ISSN: 2635 - 2826

Journal of Clinical & Biomedical Research

SCIENTIFIC Research and Community

Research Article

Open Access

Relative Concentrations of Amino Acids in the Exoskeleton of Male and Female *Neopetrolisthes Maculatus*

Adeyeye EI [1]*, Idowu OT [2], Akinsola AF [3], Olatunya AM [1], Popoola OK [1], Ayodele O [3] and Gbolagade AY [4]

[1]Department of Chemistry (Analytical Chemistry Unit), Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria

[2]Department of Chemistry, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

[3]Department of Industrial Chemistry, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria

[4]Central Laboratory, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria

ABSTRACT

Amino acid levels in the exoskeleton of the heterosexual samples of *Neopetrolisthes maculatus* were evaluated. Twenty parameters were reported on; 13 of these values were greater in the female than the male (i.e. 13/20 or 65.0%). Values in female > male were observed in Val, Thr, Lys, Met, Phe, Trp, Gly, Ala, Asp, Arg, Cys, total amino acids and protein. The variations between the male and female amino acid values were low having coefficient of variation percent range of 0.0579-16.9. The amino acid levels were significantly different between the heterosexuals at $r=_{0.01}$. Limiting amino acids on the various scoring standards were egg (Cys=0.3172 and 0.3511), provisional essential amino acid scoring pattern (Lys = 0.4145 and 0.4746), preschool child requirements (Lys =0.3930 and 0.4501) and all the three scores were significantly different at $r=_{0.01}$. The $EAAI_1$ (compared to soybean) were 1.18 (male) and 1.16 (female), $EAAI_2$ (compared to egg) were 88.5 (male) and 88.3 (female); their corresponding BV were 84.7 and 84.5. P-PER1 range was 1.86 (male) - 1.24 (female) and $P\text{-}PER_2$ range was 2.02 (male) - 1.41 (female). Total EAA was 37.7g/100g protein (male) and 37.0g/100g protein (female). In the estimates of amino acid requirements of school boys at ages 10-12years (mg/kg/day), male exoskeleton would provide more than the requirement in Phe + Tyr (186%), Trp (25.8%) and Val (48.1%) whereas in female the following were higher than the standards: Phe + Tyr (218%), Thr (8.86%), Trp (35.0%), Val (61.6%) and TEAAs (4.01%).

***Corresponding author**

Adeyeye EI, Department of Chemistry (Analytical Chemistry Unit), Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria. Phone: +2348035782925

E-mail: adeyeyeilesanmi2012@gmail.com

Received: July 01, 2021; **Accepted:** July 26, 2021; **Published:** July 31, 2021

Keywords: *Neopetrolisthes Maculatus*, Heterosexual Samples, Amino Acids.

Amino acids encountered in this work: Lysine (Lys) [PubChem C6H14N202, CID: 5962]; Glutamic acid (Glu) [PubChem C5H9NO4, CID: 33032]; Methionine (Met) [PubChem C5H11NO2S, CID: 6137]; Alanine (Ala) [PubChem C3H7NO2, CID: 5950]; Arginine (Arg) [PubChem C6H14N4O2, CID: 6322]; Valine (Val) [PubChem C5H11NO2, CID: 6287]; Leucine (Leu) [PubChem C6H13N02, CID: 6106]; Aspartic acid (Asp) [PubChem C4H7NO4, CID: 5960]; Threonine (Thr) [PubChem C4H9N03, CID 6288]; Tryptophan (Trp) [PubChem C11H12N2O2, CID: 6305]; Isoleucine (Ile) [PubChem C6H13NO2, CID: 791]; Phenylalanine (Phe) [PubChem C9H11NO2, CID: 6925665]; Histidine (His) [PubChem C6H9N3O2, CID: 6274]; Tyrosine (Tyr) [PubChem C9H11NO3, CID: 6057]; Cystine (Cys) [PubChem C6H12N2O4S2, CID: 67678]; Serine (Ser) [PubChem C3H7NO3, CID: 5951]; Glycine (Gly) [PubChem C2H5NO2, CID: 750]; Proline (Pro) [PubChem C5H9NO2, CID: 145742].

Pubchem CID

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Centre for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institute of Health (NIH) (PubChem and the American Chemical Society) [1]. Hence we can talk of PubChem Compound ID (CID).

Introduction

One of the greatest problems facing the developing countries is that of providing food of adequate quality to its increasing populations. This is because the malnutrition situation is more intense with low level of protein and mineral intake, calling for urge and urgent need to find a way of raising the protein and mineral intake of the average citizens from 5.5g/head/day as recommended by FAO to 35g/head/day as suggested by Indufueke, more than 41% of the total animal protein is obtained from fishery products in Nigeria, this is because fishery products are relatively cheaper than meat and the total fish consumption rate has risen to 2.66 million metric tonnes annually [2-5].

Crabs are part of the basic components of the ecosystem and they are consumed as food in many countries Over 100 species of crabs are known worldwide with nine species common to West African countries especially Nigeria. Crabs are known to be mostly

occurring at the mouth of estuaries and along the course of many main rivers. Crabs constitute one of the most important members of estuarine food chain.

Porcellanide family is a group of crab-shaped anomuran crustaceans that belong to the superfamily Galatheoidea together with three other families Galatheoidae, Munididae and Munidopsidae. They are commonly found in rocky and coral reefs of temperate and tropical coasts [6-9].

The World Register of Marine Species (WoRMS) has given the taxonomic details of *Neopetrolisthes maculatus* [10]. Classsification: Biota > Animalia (Kingdom) > Arthropoda (Phylum) > Crustacea (Subphylum) > Multicrustacea (Superclass) > Malacostraca (Class) > Eumalacostraca (Subclass) > Eucarida (Superorder) > Decapoda (Order) > Pleocyemata (Suborder) > Anomura (Infraorder) > Galatheoidea (Superfamily) > Porcellanidae (Family) > *Neopetrolisthes* (Genus) > *Neopetrolisthes maculatus* (Specie).

Patent: *Neopetrolisthes* Miyake, 1937
Original name: *Porcellana maculata* H. Milne Edwards, 1837
Synonymized names Neopetrolisthes *oshimai* Miyake, 1937 (Synonym) *Petrolisthes ohsimai* (Miyake, 1937) (junior synonym) *Porcellana maculata* H. Milne Edwards, 1837

In distribution, porcellanide are widely distributed in the Indo-West Pacific. They are found from east coast of Africa to Christmas Island and Western Australia, Bismarck Archipelago, Queensland, Moluccas, Palau, Taiwan, Southern Japan (Ryukyu Islands), New Caledonia, Marshall and Fiji Islands. They live in shallow subtidal water; coral and rocky reefs, being associated with large sea anemones (*Cryptodendrum, Entacmaea, Gyrostoma, Heteractis* and *Stichodactyla*), typically found in a heterosexual pair [11-13].

Neopetrolisthes maculatus is a spotted crab. There are two different colour forms, although the ground colour of bodies of both forms is white. In one form, carapace and chelipeds are white, with an uneven pattern of irregular sizes of red blotches; ambulatory legs also white, with some small red spots on meri of first pair (second pereopod). In other form, the carapace and chelipeds have a uniform pattern of numerous small, reddish purple spots; meri of ambulatory legs also with numerous small, reddish purple spots [14].

Crab is consumed by many individuals as it is often recommended for pregnant women. Utilization of both fresh water and marine resources for human consumption has increased tremendously worldwide. Aquatic food products, including crustacean shellfish, have been landed for their health promoting characteristics. Shellfish are known to be nutritionally valuable source of various mineral and high quality proteins [15, 16, 17].

The nutritional status and chemical composition of different species of crabs had been reported extensively in various parts of the world [6, 15,18-28]. This work is part of the ongoing report on the nutritional qualities of *N. maculatus*; this being on the amino acid composition of the exoskeleton of the heterosexual pairs of *N. maculatus*. It is hoped that this will further contribute to the information on Food Composition Tables. The colour pattern of the samples is of large and uneven blotches that resemble the Pacific Ocean *Neopetrolisthes maculatus* population [29].

Materials and Method

Collection of Samples

Samples were collected from trawler catches from the Atlantic Ocean at Orimedu beach in Ibeju-Lekki area of Lagos State, Nigeria. The experiment took place between July and August, 2017. The wet crabs were separated fresh and were washed with distilled water to remove adhering contaminant and transported in ice crushed containers to the laboratory for authentication and preservation prior to the analyses. The crabs were authenticated in the Department of Forestry, Wildlife and Fisheries Management of Ekiti State University, Ado-Ekiti, wrapped in aluminium foil and stored in a cool chamber at about 37°F (2.8°C) for about three days prior to analyses.

Sample Treatment

Number of matured *N. maculatus* crabs caught was 16 but 13 samples were used for this study. The 13 whole crabs separated; there were six males and seven females. Typically a crab is killed by boiling it alive; however in an attempt to give the crab a quick death, it was preserved under cold temperature. Whilst the internal organs were discarded, the other separated parts were dried in the oven at 1050C. For the purposes of analyses; the separated parts were the carapace and cheliped exoskeleton (to constitute the exoskeleton) and the muscle from the thoracic sterna and cheliped (to constitute the endoskeleton). The exoskeleton (used in this work) from each sex was separately blended.

Extraction and Analyses

Extraction and the instrumentation analysis were carried out by following AOAC method and Danka et al. [30,31].

The dried pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. The sample of 10.0g was weighed into the 250ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30ml of petroleum spirit three times with Soxhlet extractor that was equipped with thimble. The sample was hydrolyzed three times for complete hydrolysis to be achieved for the totality of amino acids recovery.

The pulverized and defatted sample was soaked with 30ml of 1M potassium hydroxide solution and was incubated for 48 hours at 110°C in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to get pH in the range of 2.5-5.0. The solution was purified by cation-exchange solid-phase extraction. The amino acids in purified solutions were derivatised with ethylchloroformate by the established mechanism:

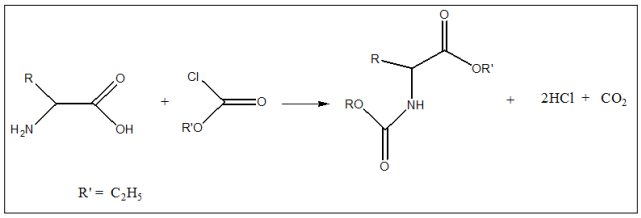

Figure 1: Derivatization process of amino acid

The derivatising reagent was removed by scavenging with nitrogen. The derivatized amino acid was made up to 1ml in a vial for gas chromatography analysis. The gas chromatographic conditions for the amino acids analysis were as follows: GC: HP6890 powered with HP ChemStation rev. A09.01 [1206] software; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0ml/min; inlet temperature: 250°C; column type: EZ; column dimensions: 10m x 0.2mm x 0.25µm; oven programme: initial @ 110°C, first ramp @ 27°C/min to 320°C ; second constant for 5mins at 3200C; detector: PFPD; detector temperature: 320°C; hydrogen pressure: 20psi; compressed air: 35 psi.

Determination of Amino Acid Quality Parameters

(i) Estimation of isoelectric point (*pI*): The estimation of isoelectric point (*pI*) for a mixture of amino acids was carried out using the following equation [32].

$$IPm = \sum_{i=1}^{n} I_i P_i X_i \quad \text{---------- (1)}$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mass or mole fraction of the i^{th} amino acid in the mixture.

(ii) Estimation of predicted protein efficiency ratio (P-PER): Computation of protein efficiency ratio (C-PER or P-PER) was carried out using the equations [33].

$P\text{-}PER_1 = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr)$ ---------- (2)
$P\text{-}PER_2 = -0.684 + 0.456\ (Leu) - 0.047\ (Pro)$ ---------- (3)

(iii) Leucine/isoleucine ratio: The leucine/isoleucine ratios, their differences and their percentage differences were calculated.

(iv) Determination of essential amino acid index ($EAAI_2$): The method of $EAAI_1$ calculation was due to Oser using the egg protein amino acids as the standard [34].

(v) Estimation of essential amino acid index ($EAAI_1$): The essential amino acid index was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus histidine in the equation 4 [35].

$$\text{Essential amino acid index} \quad \sqrt[9]{\frac{\text{mg Lysine in 1g test protein}}{\text{mg Lysine in 1g reference protein}}} \quad x \quad \text{etc. for all 8 essential amino acids + His} \quad \text{---- (4)}$$

(vi) Calculation of biological value (BV): Computation of biological value (BV) was calculated following the equation of Oser [34].

$$\text{Biological value} = 1.09\ (EAAI_1) - 11.73 \quad \text{---------- (5)}$$

(vii) Computation of Lys/Trp and Met/Trp: The ratios of Lys/Trp (L/T) and Met/Trp (M/T) were computed.

(viii) Computation of amino acid scores: The amino acid scores were computed using three different procedures:

- Scores based on amino acid values compared with whole Hen's egg amino acid profile [36].
- Scores based on essential amino acid scoring pattern [37].
- Scores based on essential amino acid suggested pattern of requirements for pre-school children [38].

(ix) Estimates of amino acid requirements at different ages (mg/kg/day): These estimates were based on the essential amino requirements in mg/kg/day of 30kg body weight of 10-12 years school boys The proposed formula for this calculation could be any of these two [37].

- Essential amino acid x 1000/100 x protein(g/100g) ----- (6)
-Essential amino acid x 10 x appropriate corresponding protein ----- (7)

(x) Other calculations: Other determinations such as total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total essential aliphatic amino acid (TEAlAA), e.t.c. and their percentages were made. Total sulphur amino acid (TSAA), percentage of cystine in TSAA (% Cys in TSAA) were also calculated. The various amino acid groups into classes I-VII were also calculated [39].

Statistical Evaluation

Data results in this work were subjected to both descriptive and inferential statistics. Results subjected to both types of statistics were the raw data of the amino acid profiles and the various amino acid scores.

Results and Discussion

The concentration of amino acids of *Neopetrolisthes maculatus* heterosexual (dry weight) exoskeleton in g/100g protein is depicted in Table 1. Among the amino acids (AA) investigated, glutamic acid (Glu) was the most concentrated with range values of 11.6-11.5g/100g protein (male/female) with percentage difference of +1.03 and closely followed by aspartic acid (Asp) with respective values of 10.3-11.5g/100g protein and percentage difference of -11.0%. Both AA are acidic amino acids (AAA). Whereas the Glu in these samples were lower than those in the heterosexual flesh of *N. maculatus* (17.7-17.8g/100g protein), the values of Asp in the flesh were lower than the present values, the values being 10.0-9.90g/100g protein [25]. The observation made in the present report for Glu and Asp also corroborated with the observation in flesh of female West African fresh water crab (*Sudananautes africanus africanus*) with Glu (130.2 mg/g protein) > Asp (72.5mg/g protein) [40]. The most (third) concentrated essential amino acid (EAA) was valine (Val) having values of 7.72-7.84g/100g protein (male/female) with percentage difference of -1.62%. Other high concentrated EAA (g/100g protein) were: Thr (5.41-5.60, % difference of -3.59), Leu (6.59-5.23, % difference of + 20.7), His (4.43-4.01, % difference of +9.68) and Phe (5.84-6.32, % difference of -8.28), whereas non-essential amino acids (NEAA) of high concentrations were: Gly (5.81-5.82, % difference of -0.1810; lowest difference value), Ala (3.62 – 3.94, % difference of -9.09), Ser (3.79-3.09, % difference of +18.4), Pro (6.50-6.09, % difference of +6.29), Arg (4.10 - 5.22, % difference of -27.2) and Tyr (6.36-6.29, % difference of +1.11). The least concentrated AA in the two samples was Cys (5.71e-1 to 6.32e-1, % difference of -10.7). Both the total AA (90.4-91.0, % difference of -0.7216) and protein (19.0-20.4, % difference of -7.37) were close in both samples respectively. The Leu values in these samples had close concentrated values when compared with Leu in *S. africanus africanus* (66.0mg/g protein) [40].

The general concentration differences in the AA profiles of the two samples can also be seen in Table 1 in columns 5 and 6 (for differences and percentage differences respectively). Twenty parameters were evaluated for in Table 1. Out of these 20 parameters, 13 parameters or 13/20 (65.0%) were positive towards the female exoskeleton or to say that 65.0% of the parameters were more concentrated in the female than the male. It also showed that seven parameters or 7/20 (35.0%) were more positive towards the male exoskeleton. On the other hand, it was 60% (positive for female) and 40% positive for male in *N. maculatus* heterosexual flesh in the *N. maculatus* heterosexual innards, it was 10/19 (52.6%) positive towards the female AA concentration and 47.4% (9/19) positive towards the male [25,26].

Table 1: Amino acid profiles (g/100g protein) of the male and female exoskeleton of *Neopetrolisthes maculatus*

Amino acid	CID	Male exoskeleton	Female exoskeleton	Difference	% difference
Val	6287	7.72	7.84	-0.1247	-1.62
Thr	6288	5.41	5.60	-0.1943	-3.59
Ile	791	2.62	2.49	+0.1233	+4.71
Leu	6106	6.59	5.23	+1.36	+20.7
Lys	5962	2.28	2.61	-0.3309	-14.5
His	6274	4.43	4.01	+0.4294	+9.68
Met	6137	2.03	2.11	-0.0783	-3.86
Phe	6925665	5.84	6.32	-0.4835	-8.28
Trp	6305	7.93e-1	7.95e-1	-0.0020	-0.2520
Gly	750	5.81	5.82	-0.0105	-0.1810
Ala	5950	3.62	3.94	-0.3287	-9.09
Ser	5951	3.79	3.09	+0.6957	+18.4
Pro	145742	6.50	6.09	+0.4084	+6.29
Asp	5960	10.3	11.5	-1.13	-11.0
Glu	33032	11.6	11.5	+0.1191	+1.03
Arg	6322	4.10	5.22	-1.11	-27.2
Tyr	6057	6.36	6.29	+0.0704	+1.11
Cys	67678	5.71e-1	6.32e-1	-0.0612	-10.7
Total		90.4	91.0	-0.6521	-0.7216
Protein		19.0	20.4	-1.40	-7.37

In Table 2, we have the descriptive statistics of the data from Table 1. The standard deviation (SD) and the coefficient of variation (CV%) were both low. The CV% ranged between 0.0579-16.9. This showed that the parameter values were close and generally homogenous. In the flesh heterosexual *N. maculatus* the parameter values were less homogenous than the exoskeleton as the flesh CV% values ranged from 0.124-32.0 and observation in the innards as in the flesh: CV% of 0.295-30.1. The inferential statistics of data in Table 1 can be found in Table 3. The mean, SD and CV% were generally close in values between male and female samples. Also, the correlation coefficient (r_{xy}) was significantly high at 0.9820; this was followed by high level (0.9648) of coefficient of determination (r_{xy}^2). The regression coefficient (Rxy) showed that for every 1.00g/100g protein rise in the overall value of male exoskeleton AA, there was a corresponding increase of 1.01g/100g protein in the female concentration of AA; this further attested to the observation showing female AA > male AA. The coefficient of alienation or no relationship (CA) was low at 0.1877 (18.77%) whilst the index of forecasting efficiency (IFE) was high at 0.8123 (81.23%). Whereas the CA is an opposite of IFE but CA + IFE = 1.00 or 100%. Whilst the IFE is a reduction in the error of prediction of the relationship between two entities, the CA represents the value of the error of prediction between two relationships. When CA is high, the IFE is low and prediction of relationship is difficult, but if IFE is high or > CA then prediction of relationship is easy. In the samples under discussion their IFE value was high (0.8123 or 81.23%) and the error was just 18.77% (the CA value). Since IFE0.8123 >> CA0.1877, this means that male exoskeleton sample can conveniently carry out the biochemical functions of female exoskeleton and vice versa.

Table 2: Descriptive statistics of the data from Table 1 pertaining to amino acid profile of male and female exoskeleton of *N. maculatus*

Amino acid	CID	Mean	Standard deviation	Coefficient of variation (%)
Val	6287	7.78	0.0882	1.13
Thr	6288	5.50	0.1374	2.50
Ile	791	2.55	0.0872	3.41
Leu	6106	5.91	0.9648	16.3
Lys	5962	2.45	0.2334	9.57
His	6274	4.22	0.3036	7.20
Met	6137	2.07	0.0554	2.68
Phe	6925665	6.08	0.3419	5.62
Trp	6305	0.7936	0.0014	0.1779
Gly	750	5.83	0.0034	0.0579
Ala	5950	3.78	0.2324	6.15

Ser	5951	3.44	0.4919	14.3
Pro	145742	6.29	0.2888	4.59
Asp	5960	10.9	0.8021	7.35
Glu	33032	11.5	0.0842	0.7303
Arg	6322	4.66	0.7881	16.9
Tyr	6057	6.32	0.0498	0.7876
Cys	67678	0.6015	0.0432	7.19
Total		90.7	0.4611	0.5084
Protein		19.7	0.9899	5.03

Table 3: Statistical analysis of the data from Table 1 concerning the amino acid profile of male and female exoskeleton of *N. maculatus*

Statistics	Male exoskeleton		Female exoskeleton
Total amino acid value	90.4		91.0
Mean	5.02		5.06
Standard deviation	2.98		3.07
Coefficient of variation (%)	59.4		60.7
Correlation coefficient (r_{xy})		0.9820	
Variance (r_{xy}^2)		0.9648	
Regression coefficient (Rxy)		1.01	
Coefficient of alienation (C_A)		0.1877	
Index of forecasting efficiency (IFE)		0.8123	
Remark		*	

* = results significantly different at n-2 and $r=_{0.01}$ (critical value = 0.590). [NOTE: n-2 = 18-2 = 16 (df)]

The various AA have different types of functions in the human body. Phenylalanine, a precursor for neurotransmitters which helps in the production of other amino acids and their functioning. Valine helps in stimulating muscle growth, regeneration and it produces energy. Threonine is a principal component of structural proteins such as collagen and elastin which are present in skin and connective tissues, helps in fat metabolism and immune function. Tryptophan is a precursor to serotonin, a neurotransmitter that helps in appetite, sleep and mood regulation. Methionine plays a major role in metabolism, detoxification, helps in tissue growth and in the absorption of minerals such as zinc and selenium needed by the body. Leucine helps in regulating blood sugar levels, enhances wound healing and stimulates growth hormones. Isoleucine helps in muscle metabolism, immune function, haemoglobin production and energy regulation. Branched-chain AA are Val, Leu and Ile. Lysine helps in protein synthesis, calcium absorption, immune function, energy production, hormone production and in collagen production. Histidine, a neurotransmitter helps in maintaining the protective barrier called myelin sheath that surrounds the nerve cells, helps in digestion, immune response, sleep-wake cycles and sexual functions [41].

