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The Relationship in the Amino Acid of the Whole Body, Flesh and Exoskeleton of Common West African Fresh Water Male Crab *Sudananautes africanus africanus*

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Abstract: The amino acid composition of the whole body, flesh and exoskeleton of the common fresh water West African male crab, *Sudananautes africanus africanus* was investigated. Glu was the most concentrated acid (128.0-130.0 mg/g crude protein) and Leu (50.0-60.9 mg/gcp) was the highest essential amino acid. Total amino acid range was 635.8-749.2 mg/gcp and total essential amino acid range was 298.2-356.6 mg/gcp or 46.9-47.9%. Val was the limiting amino acid in whole body and flesh whereas Lys was limiting amino acid in the exoskeleton and Phe+Tyr scores range was 1.0-1.2. Significant differences existed between essential amino acid and non-essential amino acids at $p<0.05$ among the samples. Percentage neutral amino acids was 54.9-57.6%, total acidic amino acids was 26.2-29.7% and total basic amino acids was 15.4-17.3%.

Key words: *Sudananautes africanus africanus*, male crab, amino acid composition

Introduction

A crab is characterized by a flattened, broad body covered by a shell or carapace. Crabs belong to the order Decapoda, Class Crustacea. Phylum Arthropoda. The order Decapoda consists of two sub-orders (a) Natantia-the swimmers and (b) Reptantia-the crawlers. Reptantia includes the crabs, lobsters and hermit crabs. They have heavy legs that can support the body for crawling. True crabs are the most successful decapodas with about 4500 species (Yoloye, 1988). Crabs are found throughout the world, chiefly in marine waters, but they also inhabit fresh water and land where they dig or inhabit burrows.

Several crabs are prized as food. They include the Alaska king crab (*Paralithodes camtschatica*); the blue crab (*Callinetes sapidus*), which is the commercially important crab occurring along the East and Gulf coasts of the United States (Bullough, 1958); the Dungenes crab (*Cancer magister*), which are present in Europe-non-swimming crabs used as food (Bullough, 1958); and the giant mangrove swimming crab (*Scyllia serrata*) which is popular in pacific waters from the East Coast of Africa to India and as far away as Japan (Muller and Tobin, 1980). Soft-shelled crabs are newly moulted crabs whose new shells have not yet hardened.

The crab is usually consumed by individuals and it is often recommended for pregnant women. Literature is available on the chemical composition of the nutritionally valuable parts of male and female common West African fresh water crab, *Sudananautes africanus africanus* (Adeyeye, 2002). There is however paucity of information on the amino acids profile of *S. africanus africanus*. The study reported in this article is an attempt to assess the quality of the amino acids of the whole body (edible parts), flesh and exoskeleton of the male common West Afican fresh water crab *S. africanus africanus*.

Materials and Methods

Collection and treatment of samples: *Sudananautes africanus africanus* samples were collected from the banks of River Osun, located at Ikere Ekiti, Ekiti State, Nigeria during the onset of the rainy season (they normally hibernate in the dry season). Six pieces of matured fresh crabs were selected from more than 12 crabs caught in holes along the river banks. The samples were stored under freezing at-10°C.

Two whole crabs were separated fresh. While the internal organs were discarded, the other separated parts were dried in the oven at 105°C. For the purpose of analysis, the separated parts were carapace and cheliped exoskeleton (which constitute the exoskeleton sample) and the muscle from thoracic sterna and cheliped (which constitute the flesh sample). For the whole body sample, the following constituted it: cheliped (muscle and exoskeleton), carapace, thoracic sternum and the other four pairs of walking legs and then dried at 105°C. The samples: whole body, exoskeleton and the flesh were separately blended.

Analysis of the samples: About 2.0g of each sample was weighed into the extraction thimble and the fat extracted with chloroform/methanol mixture using a soxhlet apparatus (AOAC, 1995). The extraction lasted for 5-6h.

About 30mg of the defatted sample was weighed into glass ampoules. Seven milliliters of 6MHC1 were added and oxygen expelled by passing nitrogen gas into the

Table 1: Amino acid composition of male fresh water crab (mg/g crude protein) dry weight

Amino acid	Whole body	Flesh	Exoskeleton	Mean	SD[a]	CV%[b]
Lys*	58.0	50.0	40.5	49.5	7.2	14.5
His*	20.2	20.1	17.8	19.4	1.1	5.7
Arg*	40.1	51.3	39.5	43.6	5.4	12.4
Asp	59.0	68.5	60.2	62.6	4.2	6.7
Thr*	30.0	37.4	26.1	31.2	4.7	15.0
Ser	20.0	29.5	15.5	21.7	5.8	26.9
Glu	130.0	128.0	128.5	128.8	0.85	0.66
Pro	32.6	29.4	26.5	29.5	2.5	8.4
Gly	41.1	61.7	50.0	50.9	8.4	16.6
Ala	33.0	28.9	23.0	28.3	4.1	14.5
Met*	21.1	22.8	20.1	21.3	1.1	5.2
Cys	11.0	10.2	8.9	10.0	0.9	8.7
Val*	29.0	37.9	38.5	35.1	4.3	12.4
Ile*	28.0	39.0	30.2	32.4	4.8	14.7
Leu*	60.9	60.0	50.0	57.0	4.9	1.8
Phe*	40.5	38.1	35.5	38.0	2.0	5.4
Tyr	29.5	36.4	25.0	30.3	4.7	15.5
Try*	-	-	-	-	-	-
Protein (g/100g)+	32.5	24.8	24.2	27.2	3.8	13.9

*Essential ammo acid. [a]Standard deviation. [b]Coefficient of variation. +Dry weight and fat free

samples. The glass ampoules were sealed with a bunsen flame and put into an oven at 105±5°C for 22h. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5ml acetate buffer (pH = 2.0) and stored in a plastic specimen bottle and kept in the deep freezer.

Amino acid analysis was by Ion Exchange Chromatography (IEC) (FAO/WHO, 1991) using the Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The period of analysis lasted for 76min for each sample. The column flow rate was 0.50ml/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart record of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Tryptophan was not determined. Norleucine was the internal standard.

Estimation of the quality of dietary protein: The amino acid score was calculated using the following formula (FAO/WHO, 1973): Amino acid score = Amount of amino acid per test protein [mg/g] /Amount of amino acid per protein in reference pattern [mg/g].

Determination of the Total Essential Amino Acid (TEAA) to the Total Amino Acid (TAA), i.e. (TEAA/TAA); Total Sulphur Amino Acid (TSAA); percentage cystine in TSAA (%Cys/TSAA); total aromatic amino acid (TArAA), etc.; the Leu/Ile ratios were calculated while the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al*. (1974), i.e. P-PER = -0.468+0.454 (Leu) -0.105 (Tyr).

Statistical analysis: Calculations made were the grand mean, standard deviation, coefficients of variation in percent and F test setting the confidence level at 95% (Christian, 1977).

Results and Discussion

Table 1 shows the Amino Acid (AA) composition. Glutamic (128.0-130.0mg/g crude protein, cp) and aspartic (59.0, 68.5, 60.2mg/g cp) were the most concentrated AA within their groups. Both of these acids are acidic AA. Phenylalanine with its sparing partner tyrosine constituted the highest Essential Amino Acid (EAA) in all the samples with values of 70.0mg/gcp (whole body, wb), 74.5mg/gcp (flesh, fl) and 60.5mg/gcp (exoskeleton, exosk).

Table 2 shows the Total AA (TAA), the Total Essential AA (TEAA), Total Acidic AA (TAAA), Total Neutral AA (TNAA), Total Sulphur AA (TSAA), total aromatic AA (TArAA) and their percentage levels. The predicted protein efficiency ratio (P-PER) were 2.9, 2.8 and 2.4. The Leu/Ile ratio range was 1.5-2.2, the difference was 19.8-32.9mg/gcp and percentage difference of 35.0-54.0.

Table 3 shows that valine had the lowest Essential Amino Acid Score (EAAS) of 0.6 (60.0%) in wb, 0.8 (80.0%) in flesh and Lys of 0.7 (70.0%) in exoskeleton.

Glu, Asp and Phe+Tyr trends in the current shell fish samples report followed the trend as observed in *Gymnarchus niloticus* (Trunk fish) (Adeyeye and Adamu, 2005). Arginine (39.5-51.3mg/gcp) is essential for children and reasonable levels were present in the samples. The lysine contents of the samples (40.5-58.0mg/gcp) were close to the content of the reference egg protein (63mg/gcp), they will therefore serve as good sources for fortification of cereal weaning foods.

Table 2: Essential, non-essential, acidic, neutral, sulphur, aromatic (mg/g crude protein) of male fresh water crab

Amino acid	Whole body	Flesh	Exoskeleton	Mean	SD	CV%
TAA	684.0	749.2	635.8	689.7	46.5	6.7
TNEAA	356.2	392.6	337.6	362.1	22.8	6.3
TEAA						
-with His	327.8	356.6	298.2	327.5	23.8	7.3
-no His	307.6	336.5	280.4	308.2	22.9	7.4
%TNEAA	52.1	52.4	53.1	52.5	0.4	0.8
%TEAA						
-with His	47.9	47.6	46.9	47.5	0.4	0.9
-no His	45.0	44.9	44.1	44.7	0.4	0.9
TNAA	376.7	431.3	349.3	385.8	34.1	8.8
%TNAA	55.1	57.6	54.9	55.9	1.2	2.2
TAAA	189.0	196.5	188.7	191.4	3.6	1.9
%TAAA	27.6	26.2	29.7	27.8	1.4	5.2
TBAA	118.3	121.4	97.8	112.5	10.5	9.3
%TBAA	17.3	16.2	15.4	16.3	0.8	4.8
TSAA	32.1	33.0	29.0	31.4	1.7	5.5
%TSAA	4.7	4.4	4.6	4.6	0.1	2.7
%Cys/TSAA	14.6	13.3	15.9	14.6	1.1	7.3
TarAA	70.0	74.5	60.5	68.3	5.8	8.5
%TarAA	10.2	9.9	9.5	9.9	0.3	2.9
P-PER	2.9	2.8	2.4	2.7	0.2	8.0
Leu/Ile	2.2	1.5	1.7	1.8	0.3	16.4
Leu-Ile	32.9	21.0	19.8	24.6	5.9	24.0
%Leu-Ile	54.0	35.0	39.6	42.9	8.1	18.9

The CV% levels were all low with the highest being for serine with a value of 26.9. With the exception of glycine, the AA compositions of the current study were lower than the AA values in ostrich (Sales and Hayes, 1996), beef (USDA, 1986), chicken (USDA, 1979).

The TEAA of 327.8, 356.6, 298.2mg/gcp without tryptophan (which was not determined) (Table 2) were close to the value of egg reference (566mg/gcp) (Paul *et al.*, 1980). The current TEAA are comparable to some literature values: it is (mg/gcp): 351 (*Zonocerus variegatus*) (Adeyeye, 2005a), 350.3 (*Macrotermes bellicosus*) (Adeyeye, 2005b), 428 (*Limicolaria* sp), 361 (*Archatina archatina*), 450 (*Archachatina marginata*) (Adeyeye and Afolabi, 2004) and 354.8 (G. *niloticus)* (Adeyeye and Adamu, 2005). The TSAA were generally lower than the 58mg/gcp recommended for infants (FAO/WHO/UNU, 1985). The ArAA range suggested for ideal infant protein (68-118mg/gcp) (FAO/WHO/UNU, 1985) has current values close to the minimum, i.e., 60.5-74.5mg/gcp. The ArAA are the precursors of epinephrin and thyroxin (Robinson, 1987) which were of reasonable level in the current samples. The percentage ratio of TEAA to the TAA in the samples were 47.9, 47.6 and 46.9 which were well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985). The TEAA/TAA percent were strongly comparable to that of egg (50%) (FAO/WHO, 1990); 43.6% reported for pigeon pea flour (Oshodi *et al.*, 1993) and 43.8-44.4% reported for beach pea protein isolate (Chavan *et al.*, 2001).

Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. For examples (Cys/TSAA) % were: 36.3% in *M. belicosus* (Adeyeye, 2005b); 25.6% in Z. *variegatus* (Adeyeye, 2005a); 35.3% in *A. marginata*, 38.8% in *A. archatina* and 21.0% in *Limicolaria* sp. respectively (Adeyeye and Afolabi, 2004) and 29.8% in G. *niloticus* (Adeyeye and Adamu, 2005). In contrast, many vegetable proteins contain substantially more Cys than Met, for example, in solid endosperm of ripe coconut, Cys was 5.69 mg/gcp and Met was 3.36mg/gcp and (Cys/Met+Cys)% was 62.87 (Adeyeye, 2004). Thus for animal protein diet, or mixed diets containing animal protein, cystine is unlikely to contribute up to 50% of the TSAA (FAO/WHO, 1991). FAO/WHO/UNU (1985) did not give any indication of the proportion of TSAA which can be met by Cys in man, it gave for rat, chick and pig, whose proportion Cys/TSAA% is about 50% (FAO/WHO, 1991). Information on the agronomic advantages of increasing the concentration of sulphur-containing amino acids in staple foods shows that Cys has positive effects on mineral absorption, particularly zinc (Mendoza, 2002; Sandstrom *et al.*, 1989).

The P-PER levels (2.4-2.9) were higher than 1.21 (cowpea), 1.82 (pigeon pea) (Salunkhe and Kadam, 1989); 1.62 (millet *ogi*) and 0.27 (sorghum *ogi*) (Oyarekua and Eleyinmi, 2004). It has been suggested that the Leu/Ile balance is more important than dietary excess of Leu alone in regulating the metabolism of Try and niacin and hence the disease process in the development of pellagra (FAO, 1995). The present Leu/Ile ratios (1.5-2.2) were low in value.

Although Val would have been described as the limiting amino acid for whole body and flesh (Table 3), however, the EAA most often acting in a limiting capacity are Lys,

Table 3: Essential amino acid scores of male fresh water crab

Amino acid	Whole body	Flesh	Exoskeleton	Mean	SD	CV%
Ile	0.7	1.0	0.8	0.8	0.1	15.0
Leu	0.9	0.9	0.7	0.8	0.1	11.3
Lys	1.1	0.9	0.7	0.9	0.2	18.1
Met+Cys	0.9	0.9	0.8	0.9	0.05	5.4
Phe+Tyr	1.2	1.2	1.0	1.1	0.1	8.3
Thr	0.8	0.9	0.7	0.8	0.1	10.2
Try	-	-	-	-	-	-
Val	0.6	0.8	0.8	0.7	0.1	12.9
Total	0.9	0.9	0.8	0.9	0.05	5.4

Met+Cys, Thr and Try (Bingham, 1977). Therefore in whole body, Thr (0.8 or 80%) would be the real limiting AA. In order to fulfil the days needs for all the EAA in the sample: it will be 100/80 or 1.25 times as much whole body protein, 100/90 or 1.11 times as much flesh protein and 100/70 or 1.43 times exoskeleton protein, would have to be eaten when they are the sole protein in the diet.

The data obtained for the Total Amino Acids (TAA), Total Essential Amino Acids (TEAA), Total Non-Essential Amino Acids (TNEAA) and Essential Amino Acid Scores (EAAS) were all subjected to the F test as follows: TAA/TAA, TEAA/TEAA, TNEAA/ TNEAA and EAAS (among the three samples) and TNEAA/TEAA within each sample. The following results were obtained: in the crab samples TAA/TAA, Fc (1.040-1.041) < Ft (2.40), number of degrees of freedom n = 16, results not significant; TEAA/TEAA, Fc (1.52-2.01) < Ft (3.44), n = 8, results not significant; EAAS/EAAS, Fc (1.46-3.96) < Ft (3.79) in flesh/exoskeleton (result not significant) but > Ft in whole body/exoskeleton (result significant), n = 7; TNEAA/TEAA in whole body, Fc (6.24), in flesh, Fc (8.12) and exoskeleton, Fc (13.56) meaning that all the TNEAA/TEAA results were significant at $p<0.05$; n = 7/8.

Human requirements for the amino acids are as follows: His and Ile are required by all ages; Leu, Lys and Val are required by school children and adults; Met+Cys and Phe+Tyr are required by preschool, school children and adults while Thr are only majorly required by adults. The World Health Organization recommended Val and Ile requirements for school children aged 10-12 years, of 33 and 30mg amino acid/kg body weight/day (FAO/WHO/UNU, 1985; FAO/WHO, 1973). For example, a 30kg child will require 990 and 900mg of Val and Ile per day respectively. The protein values for the samples (from proximate composition, g/100g) were 22.09 (whole body), 34.54 (flesh) and 40.21 (exoskeleton) all on dry weight basis. E.g., with whole body, 100g of it would provide about 640.6 and 618.5mg of Val and Ile respectively. If a 30kg child therefore consumes 100g of whole body per day, his WHO daily requirements of Val and Ile would be met by 64.7% and 68.7% respectively. For flesh, Val would be 1304.1mg (131.7%) and Ile was 1347.lmg (149.7%) while in exoskeleton, Val would be 1548.1mg (156.4%) and Ile was 1214.3mg (134.9%).

Conclusion: Male *Sudananautes africanus africanus* is a good source of protein particularly the essential amino acids. Its P-PER are mostly similar to the values in meat. The results show that all the three sub samples - whole body, flesh and exoskeleton will serve well in complementing cereal gruels used as weaning food for children in the tropics.

References

Adeyeye, E.I., 2002. Determination of the chemical composition of the nutritionally valuable parts of male and female common West African fresh water crab *Sudananautes africanus africanus*. Int. J. Food Sci. Nutr., 53: 189-196.

Adeyeye, E.I., 2004. The chemical composition of liquid and solid endosperm of ripe coconut. Oriental J. Chem., 20: 471-476.

Adeyeye, E.I., 2005a. Amino acid composition of variegated grasshopper, *Zonocerus variegatus*. Trop. Sci., 45: 141-143.

Adeyeye, E.I., 2005b. The composition of the winged termites, *Macrotermes bellicosus*. J. Chem. Soc. Nigeria, 30: 145-149.

Adeyeye, E.I. and A.S. Adamu, 2005. Chemical composition and food properties of *Gymnarchus niloticus* (Trunk fish). Biosci. Biotechn. Res. Asia, 3: 265-272.

Adeyeye, E.I. and E.O. Afolabi, 2004. Amino acid composition of three different types of land snails consumed in Nigeria. Food Chem., 85: 535-539.

Alsmeyer, R.H., A.E. Cunningham and M.I. Happich, 1974. Equations to predict PER from amino acid analysis. Food Technol., 28: 34-38.

A.O.A.C., 1995. Official Methods of Analysis, 16th edition. Association of Official Analytical Chemists, Washington DC, pp: 222-245.

Bingham, S., 1977. Dictionary of Nutrition. Barrie and Jenkins Ltd. London, pp: 76-281.

Bullough, W.S., 1958. Practical invertebrate anatomy. Macmillan Press, London.

Chavan, U.D., D.B. Mckenzie and F. Shahidi, 2001. Functional properties of protein isolates from beach pea *(Lathyrus maritimus* L.). Food Chem., 74: 77-187.

Christain, G.D., 1977. Analytical Chemistry, 2nd edition. John Wiley and Sons Inc., New York, pp: 69-71.

FAO, 1995. Sorghum and millets in human nutrition. FAO Food Nutrition Series, No. 27. FAO/UN, Rome, pp: 76-84.

FAO/WHO, 1973. Energy and Protein Requirements. Technical Report Series No. 522. WHO, Geneva, Switzerland, pp: 1-118.

FAO/WHO, 1990. Protein Quality Evaluation. Report of Joint FAO/WHO Consultation Held in Bethesda, U.S.A., 4-8 December, 1989. FAO, Rome, Italy, pp: 51.

FAO/WHO, 1991. Protein Quality Evaluation. Report of Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51. FAO/WHO, Rome, Italy, pp: 4-66.

FAO/WHO/UNU, 1985. Energy and Protein Requirements. Who Technical Report Series No. 724. WHO, Geneva, Switzerland, pp: 13-205.

Mendoza, C., 2002. Effect of genetically modified low phytic acid plants on mineral absorption. Int. J. Food Sci. Technol., 37: 759-767.

Muller, H.G. and G. Tobin, 1980. Nutrition and Food Processing. Avi Publishing, Westport, CT, pp: 195.

Oshodi, A.A., O. Olaofe and G.M. Hall, 1993. Amino acid, fatty acid and mineral composition of peigeon pea *(Cajanus cajan)*. Int. J. Food Sci. Nutr., 43: 189-191.

Oyarekua, M.A. and A.F. Eleyinmi, 2004. Comparative evaluation of the nutritional quality of corn, sorghum and millet *ogi* prepared by modified traditional technique. Food Agri. Eviron., 2: 94-99.

Paul, A.A., D.A.T. Southgate and J. Russel, 1980. Widdowson's The Composition of Foods. HMSO, London, UK.

Robinson, D.E., 1987. Food Biochemistry and Nutritional Value. Longman Scientific and Technical, London, UK, pp: 120.

Sales, J. and J.P. Hayes, 1996. Proximate, amino acid and mineral composition of ostrich meat. Food Chem., 36: 167-170.

Salunkhe, D.K. and S.S. Kadam, 1989. Handbook of world food legumes, nutritional chemistry, processing technology and utilization. CRC Press, Boca Raton, Florida.

Sandstrom, B., A. Almgren, B. Kivisto and A. Cederblad, 1989. Effect of protein and protein source on zinc absorption in humans. J. Nutr., 119: 48-53.

USDA, 1979. Composition of Foods: Poultry Products. Agriculture Handbook No. 8-5, United States Department of Agriculture, Washington DC, USA.

USDA, 1986. Composition of Foods: Beef Products. Agriculture Handbook No. 8-13, United States Department of Agriculture, Washington DC, USA.

Yoloye, V.L., 1988. Invertebrate Zoology. Ilorin University Press, Ilorin, Nigeria, pp: 192-230.