



Proximate, Mineral and Heavy Metal Analysis of Seafoods Sold in Otuoke Market
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Abstract

Proximate, mineral and heavy metal analysis were carried out on Pink shrimps (*Penaeus notialis*), Oysters (*Crossostrea gasar*) and Periwinkle (*Tympanotomus fuscatus*) sold in Otuoke market. The moisture, ash, crude protein, fat and carbohydrate contents of the samples were determined using standard AOAC methods. The mineral and heavy metal concentrations were determined using Atomic Absorption Spectrophotometer. *T. fuscatus* had the highest moisture content (67.61 %). *P. notialis* had the highest ash (7.73±0.07 %), protein (18.86±0.21 %) and carbohydrate (7.27 ±0.03 %) contents while the highest moisture (66.08±0.24 %) and fat (10.70±0.42 %) contents were obtained for Oysters. The Potassium contents of *C. gasar*, *P. notialis* and *T. fuscatus* were 59.44±0.68 mg/kg, 61.68±0.34 mg/kg and 61.59±0.46 mg/kg respectively. *T. fuscatus* has the highest Iron content (62.78±0.10 mg/kg) while the least value was obtained for *P. notialis* (6.26±0.25 mg/kg). The Copper contents ranged between 35.82±0.13 mg/kg (for *C. gasar*) and 39.44±0.09 mg/kg (for *T. fuscatus*). The Calcium contents were 754.98±1.16 mg/kg (*C. gasar*), 733.07±1.20 mg/kg (*P. notialis*) and 742.57±0.71 mg/kg (*T. fuscatus*). The least Magnesium content was obtained for *P. notialis* (302.25±1.98 mg/kg) while *C. gasar* had the highest (323.01±1.13 mg/kg). The results show that the samples are excellent dietary sources, however, the concentrations of Cu, Mn and Pb in shrimps, oysters and periwinkles were higher than WHO and European Commission permissible limits of 10.0 mg/kg, 5.4 mg/kg and 0.5 mg/kg for Cu, Mn and Pb respectively. From the standpoint of human health, the high levels of these heavy metals in these seafoods imply that their excessive consumption could pose health risk to the consumers.

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Introduction

Any form of life in the sea regarded as food by man is referred to as seafood. Seafood is a very important component of human diet in several countries and is a significant source of nutrient, particularly highly edible proteins (Faber *et al.*, 2010). Also, they are very good source of omega-3 fatty acids, a nutrient recommended for daily consumption by the American Heart Association because it gives protection against heart diseases and reduces the risk of developing stroke.

Accumulation of heavy metals by seafoods from water, sediments or food is very easy due to their trophic levels in the aquatic food chain (Zhao *et al.*, 2012). Though, seafood possesses very significant health benefits, consumption of seafood whose bodies have stored up very high levels of heavy metals is a very serious health risk. Heavy metals have been known to cause cardiovascular diseases, damage to the kidneys and liver and sometimes death (Rahman *et al.*, 2012). Seafoods are known to

possess very high sensitivity to metals in their environment and tend to accumulate them in their bodies in high concentrations (Aytekin *et al.*, 2019). These metals eventually travel up the food chain to man (Palaniappan and Karthikeyan, 2009). The gastropod *Tympanotomus fuscatus* (Periwinkle) is bisexual, and found mostly in the brackish water creeks and mangrove swamps of the Niger Delta area at the inter-tidal zone (Jamabo and Chinda, 2010). It is characterized by turreted, granular and spiny shells with tapering ends. As a delicacy in many riverine communities, it is a cheap source of animal protein. The shell is used as a source of calcium in animal feeds and employed for construction purposes (Jamabo and Chinda, 2010).

Crossostrea gasar, also known as the mangrove oyster, is the oyster species found in Nigeria (Ansa and Bashir, 2007). Oysters can withstand wide variations in salinity, dissolved oxygen and

temperature. This high level of tolerance makes them suitable for cultivation worldwide (Ogundiran and Fasakin, 2015). It is a source of animal protein and an important source of income in some countries around the world.