In Table 4 were reported the summary of parameters of essential, non-essential, acidic, neutral, sulphur, aromatic, etc. AA contents (g/100g protein) of the samples. The total AA (TAA) of 90.4-91.0g/100g protein (male/female exoskeleton) were lower than flesh AA at 96.6-97.1g/100g protein, lower than the innards of heterosexual *N. maculatus* at 95.4 (male)-96.5 (female) but higher than the value of 777.0mg/g protein in the *S. africanus africanus* female flesh. Columns in Table 4 included AA, members of AA, Class of AA in male and female exoskeleton and other quality parameters. Total non-essential amino acid (TNEAA) was 52.7-54.0 with corresponding percentage values of 58.3-59.4. Total essential amino (TEAA) was 37.7-37.0g/100g protein (with His) and percentage value of 41.7 – 40.6 whereas values of TEAA without His were 33.3 - 33.0 and the corresponding percentage values were 36.8 - 36.2 [25,26,40].

Table 4: Various quality parameters as they concern concentrations of essential, aromatic, non-essential, neutral, etc. amino acid (g/100g protein) of the heterosexual exoskeleton of *N. maculatus*

Amino acid	Members	Class	Male exoskeleton	Female exoskeleton
Total amino acid (TAA)	Gly, Ala, Ser, Pro, Val, Thr, Ile, Leu, Asp, Met, Glu, Lys, Phe, His, Arg, Tyr, Trp, Cys		90.4	91.0
Total non-essential amino acid (TNEAA)	Gly, Ala, Ser, Pro, Asp, Glu, Arg, Tyr, Cys		52.7	54.0
% TNEAA			58.3	59.4
Total essential amino acid (TEAA)	Val, Thr, Ile, Leu, Lys, His, Met, Phe, Trp			
-with His			37.7	37.0
-without His			33.3	33.0
% TEAA				
-with His			41.7	40.6
-without His			36.8	36.2
Total aliphatic amino acid (TAIAA)	Gly, Ala, Val, Leu, Ile	I [with aliphatic side chains (hydrogen and carbon)]	26.4	25.3
% TAIAA			29.2	27.8
Total essential aliphatic amino acid	Val, Leu, Ile		16.9	15.6
% TEAIAA			18.7	17.1
Total aromatic amino acid (TArAA)	His, Phe, Tyr, Trp	VI [containing aromatic rings]	17.4	17.4
% TArAA			19.3	19.1
Total essential aromatic amino acid (TEArAA)	His, Phe, Trp		11.1	11.1
% TEArAA			12.2	12.2
Total acidic amino acid (TAAA)	Glu, Asp	IV [with side chains containing acidic groups or their amides]	21.9	22.9
% TAAA			24.3	25.2
Total basic amino acid (TBAA)	Arg, Lys, His	V [with side chains containing basic groups]	10.8	11.8
% TBAA			12.0	13.0
Total neutral amino acid (TNAA)	Gly, Ala, Val, Leu, Tyr, Ser, Phe, Cys, Thr, Met, Pro		51.0	55.5
%TNAA			56.4	60.9
Total hydroxylic amino acid (THAA)	Ser, Thr	II [with side chains containing hydroxylic (OH) groups]	9.19	8.69
% THAA			10.2	9.55
Cyclic amino acid (CAA)	Pro	VII [amino acid]	6.50	6.09
% CAA			7.19	6.69

Cystine + methionine (TSAA)	Cys, Met	III [with side chains containing sulphur atoms]	2.60	2.74
% TSAA			2.88	3.01
% Cys in TSAA			22.0	23.1
Leu/Ile ratio			2.52	2.10
Leu-Ile (difference)			3.98	2.73
%/Leu-Ile/Leu			4.40	3.00
%/Leu-Ile/TAA			60.3	52.3
P-PER1			1.86	1.24
P-PER2			2.02	1.41
Isoelectric point (pI)			5.11	5.17
Essential amino acid index ($EAAI_1$)			1.18	1.16
Essential amino acid index ($EAAI_2$)			88.5	88.3
Biological value (BV)			84.7	84.5
Crude protein			19.0	20.4
Lys/Trp			2.88	3.29
Met/Trp			2.56	2.65
Phe/Tyr			0.9178	1.00

P-PER = predicted protein efficiency ratio

The predicted protein efficiency ratio ($P\text{-}PER_1$) was 1.86-1.24 and ($P\text{-}PER_2$) was 2.02-1.41. The *in vivo* P-PER is of the order of 2.2 [43]. Both P-PERs were lower than the report for the *N. maculatus* flesh where values of P-PER1 were 3.39-3.69 and $P\text{-}PER_2$ were 3.82-4.14 and also lower than the report for the innards of N. maculatus heterosexuals with values of $P\text{-}PER_1$ (2.83-3.01) and $P\text{-}PER_2$ (2.89-2.96) [25,26]. Each of our present P-PER value was lower than 2.2. According to Friedman's classification, the PER is poor (< 1.5), moderate (1.5-2) and superior (>2) [44]. On this classification, $P\text{-}PER_1$ and $P\text{-}PER_2$ were within the group of moderate in male sample but poor in the female sample. Further literature information had the following P-PER values: in the flesh of female *S. africanus africanus*, P-PER was 3.1 [40]. In *Callinectes latimanus* (a lagoon crab), $P\text{-}PER_1$ was 1.21 and $P\text{-}PER_2$ was 1.39 [45]. The present results showed male *N. maculatus* might be more physiologically utilized protein source than the female sample. In general, it has been found that the better the protein, the lower the level in the diet required producing the highest protein efficiency ratio. This emphasizes a clear reflection of the importance of the proper nutritive balance of all the amino acids to produce optimum metabolic efficiency. In the data in Table 4, Leu/Ile ratio range was 2.52 - 2.10, Leu-Ile (difference) was 3.98-2.73, %/Leu - Ile/Leu ranged from 60.3 - 52.3. In the flesh of *N. maculatus* Leu/Ile ratio had values of 1.60 - 1.63 in the flesh of *S. africanus africanus*, the ratio was 1.60 and the innards of *N. maculatus* heterosexual the ratios were 1.54 - 1.91 with the difference levels of 2.87-3.97g/100g protein and % (Leu - Ile)/Leu values of 35.1 - 47.7 [25, 26, 40]. From literature, the most ideal Leu/Ile is 2.36 [46]. The values of 2.52 - 2.10 were close to 2.36, hence, we might not experience concentration antagonism in the samples when consumed as protein source in food. It has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra [47]. A high Leu imbalance in the diet impairs the metabolism of Trp and niacin, and is responsible for the niacin deficiency in sorghum eaters [48]. Experiments in dogs have shown that animals fed sorghum proteins with less than 11g/100g protein Leu did not suffer from nicotinic acid deficiency [49]. The present Leu values of 6.59-5.23g/100g protein were less than 11g/100g protein and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas [50].

The total sulphur amino acid (TSAA) in the samples were made of Met + Cys. Whilst the total TSAA was 2.60-2.74g/100g protein, the percentage Cys/TSAA values were 22.0-23.1 lower to both innards (26.7-33.0) and meat of *N. maculatus* at 31.9-33.1. The present Cys/TSAA values were very low compared with other literature values of animal protein amino acids: 27.3-32.8% in S. africanus africanus; 36.3% in Macrotermes bellicosus; 25.6% in *Zonocerus variegatus* 35.5% in *Archachatina marginata marginata*; 38.8% in A. archatina and 21.0% in *Limicolaria* sp. (the last three are land snails consumed in Nigeria) [40,51,52,53]. The percentage of Cys in TSAA in the diet of rat, chick and pig is 50% but the standard value is unknown in man [46,38]. It should be noted that all the above literature results came from animal sources. It is however interesting to note that vegetable protein (e.g. coconut endosperm) has a percentage Cys/TSAA of 62.8% High percentage of Cys/TSAA had also been reported in *Anacardium occidentale* with a value of 50.51%. From all these literature values, it is obvious that the *N. maculatus* heterosexuals exoskeleton behaved like typical animal in their %Cys/TSAA ratios. The presence of cystine and cysteine in the diet would reduce the needs for Met and since all the sulphur in the diet is derived from these three amino acids the sulphur content is sometimes used as an approximate assessment of the adequacy of protein. In the present results the values range for Met and Cys were 2.03 – 2.11g/100g protein and 5.71e-1 to 6.32e-1g/100g protein respectively [54,55,56].

The essential amino acid index (EAAI) calculated were reported in two different forms of EAAI1 and $EAAI_2$. In the $EAAI_1$, the values were 1.18-1.16. The EAAI under this mode has soybean as its standard for comparison. The value of EAAI in defatted soybean flour is 1.26 but lower than 1.55 in whole hen's egg. In the amino acid composition of two fancy meats (liver and heart) of African

giant pouch rat (*Cricetomys gambianus*), the EAAI ranged from 1.20-1.31 [57, 58]. It should be noted that the absence of Trp in EAAI calculation of this mode may bear no significance in the EAAI; for example EAAI without Trp in soy flour remained 1.26 whilst it reduced to 1.54 in the whole hen's egg, i.e., a reduction of 0.01 or 0.645%. For the $EAAI_2$, values were 88.5-88.3 with their corresponding biological values (BV) of 84.7-84.5 depicting the quality of the protein of *N. maculatus* heterosexuals exoskeleton. In comparison, some literature values of EAAI and BV are as follows [34]. milk, cow (whole, nonfat, evaporated or dry), EAAI (88) and BV (84, predicted; 90, observed); human, EAAI (87) and BV (83); eggs, chicken (whole, raw or dried), EAAI (100), BV (97, predicted; 96, observed); whites (raw or dried), EAAI (95), BV (92, predicted; 93, observed); yolks (raw or dried), EAAI (93), BV (89, predicted); shellfish (shrimp, including prawns, raw or canned), EAAI (67), BV (61, predicted); also 86.9-89.9 (EAAI) and 83.0-86.3 (BV) in flesh of *N. maculatus* and 88.7-89.2 (EAAI) and 85.0-85.5 (BV) in innards of *N. maculatus* [25,26]. These literature results show the quality position of *N. maculatus* exoskeleton under discussion. EAAI is useful as a rapid tool in the evaluation of food formulation for protein quality. The isoelectric point, *pI*, was 5.11-5.17 showing the samples to be in the acidic medium of the pH range. The *pI* calculation from amino acids would assist in the quick production of certain protein isolate of organic product without evaluating the protein solubility to arrive at the *pI*.

In Table 4 are results for Lys/Trp (L/T) and Met/Trp (M/T) in the two heterosexual exoskeleton *N. maculatus* samples. In infant's protein requirements, a growth pattern of amino acid requirements was obtained by assigning value of unity to the Trp need [59]. Similar calculation of the amino acid content of mammalian tissue showed that there exists good agreement of growth needs and tissue amino acid patterns. This agreement is good for the L/T and M/T ratios of muscle proteins which constitute approximately 75% of the infant body proteins. The present results had L/T values of 2.88-3.29 and M/T of 2.56-2.65. The L/T values were less than those of innards as 3.00-5.01 and flesh as 3.31-4.27 but higher than their M/T values as: innards, 1.78-3.50 and flesh, 1.97-2.64.

Mammalian tissue patterns have the following values: L/T: muscle (6.3), viscera (5.3), plasma proteins (6.2). M/T: muscle (2.5), viscera (2.0), plasma proteins (1.1) [60]. The available evidence indicates that the utilization of dietary proteins increases as their Lys and Trp content approaches that of muscle tissues. In the present study, the male L/T value of 2.88 was less a half of virtually the standard L/T values of 6.3 (muscle), 5.3 (viscera) and 6.2 (plasma) proteins; however, L/T for the female (3.29) was above 50% of the various standard values. On the other hand, the M/T values of the present samples were all higher than the standard values of M/T of 2.5 (muscle), 2.0 (viscera), 1.1 (plasma) proteins which were all lower than the M/T values of 2.56-2.65 in the present results. The patterns of observations in the present results followed the trend as observed in the meat and innards of heterosexual N. maculatus for the L/T and M/T values. The mean minimum Phe requirement estimate in the presence of an excess of Tyr is 9.1 mg/kg/day. Hence Tyr can spare 78% of the dietary Phe need. Also the optimal proportions of dietary Phe and Tyr have been shown to be 60:40, respectively [61]. The Phe/Tyr in these results were low as seen in Table 4 and did not meet the optimal proportion of dietary Phe and Tyr of 60:40 respectively.

The amino acid groupings into classes I-VII are also depicted in Table 4 [39]. The concentration trend of the classes could be seen shown in g/100g protein: class I (26.4-25.3) > class IV (21.9-22.9) > class VI (17.4-17.4) > class V (10.8-11.8) > class II (9.19-8.69) > class VII (6.50-6.09) > class III (2.60-2.74). This trend was different in the flesh and innards of *N. maculatus* particularly classes V, VI, III and VII. A close observation would show that the percentages were close to their individual values with marginal differences; examples run thus: value (percentage): class I, 26.4-25.3 (29.2-27.8); class II, 9.19-8.69 (10.2-9.55); class III, 2.60-2.74 (2.88-3.01); class IV, 21.9-22.9 (24.3-25.2); class V, 10.8-11.8 (12.0-13.0); class VI, 17.4-17.4 (19.3-19.1) and class VII, 6.50-6.09 (7.19-6.69).

In Table 5, we presented the amino acid profile scores based on whole hen's egg amino acid profile. The following male amino acid scores showed that the male sample was more concentrated in these amino acids as depicted by their scores: Val (1.03), Thr (1.06), Phe (1.14), His (1.85), Gly (1.94), Pro (1.71) and Tyr (1.59) whereas such AA were Val (1.05), Thr (1.10), Phe (1.24), His (1.67), Gly (1.94), Pro (1.60), Asp (1.07) and Tyr (1.57) in the female *N. maculatus* exoskeleton. The scores also showed that 7/19 (36.8%) were more concentrated in the male sample than the egg concentration values, 1/19 (5.26%) had equivalent value of 1.94 (Gly) in both sexes whereas in females, eight (8/19=42.1%) AA scores had values greater than 1.0. The limiting AA (LAA) in both samples was Cys with values of 0.3172 (male) and 0.3511 (female) whereas in flesh and innards Ser was limiting with values in flesh as 0.513 (male) and 0.516 (female) but 0.511 (male) and 0.487 (female) in innards [25,26]. In order to fulfill the day's needs for all the AAs in *N. maculatus* exoskeleton samples, 100/31.72 or 3.15 times as male exoskeleton protein or 100/35.11 or 2.85 times as much female exoskeleton protein have to be consumed (or eaten) when they serve as the sole protein source in the diet. Table 6 contained the EAA scores (EAAS) of *N. maculatus* exoskeleton based on FAO/WHO standards [37]. The following scores were greater than 1.00 in both samples: Val (1.54-1.57), Thr (1.35-1.40), Phe + Tyr (2.03-2.10) and total AA (1.12-1.11). However, in the flesh of *N. maculatus* all the EAAS were greater than 1.00 whereas in the male and female innards five AA have EAAS greater than 1.00 each: Ile (1.09-1.33), Leu (1.17-1.19), Met + Cys (1.14-1.33), Phe + Tyr (1.27-1.35) and TEAA (1.09-1.10); but in addition, the female innards had Trp score of 1.50. In both present samples, Lys was limiting in both samples having scores of 0.4145 (male) and 0.4746 (female) with corresponding correction values of 100/41.45 (2.41) and 100/47.46 (2.11) respectively. In Table 7 was depicted the EAAS of the exoskeleton of *N. maculatus* samples based on requirements of pre-school child (2-5y). In both sexes six EAAS in each case had the EAAS values greater than 1.00. Like the observation in Table 6, Lys was the EAAS of least value in the heterosexuals. Values and correction values were 0.3930 (100/39.30 = 2.54 in male) and 0.4501 (100/45.01 = 2.22 in female).

Table 5: Amino acid scores of *N. maculatus* exoskeleton based on whole hen's egg amino acid

Amino acid	Male exoskeleton	Female exoskeleton	Mean	SD	CV%
Val	1.03	1.05	1.04	0.0118	1.13
Thr	1.06	1.10	1.08	0.0269	2.50
Ile	0.4672	0.4452	0.4562	0.0156	3.41
Leu	0.7942	0.6298	0.7120	0.1162	16.3
Lys	0.3677	0.4210	0.3944	0.0377	9.57
Met	0.6336	0.6581	0.6459	0.0173	2.68
Phe	1.14	1.24	1.19	0.0670	5.62
His	1.85	1.67	1.76	0.1265	7.20
Trp	0.4403	0.4414	0.4409	0.0008	0.1779
Gly	1.94	1.94	1.94	0.0025	0.1279
Ala	0.6695	0.7304	0.7000	0.0430	6.15
Ser	0.4792	0.3911	0.4352	0.0623	14.3
Pro	1.71	1.60	1.66	0.0760	4.59
Asp	0.9666	1.07	1.02	0.0750	7.35
Glu	0.9657	0.9558	0.9607	0.0070	0.7303
Arg	0.6724	0.8551	0.7637	0.1292	16.9
Tyr	1.59	1.57	1.58	0.0125	0.7876
Cys	0.3172	0.3511	0.3342	0.0240	7.19
Total	0.9047	0.9112	0.9079	0.0046	0.5084

Table 6: Essential amino acid scores of *N. maculatus* exoskeleton based on FAO/WHO [37] standards

Amino acid	Male exoskeleton	Female exoskeleton	Mean	SD	CV%
Val	1.54	1.57	1.56	0.0176	1.13
Thr	1.35	1.40	1.38	0.0344	2.50
Ile	0.6541	0.6233	0.6387	0.0218	3.41
Leu	0.9417	0.746	0.8442	0.1378	16.3
Lys	0.4145	0.4746	0.4446	0.0425	9.57
Met + Cys	0.7424	0.7830	0.0724	0.0282	3.70
Phe + Tyr	2.03	2.10	2.07	0.0487	2.35
Trp	0.7926	0.7946	0.7936	0.0014	0.1779
Total	1.12	1.11	1.11	0.0056	0.5004

Table 7: Essential amino acid scores of *N. maculatus* exoskeleton based on requirements of pre-school child (2-5years)

Amino acid	Male exoskeleton	Female exoskeleton	Mean	SD	CV%
Val	2.20	2.24	2.22	0.0252	1.13
Thr	1.59	1.65	1.62	0.0404	2.50
Ile	0.9344	0.8904	0.9124	0.0311	3.41
Leu	0.9987	0.7920	0.8954	0.1462	16.3
Lys	0.3930	0.4501	0.4216	0.0403	9.57
Met + Cys	1.04	1.10	1.07	0.0395	3.70
Phe + Tyr	1.02	2.00	1.51	0.6960	46.1
Trp	0.7205	0.7224	0.7215	0.0001	0.1779
His	2.33	2.11	2.22	0.1598	7.20
Total	1.32	1.30	1.31	0.0149	1.14

Results on scores from Tables 5, 6 and 7 were subjected to statistical analyses. The scores compared were egg (Male/Female), pre-school child (Male/Female) and provisional scoring pattern (Male/Female). In all the comparisons, all rxy values were high and positively significant (0.8577-0.9890). These values were high: rxy2 (0.7357-0.9781); Rxy (0.8806-1.04); CV%1 (50.9-54.5); CV%2 (51.0-53.3); IFE for egg (0.8256) and provisional scoring pattern (0.8521).

The summary of the amino acid profiles into factors A and B could be seen in Table 9. Factor A means constituted AAs of the two samples along the vertical axis whilst Factor B means constituted the AAs values along the horizontal axis as shown in the Table 9: both containing the EAA and NEAA. It would be observed that the mean of Factor A means and Factor B means gave a value of 45.4g/100g protein.

In Table 10, we have estimates of amino acid requirements at ages 10-12 years in mg/kg/day at the body weight of 30 kg. The protein of the male exoskeleton had values greater than the estimates in Phe + Tyr to the tune of 186%, to the tune of 25.8% in Trp and to the tune of 48.1% in Val. In female protein excess EAAs were produced in Phe + Tyr (excess = 218%), Thr (excess = 8.86%), Trp (excess = 35.0%), Val (excess = 61.6%) and TEAAs (excess = 4.01%). On these observations, female was better in 5/9 (55.56%) parameters whereas male was better in 3/9 (33.33%).

Table 8: Summary of the statistical analyses of the scores reported in Tables 5, 6 and 7

Statistics	Egg scores (Male/Female)	Pre-school child (Male/Female)	Provisional scoring pattern (Male/Female)
rxy	0.9847	0.8577	0.9890
rxy^2	0.9696	0.7357	0.9781
Rxy	0.9396	0.8806	1.04
$Mean_1$	0.9495	1.25	1.06
SD_1	0.5178	0.6594	0.5389
$CV\%_1$	54.5	52.8	50.9
$Mean_2$	0.9510	1.33	1.06
SD_2	0.4941	0.6770	0.5654
$CV\%_2$	52.0	51.0	53.3
C_A	0.1744	0.5141	0.1479
IFE	0.8256	0.4859	0.8521
Remark	Significantly diffrent	Significantly different	Significantly different

Egg score is significantly different at n-2 and r= 0.01 (critical value = 0.590); preschool child score is not significantly different at n-2 and r= 0.01 (critical value = 0.798); provisional score is significantly different at n-2 and r= 0.01 (critical value = 0.834)

Table 9: Summary of the amino acid profiles into Factors A and B

	Samples	(Factor A)	
Amino acid composition	Male exoskeleton	Female exoskeleton	Factor B means
Total essential amino acid	37.7	37.0	37.4
Total non-essential amino acid	52.7	54.0	53.4
Factor A means	45.2	45.5	45.4

Table 10: Estimates of amino acid requirements at ages 10-12 years (mg/kg/day)

Amino acid	School boys (10-12y) = R	School boys 30kg x R = S	Male exoskeleton = T	S-T (%)	Female exoskeleton = U	S-U (%)
Ile	30	900	497	+403(+44.8)	509	+391(+43.4)
Leu	45	1350	1253	+97(7.19)	1066	+284(+21.0)
Lys	60	1800	433	+1367(+75.9)	533	+1267(+70.4)
Met+Cys	27	810	494	+316(+39.0)	559	+251(+31.0)
Phe+Tyr	27	810	2317	-1507(-186)	2572	-1762(-218)
Thr	35	1050	1027	+23(+2.19)	1143	-93.0(-8.86)
Trp	4	120	151	-31(-25.8)	162	-42.0(-35.0)
Val	33	990	1466	-476(-48.1)	1600	-610 (-61.6)
Total EAAs	261	7830	7638	+192(+2.45)	8144	-314(-4.01)

+ = S > T or S > U; - = T > S or U > S; T or U was calculated as specific amino acid x 10 x appropriate corresponding protein

Conclusions

Neopetrolisthes maculatus exoskeleton samples in both male and female samples were good sources of high quality amino acids with the female total amino acids and the protein being more in value than the total male amino acids and its protein. In quality parameters, P-PER values were moderate; both EAAI and BV values were high; Lys/Trp were moderate but Met/Trp were high. The amino acid concentration levels had this class trend: class I > class IV > class VI > class V > class II > class VII > class III. In many parameter comparisons in the two heterosexual samples, the male exoskeleton was found to show superiority in quality than the female exoskeleton in these parameters: EAA in male was 37.7g/100g protein > 37.0 for EAA in female sample; $P\text{-}PER_1$ (male) was 1.86 > 1.24 in female; $P\text{-}PER_2$ in male was 2.02 > 1.41 in female; $EAAI_1$ male was 1.18 but female had 1.16; $EAAI_2$, male was 88.5 > 88.3 in female and BV value for male was 84.7 > 84.5 in female. According to the Department of Fisheries and Aquatic Resources (DFAR) data published in 2014, annual Sri Lanka total crab production is around 11,000 tons in 2012 [62]. This type of information is also important for us in Nigeria.

References

1. PubChem and the American Chemical Society (2018) Reshaping Scholarly Communication. USA: University of Califonia, 2005-05-31. Retrieved p-10-15.
2. Moruf OR, Saba AO, Joy Chuckwu-Ozazua, Elegbede IO (2019) Seasonal variation in macro-micronutrient compositions of the flesh and shell of the portunid crab Callinectes amnicola (De Rochebrune, 1883) from the coastal waters of Southwest Nigeria. Agricutura Tropica et Subtropica 1: 109 -110.
3. FAO (1998) Food and Agriculture Organization Annual Report. Rome, Italy.
4. Indufueke AD (1994) Nigerian move towards self-sufficiency in animal protein production. Proceedings of the Nigerian Veterinary medical Association Annual Conference, Lagos p-13-16.
5. Onyia L, Adebayo EF, Adewuyi K, Ekwunife E, Ochokwu I (2014) Comparative economics of fresh and smoked fish marketing in some local government areas in Adamawa State, Nigeria. IIFET 2014 Australia Conference Proceedings.
6. Fagbuaro O, Oso JA, Majolagbe FA, Oladapo AO (2013) Quality analysis of freshwater crab Cardisoma armatum and marine blue crab Callinectes ammicola collected from Yaba, Lagos, Nigeria. Nature and Science 11: 22-29.
7. Oyekanmi A (1984) Outline of food analysis. First Edn., Macmillan, London.
8. Hall D, Lee SY, Meziane T (2006) Fatty acid as tropic tracer in an experimental estuarine food chains. Tracer transfer. J. Exptal. Marine Biol. Ecol 336: 42-53.
9. Ahyoung ST, Baba K, Macpherson E, Poore GCB (2010) A new classification of the Galatheoidea (Crustacea: Decapoda: Anomura), Zootaxa 2676: 57-68.
10. Milnes EH (1837) Histoire naturelle des Crustacés, comprenant l'anatomie, la physiologie et al classification de ces animaux, Librairie Encyclopédique de Roret, Paris.
11. Vine P (1986) Red sea invertebrates. Immel Publishing, London.
12. Poupin J, Juncker M. (2010) Guide des Crustaces Decapodes du Pacifique Sud/A Guide to Decapod Crustaceans of the South Pacific. CRISP and CPS, Noumea.
13. Davie PJF (2002) Crustacea: Malacostraca: Eucarida (Part 2): Decapoda –Anomura, Brachyura, in: A. Wells, W. W. K. Houston (Eds). Zoological Catalogue of Australia, 19(3B), CSIRO Publishing, Melbourne.
14. Haig J (1965) The Porcellanidae (Crustacaea, Anomura) of Western Australia with description of four new Australian species. Journal of the Royal Society of Western Australia 48: 97-117.
15. King I, Childs MT, Dorsett C, Ostrander JG, Monsen ER (1990) Shellfish: Proximate composition, minerals, fatty acid and sterols. J. Amer. Dietic Ass 90: 677-685.
16. Skonberg DI, Perkins BL (2002) Nutrient composition of green crab (Carcinus maenas) leg meat and claw meat. Food Chem. 72: 401-404.
17. USDA (2003) US Department of Agriculture, National nutrient data base for standard reference. Release 16: Nutrient data laboratory Home page. Available http://www.nal.usda.gov/fnic/foodcomp/search
18. Jayasingbe GDTM, Jinadasa BKKK, Nethmina NAAN (2019) Determination of the fatty acid composition of blue swimmer crabs (Portunus pelagicus) by gas chromatography. Austin Journal of Nutrition and Food Science 7: 1124-1126.
19. Soundarapandian P, Varadharajan D, Jaganathan K, Ravichandran S (2015) Fatty acid composition in long-eyed swimming crab Podophthalmus vigil (Fabricius). Journal of Nutrition and Food Sciences 5: 1.
20. Martino RC, Gracinda MC (2004) Proximate composition and fatty acid content of the mangrove oyster Crassostrea rhizophorae along the year seasons. Braz. Arch. Biol. Tech. 47: 6-11.
21. Natan G, Dlua K, Yerlikayaa P (2003) Determination of proximate composition and mineral contents of blue crab (Callinectes sapidus) and swim crab (Portunus pelagicus) caught off the Gulf of Autlya. Food Chem 80: 495-498.
22. Kucukgulmez A, Celik M, Yanar Y, Ersoy B, Cikrikei M (2006) Proximate composition and mineral contents of the blue crab (Callinectes sapidus) breast meat, claw meat and hepatopancreas. Int. J. Food Sci. and Technol 41: 1023-1026.
23. Omotoso OT (2005) Chemical composition and nutritive significance of the land crabs, Cardisoma armatum (Decapoda). Afr. J. Appl. Zoo. and Envital. Biol 7: 68-72.
24. Adeyeye EI, Olanlokun JO, Falodun TO (2010) Proximate and mineral composition of whole body, flesh and exoskeleton of male and female common West African fresh water crab Sudananautes africanus africanus. Pol. J. Food Nutr. Sci 60: 213-216.
25. Adeyeye EI (2017) Amino acid profiles of the flesh of the heterosexual pairs of Neopetrolisthes maculatus. International Letters of Natural Sciences 61: 23-35.
26. Adeyeye EI (2019) Simultaneous identification and evaluation of amino acid profiles of the male and female innards of Neopetrolisthes maculatus. International Letters of Natural Sciences 75: 13-26.
27. Moronkola BA, Olowu RA, Tovide OO, Ayejuyo OO (2011) Determination of proximate and mineral contents of crab (Callinectes amnicola) living on the shore of Ojo River, Lagos, Nigeria. Sci. and Chem. Comm. 1: 1-6.
28. Jimmy UP, Arazu VN (2012) The proximate and mineral composition of two edible crabs Callinectes amnicola and Uca tangeri (Crustacea: Decapoda) of the Cross River Nigeria. Pak. J. Nutr. 11: 78-82.
29. Haig J (1975) Expèdition Rumphius II. Crustacès parasites, commonsaux, etc. (Th. Monod et R. Serene, Ed.). V. Porcellanidae (Crustacea, Decapoda, Anomura). Bulletin du Museum National d'Histoire Naturelle, Paris, 4e section A (1): 119-135.
30. AOAC (2006) Official Methods of Analysis, 18th ed., Association of Official Analytical Chemists, Washington, DC, Method p-982-30.

