

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, Margoric 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

Food availability and affordability are very important factors that determine the nutritional status of a particular population. A balanced diet which can support optimal growth and development must contain the food nutrients in their appropriate quantities. Carbohydrates are the most abundant of all food classes in nature and constitute about 60% of the diet. The second most important food class is protein. Protein sources are usually more expensive than carbohydrates hence most indigent families may not be able to provide adequate protein to meet the nutritional requirement of the home. Beef, pork, poultry, mutton and fish are the most common protein sources in Nigeria and they are relatively expensive. Unconventional protein sources such as snails are increasingly becoming popular due to their nutritional benefits. In the Niger delta region of Nigeria, there are several species of snails (aquatic and terrestrial) which are easily accessible sources of proteins to the teeming populations along the coastline. Snail meat is commonly referred to as "Congo meat" and is consumed mostly by people in the oil-rich Niger Delta region [1]. Snail meat is considered to be very nutritive and rich especially in protein. Several species of snails especially (*Archatina* and *Archachatina*) are widely consumed as meat in Nigeria however there are some species which are less consumed such as *Limicolaria flammea* (snail) and *Viviparus contectus* (acute mudsnail). *L. flammea* and *V. contectus* are mostly consumed by the low income earners especially those residing in the coastline and rainforest areas and in some cases they actually form the largest single item of animal protein in the diet of the common people in rural areas. *Egeria radiata* (freshwater clam) is a very nutritious clam abundant mostly in the rainy season. *Periophthalmus koelreuteri* (mudskipper) is a unique species of fish found in the brackish water and intertidal zones of the marine environment of the Niger Delta. These fishes are able to "walk on land" with their specialized fins. Although research has shown that various species of mudskippers are rich in nutrients especially proteins and lipids, mudskippers are still despised by most persons due to its physical appearance and habitats. In some coastal communities, this fish is not hunted for food thus the protein value not properly harnessed. It is important however to state that despite their rich nutrient store, mudskippers are also able to absorb toxicants in their tissues and affect consumers and thus are often used as bio-indicators in environmental studies. One major challenge all over the world today is that of providing quality food that will be sufficient to cater for the fast growing population. Various reports indicate that the major challenge is the provision of protein in comparison to carbohydrates and other food classes. 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. In recent times, there has been an increase in the consumption of snails and other unconventional protein sources (including mudskippers) hence there is the need to investigate these food sources to determine their nutritional contents with the view to improving their level of consumption by this making information available to the public. This study aims to investigate the mineral, fatty acid and amino acids composition of these 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. A total of 4kg of each sample was collected for analyses. Sample collection was done between May and September during the peak periods of their availability. 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) at 80 °C for 72 hours. The samples were then ground to fine powder using an electric mill (masterchef).



Fig. 1: *Limicolaria flammea* (land snail) Fig. 2: *Egeria radiata* (freshwater clam) Fig. 3: *Viviparus contectus* (acute mudsnail)

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, filtered into a measuring cylinder and made up to 100ml using metal free distilled water, ready for mineral determination. 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 minerals determined were Mn, Cu, Zn, Ca, Fe, Na, K and Mg. The actual concentration of mineral in the sample were then recorded from the AAS readout.

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 °C 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- x 125-mm screw-cap Pyrex (Barcelona, Spain) tube, 15 ml of 6N hydrochloric acid was added, and the tube was thoroughly flushed with N₂, 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 10x 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 ($p > 0.05$) 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)	<i>E. radiata</i>	<i>V. contectus</i>	<i>L. flammea</i>
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	<i>L. flammea</i>	<i>E. radiata</i>	<i>V. contectus</i>
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)	<i>E. radiata</i>	<i>V. contectus</i>	<i>L. flammea</i>
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)	<i>E. radiata</i>	<i>V. contectus</i>	<i>L. flammea</i>
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 Oghenevwarhe [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.

REFERENCES

1. Bassey SCO, Eteng MU, Eyong EU, Ofem, OE, Akanyoung EO, Umoh IB. Comparative Nutritional, and Biochemical Evaluation of *Ergeria Radiata* (Clams) and *Pomecia Palludosa* (Gastropods) Delicacies and Effects of Processing Methods. Res J Agri Biol Sci. 2011; 7(1):98-104.
2. Benson T. Cross-sectoral coordination failure: How significant a constraint in national effort to tackle malnutrition in Africa? Food Nutr Bull. 2007;28(2): 323-330.
3. WHO/FAO. Protein and amino acid requirement in human nutrition. 2007. Accessed 14 March, 2019. Available: http://whqlibdoc.who.int/ytrs/who_TRS_935_erg.pdf.
4. Olusanya JO. Essentials of food and nutrition. 2nd ed. Lagos: Apex Books Limited;2008.

5. APHA. Direct Air- Acetylene Flame Method, Standard Methods for the examination of metals, 20th ed. USA: Baltimore, Maryland;1998.
6. Horwitz W (ed.) Official Methods of Analysis of AOAC. 18th ed. USA: Washington DC.2006.
7. Akpang EI, Oscar VE. Proximate composition and mineral contents of edible part of four species of shellfishes from the Calabar River, Nigeria. *Ann Res Rev Biol.* 2018.26(1):1-10
8. Ekpo EA, George UU, Edet M. Impacts of Water Pollution with Heavy Metal on the tissue of *Egeria radiata* (Bivalvia: Tellinacea:Donacidae) (Lammark, 1804) obtained from Calabar River, Cross River State, Nigeria. *Nat. Sci.*2015;13(8):36-39
9. Nwabueze AA, Oghenevwairhe E. Heavy metal concentrations in the West African clam, *Egeria radiata* (Lammark, 1804) from Mclver market, Warri, Nigeria. *Int. J. Sci Nat.* 2012;3(2):309-315
10. Kadanga B. Determination of some heavy metals and investigation of nutritional composition in three species of snails (*Archatina fulica*, *Archachatina marginata* and *Limicolaria flammea*). M.Sc dissertation submitted to the department of chemistry Ahmadu Bello University, Zaria, Nigeria. 2015.
11. Adeyeye EI. Lipid Composition of three different types of land snails consumed in Nigeria. *Global J. Sci. Frontier Res. Chem*, 2012;12(7):8-22.
12. Okuzumi M, Fujii T. Nutritional and functional properties of squid and cuttle fish. *35th Anniversary Commemorative Publication*, 2000;223.