# ***Original Research Article***

**Mineral, fatty and amino acids composition of three species of mollusks (*Egeria radiata, Limicolaria flammea* and *Viviparus contectus*)**

**Abstract**

**The present study investigates the mineral, fatty acid and amino acids composition of *E. radiata, V. contectus* and *L. flammea*. All samples analyzed in this study were obtained from Yenagoa in Bayelsa state of Nigeria. Mineral composition was determined by Atomic Absorption Spectrophotometry (AAS), fatty acids were determined by High Performance Liquid Chromatography (HPLC) while amino acids composition was determined by gas chromatography (GC). Eight (8) nutritionally essential minerals were detected in all samples analyzed in this study; the concentration of minerals in order of increasing concentration was Cu>Mn>Mg>Na>Ca>Zn>K>Fe, Cu>Mn>Na>Mg>Ca>Zn>K>Fe and Cu>Mn>Mg>Ca>Na>Zn>Fe>K for *E. radiata, V. contectus* and *L. flammea* respectively. Lauric acid, myristic acid, Palmitic acid, Margaric acid, stearic acid, oleic acid, Linoleic acid, arachidic acid and arachidonic acid were detected in varying amounts in all samples. Palmitic acid concentration in *E. radiata* was the most abundant in all samples while oleic acid concentration in *L. flammea* was the least. A total of eighteen (18) amino acids were detected in all samples analyzed in this study: glycine, alanine, valine, leucine, isoleucine, serine, tryptophan, threonine, Methionine, phenylalanine, histidine, proline, aspartic acid, glutamic acid, tyrosine, arginine, lysine and Cysteine. In *E. radiata,* leucine had the highest concentration (21.287mg/100g) while proline was the least (2.854mg/100g); glutamic acid and methionine were the highest and least (19.389mg/100g and 2.996mg/100g respectively) in *V. Contectus* samples and Histidine and tryptophan were the highest and least respectively (11.639mg/100g and 1.415mg/100g). Aspartate, lysine and histidine were not detected in both samples. From the findings of this study, the samples analyzed in this study are good sources of fatty acids, amino acids and nutritionally essential minerals hence their consumption is encouraged.**



*Keywords: Egeria radiata, Limicolaria flammea, Viviparus contectus, minerals, fatty acids*

## 1. INTRODUCTION

Inadequate nutrition remains one of the pressing problems of most developing countries including Nigeria. This is mainly as a result of insufficiency in food production [1]. Malnutrition mainly results from poor quality and quantity of foods consumed by an individual [2]. Pre-school children, pregnant and lactating mothers are usually the worst hit in terms of nutritional problems relating to protein-energy malnutrition (PEM) [3]. There are several likely remedies to this nutritional situation such as mechanized agriculture, increasing available protein foods, conventional animal and plant foods; conventional oil seed protein, single cell proteins and fish protein concentrates. Other approaches include use of green leaves and leaf protein concentrates, use of lesser-known proteins, and fortification of local infant weaning foods [1]. Malnutrition in Nigeria is even more worrisome because it affects the low-income earners and peasants who often cannot afford good protein and other food sources. Thus, it is advised that peasants access their protein needs by consuming lesser-known protein sources which may be affordable and available to them. Proteins of animal sources have higher biological values than proteins of plant sources [4]. Micronutrient deficiency is a less investigated area in terms of nutritional problems however lack of essential minerals are known to result in severe metabolic issues [4]. Shellfishes such as blue crab, bivalve (*Egeria radiata*) and snails such as acute mudsnail (*Viviparus contectus*) and land snail (*Limicolaria flammea*) are commonly found in the Niger Delta region of Nigeria. These protein sources are consumed mostly by peasants and those dwelling along the riverine coasts. This study aims to investigate the mineral, fatty acid and amino acids composition of three species of mollusks consumed by locals along the coastal areas of the Niger Delta.

## 2. MATERIALS AND METHODS

### 2.1 SAMPLE COLLECTION AND PREPARATION

*L. flammea, E. radiata* and *V. contectus* samples were obtained and purchased from Yenagoa in Bayelsa State of Nigeria. After collection, the samples were washed properly with tap water and the soft tissues removed from the shells. The samples were thoroughly washed several times and rinsed with distilled water. The samples were dried to constant weight in a laboratory oven (TT-9023A Techmel & Techmel USA). The samples were then ground to fine powder using an electric mill (masterchef).

### 2.2 DETERMINATION OF MINERAL CONCENTRATION

Mineral concentration in the samples was determined using Atomic Absorption Spectrophotometer (Agilent FS240AA) according to the method of American Public Health Association [5].

**Sample Digestion:** Five grams (5g) of the dried sample was weighed and transferred into a digestion flask and 20ml of the acid mixture (650ml conc. $HNO_3$; 80ml perchloric acid; 20ml conc. $H_2SO_4$) was added. The mixture was heated in the digestion flask until a clear digest was obtained. It was then transferred to a 50ml volumetric flask and diluted with distilled water to the 50ml mark.

**Procedure*:*** The sample was thoroughly mixed by shaking and 100ml of it was transferred into a glass beaker of 250ml volume, to which 5ml of conc. nitric acid was added and heated to boil till the volume was reduced to about 18ml, by adding conc. nitric acid in increments of 5ml till all the residue is

completely dissolved. The mixture was then cooled, transferred into a volumetric flask and made up to 100ml using metal free distilled water. The sample solution was at this point aspirated into the burner system and the concentration of the mineral present was displayed on the recording system against the corresponding absorbance. The actual concentration of mineral/heavy metal in the sample was extrapolated from the standard graph of mineral/heavy metal plotted by the atomic absorption recorded.

## 2.3 FATTY ACIDS DETERMINATION

Fatty acids composition of the samples was determined by gas chromatography (GC) after extraction of crude fat by Soxhlet extraction [6].

**Procedure:** One milliliter (1ml) of crude fat extract was dissolved in 50ml of chloroform and transferred to a 100ml volumetric flask and diluted to the mark. Most of the chloroform was evaporated at room temperature. Next, 1ml of the reagent (20% vol benzene and 55% vol methanol) was added to the volumetric flask. It was then sealed and heated at 400 ºC in a water bath for 30 minutes. After heating, the organic sample was extracted with hexane and water, so that the final mixture of the reagent, hexane and water, was in proportion of 1:1:1 (i.e. 1ml each of hexane and water was added to the reaction mixture). The mixture was shaken vigorously by hand for 2minutes. About half of the top hexane phase was carefully transferred to a small test tube for injection. The fatty acid composition was then determined by gas chromatography (Buck 530) following the machine procedures.

### 2.4 AMINO ACIDS DETERMINATION

Amino acids determination was done by High Performance Liquid Chromatography (HPLC). Hydrolysis of samples was done as follows- A 0.1-g lyophilized sample was weighed into a 16- × 125-mm screw-cap Pyrex (Barcelona, Spain) tube, 15 ml of 6N hydrochloric acid was added, and the tube was thoroughly flushed with $N_2$, quickly capped, and placed in an oven at 110°C for 24hr. After hydrolysis, the tube contents were vacuum filtered (Whatman #541, Maidstone, England) to remove solids, the filtrate was made up to 25 ml with water, and an aliquot of this solution was further filtered through a 0.50-µm pore-size membrane (Millipore, Madrid, Spain). A standard solution containing 1.25 µmol/ml of each amino acid in 0.1N hydrochloric acid was created. Derivatization was done as follows: A standard solution (20 µl) or 50 µl of sample solution was pipetted into a 10× 5-mm tube and dried in vacuum at 65°C. To the residue, 30 µl of methanol-water-Phenylisothiocyanate (2:2:1 [v/v]) was added and then removed in vacuum at 65°C. Next, 30 µl of the derivatizing reagent methanol-water-Phenylisothiocyanate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Prior to injection, 150 µl of diluent consisting of 5mM sodium phosphate with 5% acetonitrile was added to each tube. Chromatographic procedure was done following standard procedures.

