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Amino acid composition of three fresh water fish samples commonly found in south western states of Nigeria

H.N. Ogungbenle*, A.A. Olaleye and K.E. Ayeni
Department of Chemistry, Ekiti State University, Ado-Ekiti, Nigeria.

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ABSTRACT

The amino acid components of three fresh water fishes: African catfish (*Clarias gariepinus*), snake fish (*Channa striatus*) and tilapia fish (*Tilapia zillii*) were reported. Among the amino acids investigated, aspartic and glutamic acids were found most abundant in all the three samples with values ranging from 10.4 – 10.7 g/100g crude protein, cp (aspartic acid) and 14.3-14.6 g/100g cp (glutamic acid. The most abundant essential amino acids were Lysine (Leu), Leucine (Leu), and Arginine (Arg). The total acidic and amino acids (TAAA) (24.9 – 25.2g/100g, cp) in all the samples were greater than the total basic amino acids (TBAA) (18.4 – 19.8g/100g, cp) and Leu / Ile ratio values (2.09 – 2.20) showed that Leu > Ile in all the samples. The predicted protein efficiency ratio (P-PER) values (3.46-3.59) were considerably high and this would make them good food complements. The isoelectric point (pI) ranged from 4.68 – 6.04, showing that they are in acidic medium. In essential amino acid scores (based on whole hen's egg), Lys was highest in all the sample with values ranging from 1.57 – 1.66 and their limiting essential amino acid was Valine (Val). On provisional essential amino acid scoring pattern, Lys (1.77 – 1.87) and Val (0.93) had the highest and lowest scores respectively. Essential amino acid scores based on pattern for pre-school children showed that all the samples would supply the required essential amino acids for pre-school children (2 – 5 years old). Generally, the samples are good sources of essential amino acids.

Introduction

The intake of animal protein by Nigerians has been very low due to reduced production (Olayide *et al*., 1972) and the increasing growth in the human population (Oyenuga, 1968). Fish contains significant amounts of all essential amino acids particularly lysine in which cereals are relatively poor. Fish protein can be used to complement the amino acid pattern and improve the overall protein quality of a mixed diet (FAO, 2005). Fish represents a major source of animal protein supply in Nigeria, which has a low per capital protein consumption (Afolabi *et al*., 1984). Recently, there has been expansion of aquaculture in Nigeria, especially catfish and tilapia due to their tolerance to a wide range of temperature, fast growth and adaptation to diversed eenvironments as well as to low oxygen and high salinity levels (Hecth *et al*., 1996). Snake fish has pharmaceutical value and has been used to reduce the post natal and post surgery pains (Mat Jais *et al*., 1994). It is highly priced in the market because of its good delicate taste (Qin and Fast, 1998). The present study is therefore aimed at investigating the amino acid composition of the three fresh water samples. This would provide more useful information on the health implications of the samples and would add new information to the composition tables of food ingredients.

Materials and methods

Sample Collection and Preparation

All the three fish samples: African *C. gariepinus*, *Channa striatus* and *Tilapia zillii* were obtained from Makoko and Epe landing sites in Lagos, Nigeria. The samples were separately dried, ground and sieved with 2000mm mesh size. The sieved samples were stored separately in a dry, cool place prior to use. 2g of the sample was weighed into the extraction thimble and extracted with chloroform/methanol (2:1 v/v) mixture using a Soxhlet apparatus (AOAC, 2005). The extraction lasted for 5-6h. About 30mg of the defatted sample was weighed into glass ampoules. Seven milliliters of 6 M HCl were added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with a Bunsen flame and put into an oven at 105°C for 22h. The ampoule was allowed to cool; 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

Amino acid analysis was done by ion exchange chromatography (IEC) using the Technicon Sequential Multisample (TSM) Amino acid Analyser (Technicon Intruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 ml/min at 60°C with reproducibility consistent within ± 3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations.

Quality Parameters Determination

Determination of Amino Acid Scores

Determination of the amino acid scores was first based on whole hen's egg (Paul et al., 1976). In this method, both essential and non-essential amino acids were scored. Secondly,

Tele:
E-mail addresses: lebdul@yhoo.com

amino acid score was calculated using the following formular (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)

In this method, Met + Cys and Phe + Tyr were each taken as a unit. Also, only the essential amino determined were scored. Amino acid score was only calculated based on the composition of the amino acids obtained in the sample compared with the suggested pattern of requirement for pre-school children (2-5 years). Here, Met = Cys and Phe + Tyr were each taken as a unit.

Determination of the Predicted Protein Efficiency Ratio

The predicted protein efficiency ratio (P-PER) was determined using one of the equations derived by Alsmeyer *et al*. (1974):

P-PER = - 0.468 + 0.454(Leu) – 0.105 (Tyr).

