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Simultaneous Identification and Evaluation of Amino Acid Profiles of the Male and Female Innards of *Neopetrolisthes maculatus*

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Abstract. This article reports the amino acid profiles of the innards of the male and female dry samples of *Neopetrolisthes maculatus* collected from the Atlantic Ocean at Orimedu beach in Ibeju-Lekki, Lagos, Nigeria. The analytical results showed high values of amino acids being observed in both heterosexual samples (g $100g^{-1}$ protein): 8.17-8.32 (Leu), 8.35-10.3 (Asp), 17.6-18.2 (Glu) and 7.76-9.55 (Arg) with total amino acid values being greater in female innards ($97.6g100g^{-1}$) than the male innards ($95.5g\ 100g^{-1}$). These quality parameters were instructive of the quality of the amino acids in the innards of *N. maculatus*: $P\text{-}PER_1$, (2.83-3.01), $P\text{-}PER_2$ (2.89-2.96), EAAI (88.7-89.0), BV (85.0-85.5), Lys/Trp (L/T) (3.00-5.01), Met/Trp (M/T) (1.78-3.50) and Phe/Tyr (1.04 - 1.65). The *pI* values were close at 5.46-5.57. In the amino acid groups (classes), the following trend was observed: class I > IV >V > VI > II > III > VII. For the amino acid scores: serine (0.487-0.511) was limiting in both samples on the total hen's egg scoring pattern; in provisional scoring pattern, Lys was limiting in both samples with values of 0.820-0.889 and in the pre-school amino acid requirements, Lys was also limiting at 0.778-0.843. In the statistical analyses total amino acid profiles as well as egg scores were significantly different between the two samples whereas quality scores in pre-school amino acid requirements and provisional amino acid scoring pattern were both not significantly different between the two samples all at $r_{=0.01}$. Among the EAAs, six out of nine (66.7%) were more concentrated in the male innards and three of nine (33.3%) were more concentrated in the female. Thus the overall summary showed the male innards amino acids were of better quality than in the female as shown: male innards EEA = 46.1 $g100g^{-1}$ and 46.0 $g100g^{-1}$ in the female with corresponding TNEAA of 49.3g $100g^{-1}$ and 51.6g $100g^{-1}$ respectively.

Introduction

Crab is often recommended for pregnant women as it is consumed by many individuals. Crab innards (insides) have a hepatopancreas which is a part of the crab's digestive system. The colour of the organ is often yellow and it is similar to the deep yellow colour usually found in high-vitamin butter produced from cows grazing on rapidly growing grass. This yellow fatty acid organ has been given a common term known as crab "butter" or "mustard". Due to its colour, this part of the viscera is expected to be rich in fat-soluble activators [1]. Many indigenous groups that understood the necessity for special foods prior to conception, during pregnancy and during lactation; crab used to be one of these foods.

Crab insides have been used in a variety of ways. In Japanese cuisine, one dish with crab organs involves a blended mixture of the viscera, served in the skull of the crab with a raw egg on its top. The natives of Fiji also were aware that a particular specie of spider crab fed to mothers during and prior to pregnancy would produce children "physically excellent and bright mentally".

Special foods of the sea were eaten "day to day" during the time of pregnancy. There is then a message from many wise traditions around the planet: eat crabs during the period of preconception, pregnancy and lactation – and eat the whole crab [1]. The Chinese mitten crabs *Eriocheir sinensis* had been described to have a delicious taste and unique pleasant aroma, and has high nutritional value [2]. The meat, hepatopancreas and gonads are all edible parts of the Chinese mitten crab. Whilst consumers in western societies often choose to eat the meat alone, Asiatic consumers greatly prefer consuming the hepatopancreas and gonads. Indeed, this contributes to the popularity of mitten crabs

as a delicacy in China with the hepatopancreas of males being especially prized, followed by the gonads of both male and female crabs [3].

Porcellanidae family is a group of crab-shaped anomuran crustaceans that belong to the superfamily Galatheoidea together with three other families Galatheidae, Munididae and Munidopsidae [4]. They are commonly found in rocky and coral reefs of temperate and tropical coasts.

The World Register of Marine Species (WoRMS) has given the taxonomic details of *Neopetrolisthes maculatus* (H. Milne Edwards, 1837) [5]. Further details of the taxonomy classification of *N. maculatus* had been given in Adeyeye [6]. The distribution of porcellanids had been enumerated [6, 7]. Porcellanids places of abode had also been enumerated as they are typically found in a heterosexual pair [8].

Neopetrolisthes maculatus is a spotted crab. Two different colour forms are known, although the ground colour of the bodies of both form 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 the first pair (second pereopod). In the 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 spots [9].

There is paucity of information on the nutritional data for the organs of *N. maculatus*. However, literature information can be found in recent works of Adeyeye [6, 10]. Whilst reference [6] was on the amino acid profiles of the flesh of the heterosexual pairs of *N. maculatus,* reference [10] was on the chemical composition (proximate, minerals, vitamins), mineral ratios and mineral safety index of the innards of male and female *Neopetrolisthes maculatus*. The work reported in this article is an attempt to evaluate simultaneously the amino acid profiles of the viscera of the male and female samples of *N. maculatus* and also appraise critically the quality superiority of the samples over each other. The colour pattern of the samples was of large and uneven blotches resembling the Pacific Ocean *N. maculatus* [11].

Materials and Methods

Collection of samples: Samples were collected from trawler catches from the Atlantic Ocean of Orimedu beach in Ibeju-Lekki area of Lagos State, Nigeria. The experiment took place between November 2014 and June 2015. The crabs were washed with distilled water to remove adhering contaminant and transported in ice crushed containers to the laboratory for identification and preservation prior to analysis. The crabs were identified in the Department of Forestry, Wildlife and Fisheries Management of Ekiti State University, Ado-Ekiti, wrapped in aluminium foil and frozen at -4^{o}C for 2-3 days before analysis.

Sample treatment: More than ten matured crabs were caught with the net but three samples were used in this study. The three whole crabs were separated fresh, two were males and only one was female. For the purpose of analysis, the separated parts were the carapace and cheliped exoskeleton (to constitute the exoskeleton) the muscle from the thoracic sterna and cheliped (to constitute flesh) and the viscera of each crab. The viscera from each sex was later dried and separately blended. Drying was at 105^{o}C in the oven.

Extraction and analysis: Extraction and the instrumental analysis were carried out by following AOAC method [12] and Danka et al. [13].

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 250 ml 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 KOH solution and was incubated for 48 hours at 110^{o}C in hermetically closed borosilicate glass container. After 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:

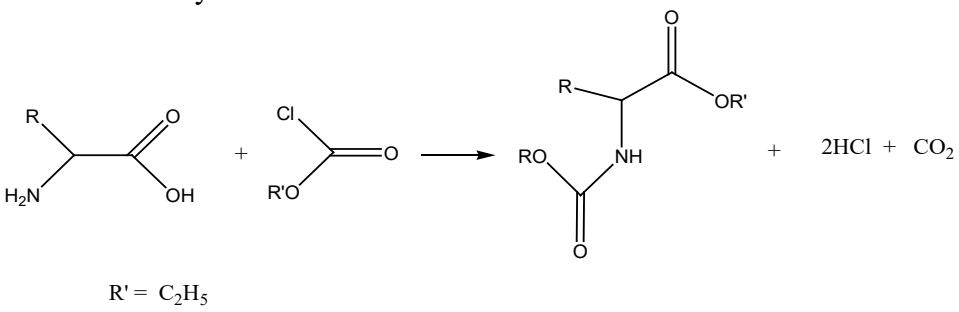


Figure 1. Derivatization process of amino acid

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 0.25 µm; oven programme: initial @110°C, first ramp @ 27°C/min to 320°C, second, constant for 5 mins at 320°C; detector: PFPD; detector temperature: 320°C; hydrogen pressure: 20 psi; compressed air: 35 psi.

Some calculations were made from the analytical data results:

(i) **Estimation of isoelectric point (*pI*)**: The estimation of the isoelectric point (*pI*) for a mixture of amino acids can be carried out by the equation of the form [14]:

$$IPm = \sum^{n}_{i=1} = IPi\ Xi \quad (1)$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric point of the ith amino acid in the mixture and Xi is the mass or mole fraction of the ith 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 done using the equations suggested by Alsmeyer et al. [15]:

$$P\text{-}PER_1 = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr) \quad (2)$$

$$P\text{-}PER_2 = -0.684 + 0.456\ (Leu) - 0.047\ (Pro) \quad (3)$$

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

(iv) **Estimation of essential amino acid index (EAAI):** The method of EAAI calculation due to Oser [16] using the egg protein amino acids as the standard.

(v) **Estimation of biological value (BV):** Computation of biological value (BV) was calculated following the equation of Oser [16]:

$$Biological\ value = 1.09\ (EAAI) - 11.73 \quad (4)$$

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

(vii) **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 [17].
- Scores based on essential amino acid scoring pattern [18].

- Scores based on essential amino acid suggested pattern of requirements for pre-school children [19].

(viii) The amino acid value differences and their percentage values were calculated between the male and female crab samples.

Statistical evaluation

Data results in Tables 1, 5, 6 and 7 were subjected to statistical analyses of correlation coefficient (r_{xy}), regression coefficient (Rxy), coefficient of determination or variance (r_{xy}^2), the coefficient of alienation (C_A) and index of forecasting efficiency (IFE). Other calculations were grand mean, standard deviation (SD) and coefficient of variation (CV%). The r_{xy} value was converted to critical Table value (r_T) to see if significant differences existed among the two heterosexual sample results at $r_{=0.01}$ [20].

Results and Discussion

The amino acid profiles of the heterosexual innards of *N. maculatus* have been depicted in Table 1. They were reported on dry weight basis at g $100g^{-1}$ crude protein (cp). Highest concentrated amino acids in both samples were acidic amino acids which were Glu (17.6-18.2g $100g^{-1}$) followed immediately by Asp (8.35-10.3 g $100g^{-1}$), the female sample was greater in both amino acids (AA). The two mostly concentrated EAAs in both samples were Leu (8.17 - 8.32g $100g^{-1}$) and Arg (7.76-9.55g $100g^{-1}$cp). Only Trp in the male sample had value of less than 1g $100g^{-1}$cp with a value of 9.75e-1g $100g^{-1}$cp whereas all other AAs had appreciable values. The overall AAs for the male sample added up to 95.4g $100g^{-1}$cp but added to 97.6g $100g^{-1}$cp in the female. However, the total AAs in the male sample might not be a true reflection of the crude protein as the crude protein in the innards male sample was 56.8g $100g^{-1}$ whereas it was 55.5g $100g^{-1}$ in the female [10]. This observation of lower crude protein (with higher total AAs) and higher crude protein (with lower total AAs) could be due to the fact that the crude protein in the male *N. maculatus* innards contained less true protein compared to the female innards crude protein. The coefficient of variation (CV%) values were generally low with the lowest value being observed in Pro which had values of 3.31-3.32g 100^{-1}cp, mean value of 3.31g $100g^{-1}$ (approximately), ±SD value of 0.010 and CV% of 0.295 whereas the highest CV% was observed in Trp with following information: concentration values 9.75e-1 to 1.50g $100g^{-1}$cp, mean value of 1.24g $100g^{-1}$ cp, ±SD value of 0.373 and CV% value of 30.1. The low CV% values showed that the concentration values were close in the samples on parameter comparisons. The values discussed above were mostly in tandem with the observations made in *N. maculatus* flesh amino acid profiles in both the male and female samples. Thus we have: Glu was mostly concentrated (17.7-17.8g $100g^{-1}$cp) closely followed by Asp (9.90-10.0g $100g^{-1}$cp) both also acidic AAs, also in the EAAs, we have Arg (8.70-9.07g $100g^{-1}$cp) and Leu (7.23-7.94g $100g^{-1}$cp) whereas the total AA values ranged from 96.6 -97.1g $100g^{-1}$cp [6]. Unlike in the innards, the flesh of *N. maculatus* has total AAs as a reflection of the crude protein values: in female flesh, crude protein was 40.2g $100g^{-1}$ (and total AAs of 96.6g $100g^{-1}$) whereas in the male we have 43.7g $100g^{-1}$ total AAs [6]. Also in the flesh of *N. maculatus*, the CV% values of the AAs were close and low with values of 0.124 (observed for Glu) - 32.0 (observed for His) [6]. The observations made in the present report for Glu and Asp also corroborated with the observations in the flesh of female West African fresh water crab *(Sudananatutes africanus africanus)* with Glu (130.2mg g^{-1} crude protein) > Asp (72.5mg g^{-1}cp) but almost similar for the positions of Arg and Leu: Leu (66.0mg g^{-1}cp)> Arg (58.9mg g^{-1}cp) [21].

The concentration differences and their percentages in the two heterosexual samples could be seen in columns seven and eight respectively in Table 1. Out of the 19 parameters recorded, nine parameters or 9/19 (47.4%) were positive towards male innards, in order words 47.4% of the parameters were more concentrated in the male than the female. It also showed that 10 parameters or 10/19 (52.6%) were more positive towards the female innards. The relative concentration values in the innards among the sexes had opposite values in the heterosexual flesh where the female had

60.0% concentration values > 40.0% concentration values in the male flesh [6]. The percentage differences ranged from -0.418 (Pro) to -54.1(Trp).

Table 1. Amino acid profiles (g $100g^{-1}$protein) of the male and female innards of *Neopetrolisthes maculatus*

Amino acid	Male innards	Female innards	Mean	SD[#]	CV%[+]	Value difference	% difference
Glycine (Gly)	6.22	5.77	5.99	0.318	5.31	+0.450	+7.24
Alanine (Ala)	5.61	4.89	5.25	0.508	9.61	+0.718	+12.8
Serine (Ser)	4.03	3.85	3.94	0.134	3.39	+0.189	+4.69
Proline (Pro)	3.31	3.32	3.31	0.010	0.295	-0.014	-0.418
Valine (Val)*	4.77	4.20	4.48	0.406	9.05	+0.574	+12.0
Threonine (Thr)*	3.73	3.80	3.77	0.050	1.32	-0.070	-1.88
Isoleucine (Ile)*	4.36	5.30	4.83	0.668	13.8	-0.944	-21.7
Leucine (Leu)*	8.32	8.17	8.25	0.108	1.31	+0.152	+1.83
Arspartic acid (Asp)	8.35	10.3	9.34	1.40	15.0	-1.98	-23.7
Lysine (Lys)*	4.89	4.51	4.70	0.267	5.68	+0.378	+7.72
Methionine (Met)*	3.42	2.68	3.05	0.523	17.2	+0.739	+21.6
Glutamic acid (Glu)	17.6	18.2	17.9	0.402	2.24	-0.568	-3.22
Phenylalanine (Phe)*	4.76	4.13	4.44	0.447	10.1	+0.632	+13.3
Histidine (His)*	3.13	2.19	2.66	0.666	25.0	+0.941	+30.1
Arginine (Arg)	7.76	9.55	8.66	1.26	14.6	-1.78	-23.0
Tyrosine (Tyr)	2.89	3.96	3.42	0.760	22.2	-1.07	-37.2
Tryptophan (Trp)*	9.75e-1	1.50	1.24	0.373	30.1	-0.528	-54.1
Cystine (Cys)	1.24	1.32	1.28	0.055	4.28	-0.078	-6.24
Total amino acid	95.4	97.6	96.5	1.60	1.66	-2.27	-2.38

*Essential amino acid; [#]SD= standard deviation; [†]CV% = coefficient of variation; + = male innards value > female innards value; - = male innards value < female innards value. All determinations were in duplicate and on dry weight.

In Table 2, we have the statistical analysis result from the data in Table 1. Table 2 showed female values to be greater than the male in the following parameters: TAAs, mean, SD and CV% although those values in the two samples were relatively close to each other. These values were also high: r_{xy} (0.9790 and significantly different since ($r_c = 0.9790 > r_T = 0.590$ at $r_{=0.01}$), r_{xy}^2 (0.9585), R_{xy} (1.06), IFE (0.7962) but low C_A (0.2038). The R_{xy} of 1.06 meant that for every unit increase in the male AA parameter, there was a corresponding increase of 1.06g $100g^{-1}$ in the female AA parameter. The values of index of forecasting efficiency (IFE) and coefficient of alienation (C_A) are always in reverse to each other. The C_A is the error of prediction of relationship between two entities whereas the IFE is the value of reduction of error in predicting the relationship between two entities; the higher the value of C_A, the higher the difficulty in predicting the relationship between two entities whereas the higher the IFE, the easier the prediction. In these results, since the IFE > C_A, it meant that the biochemical functions of the male innards could as well be performed easily by the female innards and vice versa. Table 3 depicted the summary of the concentrations of essential, aromatic, non-essential, neutral, etc. of the amino acid levels in the samples in g $100g^{-1}$. The total AAs values ranged from 95.4-97.6 $g100g^{-1}$ with the female taking the higher concentration value. These values were close to the values of AAs in heterosexual flesh of *N. maculatus* having range values of 96.6-97.1 $g100g^{-1}$ [6], but higher than the total AAs in the flesh of *S. africanus africanus* (777.0 mg g^{-1}cp) [21]. The EAA range was 46.0-46.1 $g100g^{-1}$ and CV% of 47.1-48.4 respectively; note however that the EAAs in the male were higher than those of the female indicating that AAs in the male *N. maculatus* innards would be of higher nutritional quality than in the female. This observation had been made in China with the hepatopancreas of males being especially prized, followed by the gonads of both male and female crabs [3]. The total sulphur amino acids (TSAA) of the samples were 4.00-4.66$g100g^{-1}$ which were highly comparable to the values in flesh (4.04-4.85$g100g^{-1}$cp) and also close

to the standard value of 58mg g^{-1}cp recommended for infants [19]. The total aromatic amino acid values range suggested for ideal protein (68-118mg g^{-1}cp) [19] were found to be lower than in the present results of 11.7-11.8 g100g^{-1}cp making the samples to be very good sources of ArAA and the samples might also be qualified as supplements to foods of lower ArAA values. The values of 11.7-11.8g 100g^{-1} ArAA were higher than in the flesh of *N. maculatus* where values of 7.72-9.67g100g^{-1}cp were reported [6]. The percentage ratio of EAAs to the TNEAA of 46.0-46.1 were above the 39% considered adequate for an ideal food for infants, 26% for children and 11% for adults [19]. The EAA/TAA in eggs is 50.0% [22].

In Table 3, we have two predicted protein efficiency ratio types (P-PER_1 and P-PER_2). Values range in P-PER_1 were 2.83-3.01 and 2.89-2.96 in P-PER_2. These values were lower than in the *N. maculatus* flesh where P-PER_1 range was 3.39-3.69 and P-PER_2 range was 3.82-4.14. The *in vivo* P-PER is in the order of 2.2 [23]; all the present P-PER values were greater than 2.2 attesting to the nutritional quality of the innards. Literature information had the following P-PER values: in the flesh of female *S. africanus africanus*, P-PER was 3.1 [21]; in *Callinectes latimanus* (a lagoon crab), P-PER_1 was 1.21 and P-PER_2 was 1.39 [24]. These literature values showed that *N. maculatus* might be more physiologically utilized protein source than some of quoted references. In general, it has been found that the better the protein, the lower the level in the diet required to produce the highest protein efficiency ratio. This is a clear reflection of the importance of the proper nutritive balance of all the amino acids to produce optimum metabolic efficiency. The Leu/Ile values ranged from 1.54-1.91 with the difference levels of 2.87-3.97 g100g^{-1} and % (Leu-Ile)/Leu values of 35.1-47.7. In the flesh of *N. maculatus* Leu/Ile ratio had values of 1.60-1.63 [6] and in the flesh of *S. africanus africanus*, the ratio was 1.60 [21]. In literature, the most ideal Leu/Ile is 2.36 [25]. The values of 1.54-1.91 showed that 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 [26]. A high Leu imbalance in the diet impairs the metabolism of Trp and niacin, and is responsible for the niacin deficiency in sorghum eaters [27]. Experiments in dogs showed that animals fed sorghum proteins with less than 11g100g^{-1} protein Leu did not suffer from nicotinic acid deficiency [28]. The present Leu values of 8.17-8.32 g100g^{-1} were less than 11 g100g^{-1} protein and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas [29]. The percentage Cys/TSAA values were 26.7-33.0 close to the values of 31.9-33.1% in the meat of *N. maculatus* [6]. The present Cys/TSAA values were very comparable with other literature values of animal protein amino acids: 27.3-32.8% in *S. africanus africanus* [8]; 36.3% in *Macrotermes bellicosus* [30]; 25.6% in *Zonocerus variegatus* [31]; 35.5% in *Archachatina marginata*; 38.8% in *A. archatina* and 21.0% in *Limicolaria* sp. (the last three were land snails consumed in Nigeria) [32]. The percentage Cys in TSAA in the diet of the rat, chick and pig is 50% [25] but the value is unknown in man [19]. It is however interesting to note that vegetable protein (e.g. coconut endosperm) has a percentage Cys/TSAA of 62.8% [33]. High percentage Cys/TSAA had also been reported in *Anacardium occidentale* with a value of 50.51% [34]. The % Cys/TSAA in *N. maculatus* showed that the crab behaved like a typical animal protein [34]. 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 [35]. In the present results, the values range for Met and Cys were respectively 2.68-3.42g 100g^{-1} and 1.24-1.32g 100g^{-1}.

Table 2. Statistical analysis of the data from Table 1 pertaining to amino acid profile of male and female innards of *Neopetrolisthes maculatus*

Statistics	Male innards		Female innards
Total amino acid value	95.4		97.6
Mean	5.30		5.42
SD	3.72		4.04
CV%	70.3		74.5
Correlation coefficient (r_{xy})		0.9790	
Variance (r_{xy}^2)		0.9585	
Regression coefficient (R_{xy})		1.06	
Coefficient of alienation (C_A)		0.2038	
Index of forecasting efficiency (IFE)		0.7962	
Remark		Results significantly different	

*Results significantly different at n-2 and $r_{=0.01}$ (critical value = 0.590). (NOTE: n-2 = 18-2 = 16.)

Table 3. Concentrations of essential, aromatic, non-essential, neutral, etc. amino acid (g $100g^{-1}$ protein) of the male and female *N. maculatus* innards

Amino acid	Male innards	Female innards	Mean	SD	CV%
Total amino acid (TAA)	95.4	97.6	96.5	1.56	1.61
Total non-essential acid (TNEAA)	49.3	51.6	50.5	1.63	3.22
% TNEAA	51.6	52.9	52.3	0.919	1.83
Total essential amino acid (TEAA)					
- with His	46.1	46.0	46.1	0.071	0.154
- without His	43.0	43.8	43.4	0.566	1.30
%TEAA					
- with His	48.4	47.1	47.8	0.919	1.93
- without His	45.1	44.9	45.0	0.141	0.314
Total aliphatic amino acid (TAIAA) (CLASS I)	29.3	28.3	28.8	0.707	2.46
%TAIAA	30.7	29.0	29.9	1.20	4.03
Total essential aliphatic amino acid (TEAIAA)	17.4	17.7	17.6	0.212	1.21
%TEAIAA	18.3	18.1	18.2	0.141	0.777
Total aromatic amino acid (TArAA) (CLASS VI)	11.7	11.8	11.8	0.071	0.602
% TArAA	12.3	12.1	12.2	0.141	1.16
Total acidic amino acid (TAAA) (CLASS IV)	26.0	28.5	27.3	1.77	6.49
% TAAA	27.2	29.2	28.2	1.41	5.01
Total basic amino acid (TBAA) (CLASS V)	15.8	16.2	16.0	0.283	1.77
%TBAA	16.5	16.6	16.6	0.071	0.427
Total neutral amino acid (TNAA)	52.6	51.4	52.0	0.849	1.63
% TNAA	55.2	52.6	53.9	1.84	3.41
Total hydroxyl amino acid (THAA) (CLASS II)	7.77	7.65	7.71	0.085	1.10
% THAA	8.15	7.83	7.99	0.226	2.83
Cyclic amino acid (Pro) (CAA) (CLASS VII)	3.31	3.32	3.32	0.007	0.213
% CAA	3.47	3.40	3.44	0.049	1.44
Total sulphur amino acids (TSAA) (CLASS III)	4.66	4.00	4.33	0.467	10.8
% TSAA	4.89	4.09	4.49	0.566	12.6

% Cys in TSAA	26.7	33.0	29.9	4.45	14.9
Leu/Ile ratio	1.91	1.54	1.73	0.262	15.2
(Leu-Ile) difference	3.97	2.87	3.42	0.778	22.7
% (Leu- Ile)/Leu	47.7	35.1	41.4	8.91	21.5
P-PER$_1$,i.e. -0.468+0.454 (Leu)-0.105 (Tyr)	3.01	2.83	2.92	0.127	4.36
P-PER$_2$, i.e. -0.684+0.456 (Leu)-0.047 (Pro)	2.96	2.89	2.93	0.049	1.69
Calculated isoelectric value (*pI*)	5.46	5.57	5.52	0.078	1.41
Essential amino acid index (EAAI)	88.7	89.2	89.0	0.354	0.397
Biological value (BV)	85.0	85.5	85.3	0.354	0.415
Lys/Trp or L/T	5.01	3.00	4.01	1.42	35.5
Met/Trp or M/T	3.50	1.78	2.64	1.23	46.1
Phe/Tyr	1.65	1.04	1.35	0.431	32.1

The amino acids composition of *N. maculatus* heterosexual innards showed high quality values as demonstrated by their essential amino acid index (EAAI) values of 88.7-89.2 and their corresponding biological values (BV) of 85.0-85.5. In comparison, some literature values of EAAI and BV are as follows: *N. maculatus* meat, EAAI (86.9-89.9) and BV (83.0-86.3) [6]; others are [16]: 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). These literature results show the quality position of *N. maculatus* innards under discussion. EAAI is useful as a rapid tool in the evaluation of food formulation for protein quality. The isoelectric point, *pI*, was 5.46-5.57 which showed 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 isolate of organic product without evaluating the protein solubility to get to the *pI*.

In infants protein requirements, a growth pattern of amino acid requirements was obtained by assigning value of unity to the Trp need [36]. Similar calculation of the amino acid content of mammalian tissues showed that there exists good agreement of growth needs and tissue amino acid patterns. This agreement is good for the Lys/Trp (L/T) and Met/Trp (M/T) ratios of muscle proteins which constitute approximately 75% of the infant body proteins. The present results had L/T values of 3.00-5.01 and M/T of 1.78-3.50. These values were better than in the meat of *N. maculatus*: L/T values 3.31-4.27 and M/T of 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) [37]. 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 results the male L/T value of 5.01 was close to the muscle standard of 6.3 and just below the mammalian viscera of 5.3; the M/T of male innard was 3.50 which was greater than the M/T of mammalian muscle by 1.0 (standard value being 2.50) and female innard M/T of 1.78 was close to mammalian viscera of 2.0. The patterns of observations in the present results followed the trend as observed in the meat of heterosexual *N. maculatus* for the L/T and M/T values.

The amino acid groupings into classes I to VII have been depicted in Table 4 [38]. The concentration trend of the classes could be seen as shown in g100g^{-1} protein: class I (28.3-29.3)> class IV (26.0-28.5)> class V (15.8-16.2) > class VI (11.7-11.8)> class II (7.65-7.77)> class III (4.00-4.66)> class VII (3.31-3.32). This trend was as observed in the meat of heterosexual *N. maculatus* [6]. A close observation would show that the percentages were close to their individual values with very marginal differences; examples were: value (percentage): class 1, 28.3-29.3 (29.0-30.7); class II, 7.65-7.77 (7.83-8.15; class III, 4.00-4.66 (4.09-4.89); class IV, 26.0-28.5 (27.2-29.2); class V, 15.8-16.6 (16.5 – 16.6); class VI, 11.7-11.8 (12.1-12.3) and class VII, 3.31-3.32 (3.40-3.47). All the CV% values were generally low with values ranging between 0.213 and 10.8; in the meat of *N. maculatus* the CV% range was also low at 0.251-12.8 [6].

Table 4. Amino acid groups of *N. maculatus* heterosexual innards

	Value in g 100 g^{-1} protein (% value)				
Class	Male (Innards)	Female (innards)	Mean	SD	CV%
I. [with aliphatic side chains (hydrogen and carbon)= Gly, Ala, Val, Leu, Ile]	29.3 (30.7%)	28.3 (29.0%)	28.8	0.707	2.46
II. [with side chains containing hydroxylic (OH) groups= Ser, Thr]	7.77 (8.15%)	7.65 (7.83%)	7.71	0.085	1.10
III. [with side chains containing sulphur atoms = Cys, Met]	4.66 (4.89%)	4.00 (4.09%)	4.33	0.467	10.8
IV. [with side chains containing acidic groups or their amides = Asp, Glu]	26.0 (27.2%)	28.5 (29.2%)	27.3	1.77	6.49
V. [with side chains containing basic groups = Arg, Lys, His]	15.8 (16.5%)	16.2 (16.6%)	16.0	0.283	1.77
VI. [containing aromatic rings = His, Phe, Tyr, Trp]	11.7 (12.3%)	11.8 (12.1%)	11.8	0.071	0.602
VII. [Imino acids = Pro]	3.31 (3.47%)	3.32 (3.40%)	3.32	0.007	0.213

Table 5 presented the total amino acid scores based on whole hen's egg amino acids profile. These amino acids were more concentrated in the male innards than the whole hen's egg values as shown by their scores: Gly (2.07), Ala (1.04), Leu (1.00), Met (1.07), Glu (1.47), His (1.30), Arg (1.27) whereas such acids were Gly (1.92), Glu (1.52) and Arg (1.57) in the female innards. The scores summary showed that 6/19 (31.6%) values in the male were more concentrated than the egg concentration values, 1/19 (5.26%) had equivalent value in the male with egg value in Leu whereas 3/19 (15.8%) in the females had scores greater than 1.0 each. The limiting amino acid (LAA) in both samples was Ser with values of 0.511 (male) and 0.487 (female); in the meat of *N. maculatus* Ser was also the LAA with values of 0.513 (male) and 0.516 (female) [6]. Therefore, in order to fulfill the day's needs for all the amino acids in *N. maculatus* innards samples, 100/51.1 or 1.96 times as much male innards protein, or 100/48.7 or 2.05 times as much female innards protein would have to be consumed (eaten) when they serve as the sole protein source in the diet. Table 6 contained the essential amino acid scores of *N. maculatus* innards based on FAO/WHO [18] standards. The following scores were greater than 1.00 in both samples: Ile (1.09-1.33), Leu (1.17-1.19), Met + Cys (1.14-1.33), Phe + Tyr (1.27-1.35) and total AA (1.09 - 1.10) but in addition, the female innards had Trp score greater than 1.00 (1.50). In the meat of *N. maculatus*, all the above AAs including Trp had their scores greater 1.00 each. In both samples Lys was LAA with male value being 0.889 and female value being 0.820. The correction value in the male innard protein was 100/88.9 or 1.12 whilst the correction value in the female was 100/82.0 or 1.22. In the *N. maculatus*, meat, Lys (0.804) was limiting in the female whereas Val (0.823) was limiting in the male. In Table 7 we have depicted the essential amino acid scores of the innards of *N. maculatus*, samples based on requirements of pre-school child (2-5y). Whilst two scores (Lys = 0.843; Trp = 0.887) were less than 1.00 each in the male innards protein, only Lys had score less than 1.00 in the female innards having score value of 0.778. Hence, Lys was the LAA in both samples and their respective correction values were 100/84.3 or 1.19 and 100/77.8 or 1.29.

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

Amino acid	Male innards	Female innards	Mean	SD	CV%
Gly	2.07	1.92	2.00	0.106	5.32
Ala	1.04	0.905	0.973	0.095	9. 82
Ser	0.51	0.487	0.499	0.017	3.40
Pro	0. 870	0. 874	0. 872	0.003	0.324
Val	0.636	0.559	0.598	0.054	9.11
Thr	0.732	0.746	0.739	0.010	0.134
Ile	0.778	0.946	0. 862	0.119	13.8
Leu	1.00	0.984	0.992	0.011	1.14
Asp	0.780	0.965	0. 873	0.131	15.0
Lys	0.789	0.728	0.759	0.043	5.69
Met	1.07	0. 836	0.953	0.165	17.4
Glu	1.47	1.52	1.50	0.035	2.36
Phe	0.933	0. 809	0.871	0.087	10.1
His	1.30	0.911	1.11	0.275	2.49
Arg	1.27	1.57	1.42	0.212	14.9
Tyr	0.721	0.990	0. 856	0.190	22.2
Trp	0.542	0. 835	0.689	0.207	30.1
Cys	0.691	0.734	0.713	0.030	4.27
Total	0.955	0.977	0.966	0.016	1.61

Results on scores from Tables 5, 6 and 7 were subjected to statistical analyses. In the egg's score comparison, both r_{xy} and r_{xy}^2 were high whereas IFE value was just above 50.0%. The R_{xy}, mean (for both samples), their SD values were each being less than 1.00. The CV% values were slightly low and close to each other, values being 36.4 - 38.9. The $r_c = 0.8772 > r_T = 0.798$ at $r_{=0.01}$ making the r_{xy} being significantly different in the two samples on eggs score basis. Also, the $C_{A\,(0.480)} <$ IFE $_{(0.520)}$ making the prediction of biochemical relationships easy. For the pre-school AA requirements scores and provisional EAA scoring pattern scores, their $r_c = 0.6055 < r_T = 0.798$ (pre-school scores) and $r_c = 0.4547 < r_T = 0.798$ (EAA scoring pattern) at $r_{= 0.01}$ showed that no significant differences occurred in the two samples for the two scores. Also, prediction of relationship would also be difficult among the two samples since C_A>IFE in both types of scores: $C_{A\,(0.796)}$ >IFE $_{(0.204)}$ (pre-school child scores) and $C_{A\,(0.891)}$ > IFE $_{(0.109)}$ (provisional scoring pattern).

Table 6. Essential amino acid scores of *N. maculatus* innards based on FAO/WHO (1973) [18] standards

Amino acid	**Male innards**	**Female innards**	**Mean**	**SD**	**CV%**
Val	0.954	0.840	0.897	0.081	8.99
Thr	0.935	0.950	0.943	0.011	1.13
Ile	1.09	1.33	1.21	0.170	14.0
Leu	1.19	1.17	1.18	0.014	1.20
Lys	0.889	0.820	0.855	0.049	5.71
Met + Cys	1.33	1.14	1.24	0.134	10.9
Phe + Tyr	1.27	1.35	1.31	0.057	4.32
Trp	0.975	1.50	1.24	0.371	30.0
Total	1.09	1.10	1.10	0.007	0.646

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

Amino acid	Male innards	Female innards	Mean	SD	CV%
Val	1.36	1.20	1.28	0.113	8.84
Thr	1.10	1.12	1.11	0.014	1.27
Ile	1.56	1.09	1.33	0.332	25.1
Leu	1.26	1.23	1.25	0.021	1.70
Lys	0. 843	0.778	1. 811	0.046	5.67
Met + Cys	1. 86	1.60	1.73	0.184	10.6
Phe + Tyr	1.21	1.28	1.25	0.049	3.98
Trp	0. 887	1.36	1.12	0.334	29. 8
His	1.65	1.15	1.40	0.354	25.3
Total	1.25	1.23	1.24	0.014	1.14

The summary of the amino acid profiles into Factors A and B could be seen in Table 9. Factor A means constituted amino acids of the two samples along the vertical axis whilst Factor B means constituted the amino acids values along the horizontal axis as shown in the Table: both containing the essential and non-essential amino acids. It would be observed that the mean of Factor A means and Factor B means gave a value of 48.3g $100g^{-1}$ protein. It is interesting to note that the Factors A and B means gave a value of 48.4 in the meat of *N. maculatus* heterosexual samples [6].

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)
r_{xy}	0. 8772	0.6055	0.4547
r_{xy}^2	0.7695	0.3666	0.2067
R_{xy}	0.8254	0.5540	0.6829
$Mean_1$	0.956	1.30	1.08
SD_1	0.372	0.323	0.156
$CV\%_1$	38.9	24.9	14.5
$Mean_2$	0.963	1.28	1.13
SD_2	0.350	0.296	0.235
$CV\%_2$	36.4	23.0	20.7
C_A	0.480	0.796	0. 891
IFE	0.520	0.204	0.109
Remark	Significantly different	Not significantly different	Not significantly different

Egg score is significantly different at n-2 and $r_{=0.01}$ (critical value = 0.575); pre-school child score is not significantly different at n-2 and $r_{=0.01}$(critical value = 0.765); provisional score is not significantly different at n-2 and $r_{=0.01}$ (critical value = 0.798).

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

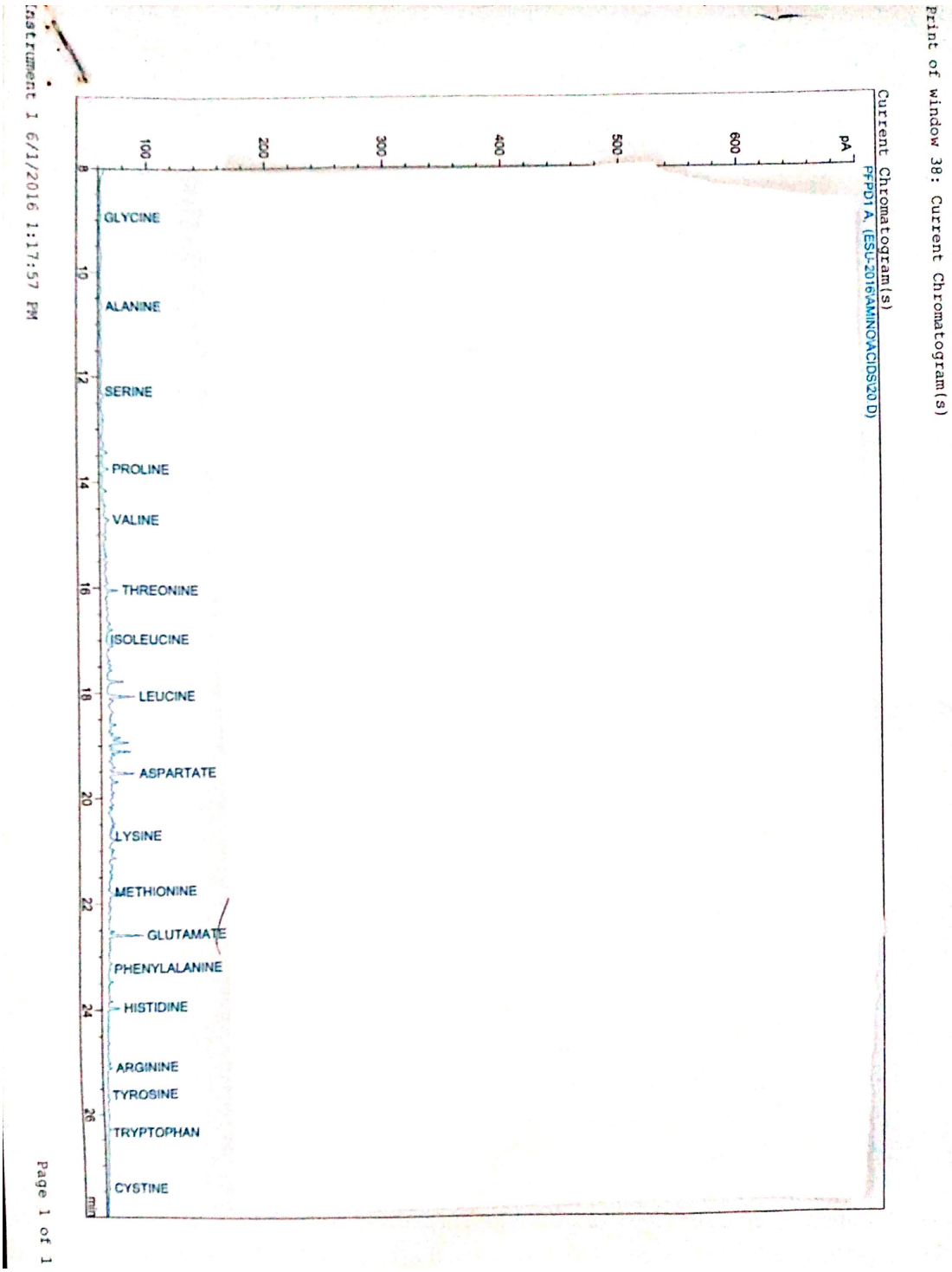
	Samples (Factor A)		
Amino acid composition	**Male innards**	**Female innards**	**Factor B means**
Total essential amino acid	46.1	46.0	46.1
Total non-essential amino acid	49.3	51.6	50.5
Factor A means	47.7	48. 8	48.3

Conclusions

Neopetrolisthes maculatus innards samples in the male and female were good sources of high quality amino acids with the female total amino acids being more in value than the total male amino acids. In quality parameters, $P\text{-}PER_1$, and $P\text{-}PER_2$ were high; both EAAI and BV values were high;

both Lys/Trp and Met/Trp were high. The amino acid concentration levels had this class trend: class I> IV > V > VI > II > III > VII. The male protein contained less true protein when compared to the female protein. The parameter comparisons in the two heterosexual samples showed the male innards to be superior in quality than the female innards in these parameters: EAA in male was 46.1g $100g^{-1}$ > EAA female of 46.0g $100g^{-1}$; P-PER_1 male was 3.01 > 2.83 in female, P-PER_2 in male was 2.96 > 2.89 in female; in the total EAA, 6/9 or 66.7% were more concentrated in male and only 3/9 or 33.3% in the female; Lys/Trp in male was 5.01 but 3.00 in female; Met/Trp was 3.50 in male but 1.78 in female. However, the female innards protein had a higher value of EAAI (89.0) but 88.7 in the male and female BV of 85.5 > 85.0 in the male.

Appendix 1. Chromatogram



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