## 3. RESULTS AND DISCUSSION

The results of the mineral, fatty acid and amino acids composition of the samples are summarized in the tables 1-4 below.

**Mineral composition**: The mineral composition of the samples is shown on table 1 below. *E. radiata* had the highest significant ($p<0.05$) concentration of Zn, Ca, Fe and K. No significant difference ($p>.05$) was observed in the concentration of Mn, Cu, Na and Mg in the three samples.

**Fatty acid composition**: *L. flammea* had the highest amount of myristic acid and Lauric acid; *E. radiata* had the highest amount of Margaric acid, stearic acid, Linoleic acid and Arachidonic acid. Linoleic acid, Arachidic acid and Arachidonic acid were not detected in *L. flammea;* Lauric acid and *myristic acid* were not detected in *E. radiata;* Lauric acid and oleic acid were not detected in *V. contectus.*

**Amino acid composition:** A total of eighteen (18) amino acids (ten essential and eight non-essential) were detected in all three samples. With the exception of threonine and proline, there was a significant difference in the amino acid composition amongst all the samples analyzed. Lysine, histidine and Aspartate were not detected in *E. radiata* and *V. contectus. L. flammea* contained all eighteen amino acids reported in this study.

**Table 1: Mineral composition of samples (mg/Kg)**

| Metal(mg/Kg) | *E. radiata* | *V. contectus* | *L. flammea* |
|---|---|---|---|
| **Mn** | 0.934 | 0.846 | 0.816 |
| **Cu** | 0.189 | 0.381 | 0.191 |
| **Zn** | 8.534 | 4.194 | 4.114 |
| **Ca** | 7.209 | 3.819 | 3.739 |
| **Fe** | 10.738 | 8.838 | 4.302 |
| **Na** | 4.673 | 3.218 | 3.988 |
| **K** | 8.817 | 6.616 | 4.676 |
| **Mg** | 3.698 | 3.299 | 3.229 |

**Table 2: Fatty acids composition of samples (%)**

| Fatty acid | *L. flammea* | *E. radiata* | *V. contectus* |
|---|---|---|---|
| **Lauric acid (C12:0)** | 25.647 | **nd** | **nd** |
| **Myristic acid (C14:0)** | 15.078 | **nd** | 8.083 |
| **Palmitic acid (C16:0)** | 33.171 | 12.900 | 53.341 |
| **Margaric acid (C17:0)** | 10.645 | 18.126 | 6.464 |
| **Stearic acid (C18:0)** | 15.040 | 21.323 | 16.315 |
| **Oleic acid (18:1)** | 0.417 | 6.288 | **nd** |
| **Linoleic acid (18:2)** | **nd** | 4.658 | 2.445 |
| **Arachidic acid (C20:0)** | **nd** | 1.107 | 1.634 |
| **Arachidonic acid (20:4)** | **nd** | 15.595 | 11.690 |

nd-not detected

**Table 3: Essential amino acids composition of samples**

| Amino acids(mg/100g) | *E. radiata* | *V. contectus* | *L. flammea* |
|---|---|---|---|
| **Valine** | 10.412 | 8.484 | 4.484 |
| **Threonine** | 4.024 | 3.684 | 3.684 |
| **Isoleucine** | 11.099 | 9.570 | 9.564 |
| **Leucine** | 21.287 | 18.474 | 6.475 |
| **Lysine** | **nd** | **nd** | 9.720 |
| **Methionine** | 4.245 | 2.996 | 1.496 |
| **Phenylalanine** | 19.787 | 15.389 | 8.489 |
| **Histidine** | **nd** | **nd** | 11.639 |
| **Arginine** | 18.065 | 16.553 | 6.545 |
| **Tryptophan** | 3.583 | 1.115 | 1.415 |