Results and discussion

Table 1 presents the amino acid compositions of the three fish samples: Cat fish *(Clarias gariepinus)*, snake fish (*Channa striatus)* and tilapia fish (*Tilapia zillii)*. Valine in the *Clarias gariepinus* had similar value (4.66g/100g, crude protein, cp) with that of and *Tilapia zillii* little variation in *Channa striatus* (4.65g/100g cp). Valine daily requirement is 23mg/kg and its deficiency leads to locomotor dysfunction in young rats (Adeyeye, 2009). Threonine ranged between 4.13 – 4.39g/100g cp with the highest concentration (4.39g/100g cp) recorded for snake fish. Threonine was the last of the 20 amino acids to be discovered. The values of methionine in the samples (2.82 – 3.06g/100g cp) were comparatively higher than those recorded for fin fishes (2.04 – 2.40g/100g cp) (Adeyeye, 2009). Methionine contains sulphur in the thioether linkage. The sulphur atom of cystine is obtained uniquely from the essential amino acid methionine. Methionine is needed for the synthesis of other important substances, including choline. Administration of methionine prevents the fatty liver and causes resumption of growth (Adeyeye, 2009). Arginine and histidine in the samples are high: 5.50 – 6.55g/100g (Arginine) and 3.02 – 3.37g/100g cp (histidine) and are therefore good for children. Histidine is a semi-essential amino acid. Children do not grow if it is absent from their diet, but adults can probably synthesise enough for their daily needs. It is a precursor of histamine, a substance normally present in small amounts in cells. The presence of substantial amounts of arginine in diets enhances Ca^{2+} absorption, but under most physiological circumstances, this is of little consequence (white *et al*., 1973).

Generally, in all the three samples, aspartic (Asp) and glutanic (Glu) acids were found to be the most abundant. Also, leucine (Leu), Lysine (Lys) and arginine (Arg) were high in the samples and were observed to be the most abundant essential amino acids.

Table 2 reveals the concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) and their percentage values. Leu/Ile ratios, the predicted protein efficiency ratio (P-PER) and the isoelectric point (pI) are also depicted in Table 2. The contents of TEAA (48.7 – 49.3g /100g cp) without tryptophan in the samples were fairly close to the value for egg reference protein (56.6g/100g cp) (Paul *et al*., 1976). The total sulphur amino acids (4.22 – 4.49g/100g cp) were fairly lower than the 5.8g/100g cp recommended for infants (FAO/WHO/UNU, 1985). The total aromatic amino acid (7.54g/100g cp) recorded for *Clarias gariepinus* was higher than the suggested minimum (6.8g/100g cp) for ideal infant protein (FAO/WHO/UNU, 1985) whereas the 6.30g/100g cp and 6.57g/100g cp for *Channa striatus* and *Tilapia zillii* respectively were comparable. The range of values for percentage total essential amino acid (%TEAA) (50.3 – 51.5%) were much higher than the 39% considered adequate in food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985). However, the % TEAA values in the present report compare favourably with that of egg (50%) (FAO/WHO, 1990). In all the samples, the total acidic amino acid (24.9 – 25.2g/100g cp) were found to be higher than the total basic amino acid, (18.4 – 19.8g/100g cp), this showed that the sample's protein were probably more acidic in nature. The value for Leucine / Isoeleucine ratio was 2.09 for *Clarias gariepinus*, 2.17 for *Channa striatus* and 2.20 for *Tilapia zillii*. These ratios revealed that Leu>Ile in all the samples. Leu/Ile imbalance from excess leucine might be a factor in the development of pellagra particularly in maize (FAO, 1995). Also, clinical, biochemical and pathological observations in experiments conducted in humans and laboratory animals showed that high leucine in diets impairs the metabolism of tryptophan and niacin and is responsible for niacin deficiency in sorghum eaters (FAO, 1995). High leucine is also a factor contributing to the pellagragenic properties of maize (Belavady and Gopalan, 1969). The P-PER ranged between 3.46 – 3.59. Protein efficiency ratio (PER) is defined as the gain in weight per gram of ingested protein. The P-PER values in the present study vary between 0.0 for a very poor protein food and a maximum possible of just above 4.0 for good protein food. The present values were far above average. Literature revealed that P-PER is 0.27 in sorghum *ogi* and 1.62 in millet *ogi* (Oyarekua and Eleyinmi, 2004). This implies that the samples will be good complements of the *ogi* samples. The isoelectric point (pI) in the present report was 5.72 for cat fish, 5.68 for snake fish and 6.04 for tilapia fish. The pI values in the acidic medium showed that the samples may be useful in formulating very low-acid foods such as meat products (Olaofe *et al*., 1993). The pI values will be useful in the preparation of a protein isolate of an organic compound.

Table 3 depicts the amino acid scores of the samples based on whole hen's egg amino acids. Generally, valine had the lowest score of 0.62 in all the essential amino acids of the samples. This means, to correct for the amino acid needs from the samples, 100/62 or 1.61 times as mush *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii* have to be taken when they are sole protein sources in the diet (Bingham, 1977).

The amino acid scores of the fish samples based on provisional essential amino acid scoring pattern was shown in Table 4. Valine also had the lowest score (0.93 or 9.3%) in all the samples. Therefore, the EAAs showed that valine was limiting across board and would require 100/93 or 1.08 correction factor.

Table 5 shows the amino acid scores of the samples based on the suggested amino acid requirements for pre-school children. The Table revealed that all the samples would be able to supply the required essential amino acids for the pre-school children (2-5 years). This is because virtually all the essential amino acid scores were above the 100% requirement each.

Contrary to the present report, the EAAs most often acting in a limiting capacity are (a) Lysine; (b) Met + Cys; (c) