers and food vendors (handlers) should be educated about the risk associated with sea food consumption and all that should be done to ensure food safety and Consumers should be advised to cook seafood properly (a minimum of 160F for 15mins). Shell oyster should be cooked until the shells open or begin to curl (all shells that do not open or curl during the cooking process must be discarded). Sea food habitats should be properly protected by the government, environmentalists and individuals against pollution especially fecal pollution.

5. Conclusion

Seafood safety relies on environmentalists, the government, food vendors, buyers, processors, sellers and consumers to ensure that proper attention is given to the handling and consumption of periwinkle, oyster and whelk to improve food safety and reduce the risk of food poisoning associated with seafood.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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