Pink shrimp, *Penaeus notialis* belongs to the family *Penaeidae*. They are distinguishable from other shrimp families by their antennae, which are longer than their body lengths. The overall body colour of this species is variable but generally gray, bluish or red-brown. The integument is thin and translucent while the carapace has a medial carina that extends nearly to the posterior end of the carapace and is bordered by a broad, rounded groove on either side. The rostrum may have 1- 3 teeth and the abdomen has 4 – 6 carinate segments, with the carina of the sixth segment ending in a spine. A dark, distinctive spot on the pleural junction between the 3rd and 4th abdominal segments is a feature that can be used to distinguish this species from other members of the genus.

The health benefits of seafood consumption have made it imperative to determine the mineral and heavy metals concentrations in these kinds of seafood so that the consumers may be properly guided in the choice of aquatic products to be consumed, hence this study.

Materials and Methods

Sample collection and preparation

Fresh samples (*P. notialis*, *C. gasar* and *T. fuscatus*) used in the current work were bought from Otuoke market. Samples were placed in glass bottles filled with water and were preserved in a cooler containing ice until use. After carefully removing the periwinkles from their shells, all samples (*P. notialis*, *C. gasar* and *T. fuscatus*) were dried in an oven for 6 hours, after which they were ground using an electric blender.

Proximate Analysis of Samples

Percentage moisture crude protein, crude fat, crude fiber and ash content of the formulation was determined based on the official methods of analysis (AOAC, 2012). Percentage carbohydrate was determined by subtracting the measured protein, fat, ash, and water from the total weight.

Determination of Moisture Content

A crucible was dried in an oven and cooled in a desiccator. The weight of the crucible was recorded (W_1). 1 g of the sample was weighed into the crucible and the weight of sample and crucible taken (W_2). The crucible (with the sample) was placed in an oven at 105°C for about 2-3hrs until constant weight of the dried sample and crucible was observed. The weight of the dried sample and

the crucible was noted (W_3). The % moisture content in sample is calculated from:

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Determination of Ash Content

A crucible was dried in an oven and cooled in a desiccator. The weight of the crucible was recorded as W_1 . A 2 g portion of the sample was weighed into the crucible and the total weight (sample + crucible) recorded (as W_2), placed in a muffle furnace, and heated to between 500 and 600°C for between 4-5hrs until the sample turned slightly whitish. The weight (of crucible and sample) after ashing is recorded (as W_3). The % ash is calculated from:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

The weight of the ash was determined after incineration of the sample in a muffle furnace at 550°C until ash was obtained. The percentage of material burnt off was regarded as organic matter.

Determination of Crude Protein Content

Protein in the sample was determined by Kjeldahl method. 0.5-1.0 g of dried samples were taken in a digestion flask. 10–15 ml of concentrated H_2SO_4 and 8 g of digestion mixture i.e. K_2SO_4 : CuSO_4 (8: 1) were added. The flask was swirled to mix the contents thoroughly then placed on a heater to start digestion till the mixture become clear (blue-green in color). This process took about 2 hrs to complete. The digest was cooled and transferred to 100 ml volumetric flask and volume were made up to mark by the addition of distilled water.

Distillation of the digest was performed in Markam Still Distillation Apparatus. Ten- milliliter of digest was introduced in the distillation tube then 10 ml of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 20 ml of 4% boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appears due to NH_4OH . The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the following formula:

$$\% \text{ Crude Protein} = 6.25 * \text{ \%N } (*. \text{ Correction factor})$$

$$\% \text{ N} = \frac{((S-B) \times N \times 0.014 \times D \times 100)}{(\text{Wt. of sample} \times V)}$$

where: S = Sample titration reading; B = Blank titration reading; N = Normality of HCl

D = Dilution of sample after digestion; V = Volume taken for distillation; 0.014 = Milli equivalent weight of Nitrogen

Determination of Fat Content

0.5g of the sample was weighed into Whatman filter papers. The sample was held tightly inside a soxhlet extractor. The weight of a 250 ml of round bottom flask was determined (W_1). Petroleum ether (40- 60°C b.p) was added up to about two-third volume of the flask and the weight (flask + petroleum ether) measured (as W_2). The set up was allowed to boil on heating mantle for between 4-6hrs. The petroleum ether siphoned over the barrel and the condenser was detached. The weight of the flask after extraction was taken (as W_3).

$$\% \text{ Fat} = \frac{W_3 - W_2}{W_2 - W_1} \times 100$$

Determination of Carbohydrate Content

The carbohydrate content was determined by difference by subtracting the measured protein, fat, ash, and water from the total weight.

Determination of Mineral and Heavy Metal Content Of Samples

Platinum or silica dishes were cleaned, dried and ignited at 500°C for 30 minutes in the furnace. They were then cooled, placed in a dessicator and weighed until a constant weight was obtained. About 2 g of sample was then accurately weighed into the dish and burnt at 500°C. Sample was checked periodically to ensure complete ashing. The percentage ash content was determined using the formula:

$$\% \text{ Ash Content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

The ash obtained was thereafter dissolved by adding 5mls of 10% HCl solution and warming on a water bath. Where the ash did not totally dissolve, it was further treated with 5mls of 10% nitric acid and warmed on water bath. The sample is then transferred quantitatively using a stirring rod, through a funnel into a clean, dry 20ml Standard volumetric flask. The solution of ash is then aspirated on presentation to the Atomic Absorption Spectrophotometer where the concentration of the individual metal(s) is determined.

Results and Discussion

The results of the proximate analyses of the samples are shown in Table 1. Of the three samples, *T. fuscatus* (67.61 %) had a slightly higher moisture content than *C. gasar* (66.08 %) and Pink shrimps (63.71 %). High moisture content allows enzymatic reactions go on smoothly in living organisms. However, high moisture content may also make the samples susceptible to spoilage by microbes, increasing oxidative degradation of polyunsaturated fatty acids and ultimately decreasing the quality of the samples thereby reducing their preservation time (Omolara and Omotayo, 2009). The moisture contents in all the

three species were within the acceptable level (60-80 %), which could be due to the stable water levels in the location where the samples were collected (Adewumi *et al.*, 2014). The percentage of water is also a good indicator of its relative content of energy, protein and lipid (Olagunju., *et al.* 2012). The moisture content obtained for *C. gasar* and *T. fuscatus* are slightly lower than the values of 77.37 % and 84.80 % respectively previously reported for both samples (Kiin-Kabariet *al.*, 2017).

P. notialis had a higher ash content (7.73 %) than *C. gasar* (5.22 %) and *T. fuscatus* (6.09 %). Ash measures the mineral content in an organism. These values are slightly less than those reported by Kiin-Kabari *et al.* (2017) for *C. gasar* and *T. fuscatus*. The differences in the concentration of minerals may be dependent on several factors including seasonal changes, age, sex, size, and sexual maturity, food source, and availability in the respective habitat of organisms and other factors such as water chemistry, salinity, temperature, and contaminants (Kucukgulmez *et al.*, 2006).

P. notialis had the highest crude protein content (18.86 %) while *C. gasar* had the least (14.13 %). Protein is the major structural component of cells and is responsible for the building and repair of body tissues. The result had shown that the samples constitute a rich source of protein. Values of 13.31 and 9.97 were obtained by Kiin-Kabari *et al.* (2017) for *C. gasar* and *T. fuscatus* respectively.

The highest fat content was obtained for *C. gasar* (10.70 %) while the least was obtained for *P. notialis* (2.58 %). These values are generally low. This could be beneficial as it could contribute to reducing the risk of hypercholesterolemia which is capable of causing cardiovascular disease, due to its high omega-3 fatty acid content (Kiin-Kabariet *al.*, 2017). Lipids are an alternative energy source in times of fasting and starvation. Fats are important in the structural and biological functioning of the cells. In crustaceans, not only do lipids function as the main organic reserve and source of metabolic energy but are also indispensable in maintaining cellular integrity. Generally, lipids act as major food reserve together with protein and may fluctuate periodically due to environmental variable like temperature (Varadharajan and Soundarapandian, 2014). The lipid content of *C. gasar* and *T. fuscatus* obtained in the current samples are higher than those obtained from samples obtained from Rivers State (Kiin-Kabariet *al.*, 2017). *P. notialis* had the highest carbohydrate content (7.70 %) while *C. gasar* had the least (3.69 %). Low carbohydrate content indicates that high consumption of these seafoods must be supplemented with energy-rich

foods to balance the energy-protein intake requirement. Carbohydrates are sources of instant energy, which can be used in the body's development and growth (Olagunjuet *al.*, 2012).

Table 1: Proximate composition of *P. notialis*, *C. gasar* and *T. fuscatus*

| Parameters | <i>C. gasar</i> | <i>P. notialis</i> | <i>T. fuscatus</i> |
|-------------------|--------------------------|-------------------------|-------------------------|
| Moisture (%) | 66.08±0.24 ^a | 63.71±0.63 ^b | 67.61±0.55 ^c |
| Ash (%) | 5.22±0.17 ^a | 7.73±0.07 ^b | 6.09±0.19 ^c |
| Crude Protein (%) | 14.13±0.33 ^a | 18.86±0.21 ^b | 17.67±0.12 ^c |
| Fat (%) | 10.70±0.42 ^a | 2.58±0.04 ^b | 3.28±0.20 ^c |
| Carbohydrates (%) | 3.69 ± 0.02 ^a | 7.27±0.03 ^b | 5.20 ±0.18 ^c |

Values are reported as mean ± standard deviation. Values with different superscripts (along the row) are significantly different (at p < 0.05)

The results of the mineral and heavy metal contents of the samples are shown in Table 2. The results show that the samples are rich in calcium, magnesium, potassium and sodium. The potassium content of the samples ranged between 59.44 mg/kg (for *C. gasar*) and 61.68 mg/kg for *P. notialis*. However, there is no significant difference between the potassium content values obtained for *P. notialis* and *T. fuscatus*. Potassium is needed in fluid balance and regulation of nerve impulse conduction, regular heart beat and cell metabolism (Fagbuaet *al.*, 2006). Among the samples studied the lowest iron content was obtained for *P. notialis* (6.26 mg/kg) while *C. gasar* and *T. fuscatus* had values 38.62 mg/kg and 62.78 mg/kg respectively. Iron content values between 9.69-29.50mg/100g have been previously reported for *C. gasar* and *T. fuscatus* (Davies and Jamabo, 2016).

Iron is important for red blood formation, therefore *C. gasar* and *T. fuscatus* can be recommended for pregnant women and children. The samples are

very rich in calcium with values ranging between 733.07 mg/kg for Pink shrimps and 754.98 mg/kg for *C. gasar*. Calcium in addition with other micro minerals and protein can help in bone formation with calcium acting as principal contributor. Calcium is important in blood clotting, muscles contraction and in certain enzymes in metabolic processes (Abuludeet *al.*, 2006). The magnesium content of the samples ranged between 302.25 mg/kg and 323.01 mg/kg. They are therefore good sources of magnesium, an essential micronutrient needed for nervous system health (Mottonen and Uhari, 1997). A sodium content of 47.38 mg/kg, 48.24 mg/kg and 47.46 mg/kg was obtained for *C. gasar*, *P. notialis*, and *T. fuscatus* respectively. However, no significant difference was obtained among these values. Sodium plays a vital role in regulating the pH, osmotic pressure, water balance, nerve impulse transmission and active transport of glucose/amino acid (Davies and Jamabo, 2016).

Table 2: Trace / Heavy Metal Analysis of Seafood

| Parameters | <i>C. gasar</i> (mg/kg) | <i>P. notialis</i> (mg/kg) | <i>T. fuscatus</i> (mg/kg) |
|---------------|----------------------------|-------------------------------|-------------------------------|
| Potassium, K | 59.44±0.68 ^a | 61.68±0.34 ^b | 61.59±0.46 ^b |
| Iron, Fe | 38.62±0.55 ^a | 6.26±0.25 ^b | 62.78±0.10 ^c |
| Copper, Cu | 35.82±0.13 ^a | 38.91±0.12 ^b | 39.44±0.09 ^c |
| Cadmium, Cd | 0.63±0.02 ^a | 0.38±0.01 ^b | 0.35±0.12 ^b |
| Calcium, Ca | 754.98±1.16 ^a | 733.07±1.20 ^b | 742.57±0.71 ^c |
| Magnesium, Mg | 323.01±1.13 ^a | 302.25±1.98 ^b | 318.11±1.30 ^c |
| Manganese, Mn | 23.44±0.87 ^a | 12.32±0.32 ^b | 21.93±0.32 ^c |
| Sodium, Na | 47.38±0.67 ^a | 48.24±0.61 ^a | 47.46±0.99 ^a |
| Lead, Pb | 6.49±0.39 ^a | 1.55±0.09 ^b | 2.07±0.08 ^c |