31. Danka PO, Dobrina DT, Kalin VI (2012) Simultaneous identification and determination of total content of amino acids in food supplements – tablets by gas chromatography. Asian J. Pharm. Clin. Res 5: 57-68.
32. Olaofe O, Akintayo ET (2000) Prediction of isoelectric point of legume and oil seed proteins from their amino acid composition. The J. Technosci 4: 49-53.
33. Alsmeyer RH, Cunningham AE, Happich ML (1974) Equations to predict PER from amino acid analysis, Food Technol 2: 24-38.
34. Oser BL (1959) An Integrated essential amino acid index for predicting the biological value of proteins, in: AA Albanese (Ed.), Protein and Amino Acid Nutrition. Academic Press, New York, pp. 281-295.
35. Steinke FH, Prescher EE, Hopkins DT (1980) Nutritional evaluation (PER) of isolated soybean protein and combinations of food proteins. Journal of Food Science 45: 323-327.
36. Paul AA, Southgate DAT, Russsel J (1978) First supplement to McCance and Widdowson's The Composition of Foods. HMSO, London.
37. FAO/WHO (1973) Energy and protein requirements. Technical Report Series No 522, WHO, Geneva.
38. FAO/WHO/UNU (1985) Energy and protein requirements. Technical Report Series No 724, WHO, Geneva.
39. Nieman DC, Butterworth DE, Nieman CN (1992) Nutrition. Wc Brown Publishers, Dubuque.
40. Adeyeye EI (2008) Amino acid composition of whole body, flesh and exoskeleton of female common West African fresh water crab Sudanautes africanus africanus. Pakistan Journal on Nutrition 7: 748-752.
41. Walther B, Schmid A, Sieber R, Wehmuller K (2008) Cheese in nutrition and health. Diary Science & Technology 88: 389-405.
42. FAO/WHO (1990) Protein quality evaluation. Report of Joint FAO/WHO Consultation, Bethesda, MD, 4-8 December, 1989. FAO/WHO, Rome.
43. Muller HG, Tobin G (1980) Nutrition and food processing. Avi Publishing, Westport, CT.
44. Friedman M (1996) Nutritional value of proteins from different food sources – A review. Journal of Agriculture and Food Chemistry 44: 6-29.
45. Adeyeye EI, Oyarekua MA, Adesina AJ (2014) Proximate, mineral, amino acid composition and mineral safety index of Callinectes latimanus. International Journal of Development Research 4: 2641-2649.
46. FAO/WHO (1991) Protein quality evaluation. Report of Joint FAO/WHO Expert Consultation. Food and Nutrition Paper 51, FAO, Rome.
47. FAO (1985) Sorghum and millets in human nutrition. FAO Food Nutrition Series No 27, FAO/UN, Rome.
48. Belavady B, Srikantia SG, Gopalan C (1963) The effect of oral administration of leuane on the metabolism of tryptophan. Biochem. J 87: 652-655.
49. Belavady B, Rao PU (1979) Leucine and isoleucine content of jowar and its pellagragenicity, Indian Exp. Biol 17: 659-661.
50. Deosthale YG (1980) Nutrition dimension of high yielding and hybrid crop varieties: Locational and varietal differences in nutritional value; in: FAO, FAO Food and Nutrition Series, No. 27, Sorghum and Millets in Human Nutrition, FAO p- 82.
51. Adeyeye EI (2005a) Amino acid composition of variegated grasshopper, Zonocerus variegatus. Tropical Science 45: 141-143.
52. Adeyeye EI (2005b) The composition of the winged termites, Macrotermes bellicosus. Journal of Chemical Society of Nigeria 30(2): 145-149.
53. Adeyeye EI (2004) The chemical composition of liquid and solid endosperm of ripe coconut. Oriental Journal of Chemistry 20: 471-476.
54. Adeyeye EI, Afolabi EO (2004) Amino acid composition of three different types of land snails consumed in Nigeria. Food Chemistry 85: 535-539.
55. Adeyeye EI, Asaolu SS, Aluko AO (2007) Amino acids composition of two masticatory nuts (Cola acuminata and Garcinia kola) and snack nut (Anacardium occidentale). International Journal of Food Science and Nutrition 58: 241-249.
56. Bingham S (1977) Dictionary of Nutrition. Barrie and Jenkins, London.
57. Cavins JC, Kwolek DF, Inglett GE, Cowen JC (1972) Amino acid analysis of soybean meal: interlaboratory study. Journal of Association of Official Analytical Chemists 55: 686-694.
58. Adeyeye EI, Aremu MO (2011) Amino acid composition of two fancy meats (Liver and Heart) of African giant pouch rat (Cricetomys gambianus). Oriental Journal of Chemistry 27: 1409-1419.
59. Albanese AA (1950) Protein and amino acid requirements of mammals, Academic Press, New York.
60. Mitchell HH (1950) a method for determining the biological value of protein, in: AA Albanese (Ed.), Protein and amino acid requirements of mammals. Academic Press, New York p- 1-32.
61. Pencharz PB, Hsu Jean. W-C, Ball RO (2007) Aromatic amino acid requirements in healthy human subjects. The Journal of Nutrition p-1-3.
62. Steve C (2014) Sri Lankan blue swimming crab fishery assessment. Sri Lanka: Seafood exporters' association of Sri Lanka, Colombo.