nd- not detected

**Table 4: Non-essential amino acids composition of samples**

| Amino acids(mg/100g) | *E. radiata* | *V. contectus* | *L. flammea* |
|---|---|---|---|
| **Glycine** | 10.265 | 7.262 | 3.787 |
| **Alanine** | 5.788 | 4.679 | 4.680 |
| **Serine** | 10.264 | 9.878 | 5.672 |
| **Proline** | 2.854 | 3.027 | 3.201 |
| **Aspartate** | **nd** | **nd** | 9.394 |
| **Glutamic acid** | 20.852 | 19.389 | 13.392 |
| **Tyrosine** | 9.856 | 7.787 | 5.877 |
| **Cysteine** | 6.257 | 5.424 | 1.445 |

nd- not detected

Population growth has resulted in acute food shortage and micronutrient deficiency especially in sub-Saharan Africa. Women and children are usually most affected in terms of food shortage and nutrient deficiency. Investigation of available food sources to ascertain their nutritional qualities will improve the nutritional status of affected populations. The present studies investigated the fatty acid, mineral and amino acid concentration of species which form the basis of protein source for most coastline families.

A total of eight nutritionally essential metals are reported in all three samples analyzed in this study. The findings of this study on the concentration of Na, Ca and Mn in *E. radiata* corroborates the findings of Akpang and Oscar [7]. Ekpo *et al.,* [8] reported a range of 0.129-0.00 mg/Kg for Fe, Cu, and Zn; this is quite low compared to the results in the present study which indicate a much higher range. Nwabueze and Oghenevwairhe [9] reported a mean range of 2.708-25.678 mg/kg for the concentration of Mn, Cu and Fe in *E. radiata;* these values are much higher compared to the results of this present study. The concentration of minerals in order of increasing concentration was Cu>Mn>Na>Mg>Ca>Zn>K>Fe and Cu>Mn>Mg>Ca>Na>Zn>Fe>K for *V. contectus* and *L. flammea* respectively. The findings of this study on the concentration of Zn and Cu in *L. flammea* are similar to reports by Kadanga [10]. Fats are complex biomolecules which are essentials to life. Short chain fatty acids contribute to the health of the immune

system, medium-chain fatty acids have eight to twelve carbon atoms and are common in butterfat and the tropical oils while long-chain fatty acids usually contain from 14 to 18 carbon atoms and can be either saturated, monounsaturated or polyunsaturated [11]. Fatty acids serve several functions in the body such as shock absorbers, energy sources and reservoirs, maintenance of immune system, synthesis of biomolecules etc. Myristic acid (14:0) is a ubiquitous component of lipids in most living organisms and is found very specifically in certain proteolipids, where it is linked via an amide bond to an N-terminal glycine residue, and is essential to the function of the protein components; oleic acid is by far the most abundant monoenoic fatty acid in plant and animal tissue, both in structural lipids and in depot fats; oleic acid is the biosynthetic precursor of a family of fatty acid with the (n-9) terminal structure and with chain-lengths of 20-24 or more [11]. Lauric acid, myristic acid, Palmitic acid, Margaric acid, stearic acid, oleic acid, Linoleic acid, arachidic acid and arachidonic acid were detected in varying amounts in all samples. Palmitic acid concentration in *E. radiata* was the most abundant in all samples while oleic acid concentration in *L. flammea* was the least. The abundance of the fatty acids in the samples indicates that they are very good sources of fatty acids. Protein is an essential substrate for the sustenance of life and exists in the largest quantity of all the nutrients as a component of the human body [12]. The quality of a protein source is a function of the constituent amino acids (building blocks of proteins) [4]. A total of eighteen (18) amino acids were detected in all samples analyzed in this study: glycine, alanine, valine, leucine, isoleucine, serine, tryptophan, threonine, Methionine, phenylalanine, histidine, proline, aspartic acid, glutamic acid, tyrosine, arginine, lysine and Cysteine. This study corroborates the findings of Kadanga [10] on the amino composition of *L. flammea.* There is limited literature on the amino composition of *E. radiata* and *V. contectus.* Essential amino acids are very important in the diet for the maintenance of health.

## 4. CONCLUSION

The findings of this study indicate that the snail species are very good sources of minerals, fatty acids and amino acids. Consumption of these protein sources are therefore encouraged because they are affordable and readily available especially during the rainy season.

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