Values are reported as mean ± standard deviation. Values with different superscripts (along the row) are significantly different (at p < 0.05)

The high chance of bioaccumulation of heavy metals in seafood samples makes it necessary to measure their levels in the samples alongside determining their nutritive values. Table 2 shows the heavy metal contents (Pb, Mn, Cu and Cd) of

the studied samples. The concentration of Copper is highest in *T. fuscatus* (39.44 mg/kg) and lowest in *C. gasar* (35.82 mg/kg). These values are far higher than the WHO permissible level for Copper (10µg/g) (Ogundiran and Fasakin, 2015). Copper is

an essential component of the human body and other lower organisms. Nevertheless, they are toxic when present beyond the concentrations required for biological processes. Copper is required by living organisms as an essential part of their oxygen-carrying pigment haemoglobin and several enzymes (Sivaperumalet *et al.*, 2007). The lead concentrations in the samples ranged between 1.55mg/kg (in *P. notialis*) and 6.49 mg/kg (in *C. gasar*). The lead levels reported in the current study are higher than the EC permissible level of 0.5µg/g for Pb (EC, 2011). Lead affects the nervous system and may lead to impaired learning ability. Lead can cause fetal injury and have an adverse effect on fertility. Children are extra sensitive to lead because they absorb more lead than adults. Lead also affects enzyme activity in the blood and the transport of oxygen around the body. It also accumulates in the bones (NIFES, 2016). Cadmium has a long half-life in humans and can be carcinogenic (Jaishankar *et al.*, 2014). It can be accumulated in the kidneys and caused kidney failure. Cadmium can also have an adverse effect on fertility. Cadmium levels of 0.35 mg/kg, 0.38 mg.kg and 0.63 mg/ kg were obtained for *T. fuscatus*, *P. notialis* and *C. gasar* respectively. No significant difference was obtained for Cadmium levels in *P. notialis* and *T. fuscatus*. Cadmium levels were lower than the WHO's permissible levels of 0.5-1.0 mg/kg (Okparanta and Daminabo, 2018). Although manganese (Mn) is micronutrients in human nutrition as co-enzyme, it is considered as a heavy metal because its density equal 7.0 - 9.99. All studied samples exceeded the permissible limits of 5.4 ppm stated by European Commission (EC, 2011).

T. fuscatus dwell at the bottom of rivers and oceans which acts as sink for heavy metal contaminants. The accumulation of Pb in *T. fuscatus* observed in this study is consistent with previous documentations (De Wolf *et al.*, 2000). Lead concentrations ranging from 0.05-9.02µg/g and Cd from 0.07-2.85µg/g were observed in periwinkle that was employed as a biomonitor to assess metal pollution in Korean coastal water (Kang *et al.*, 2000). Heavy metal pollutants are introduced into the marine environment via industrial and agricultural inputs and runoff from land. These metals can be released and become available to estuarine living organisms (De Wolf *et al.*, 2000). Generally, all the marine organisms studied very high levels of Cu, Zn and Pb. Therefore, the safeguarding of the environment from contamination to keep these metals at the minimum required level for biological processes will be highly beneficial.

Conclusion

This study was carried out to determine the proximate, mineral and heavy metal levels in some seafood sold in Otuoke market. The results of the proximate and mineral analysis of the samples show that they are excellent dietary sources if uncontaminated by environmental pollutions. However, the concentrations of Cu, Mn and Pb in shrimps, oysters and periwinkles were higher than WHO permissible limits (10.0 mg/kg, 5.4 mg/kg and 0.5 mg/kg for Cu, Mn and Pb respectively) in crustaceans and snails. The high levels of these heavy metals in the samples investigated suggest that excessive consumption of these seafoods may pose health risks to the consumers.

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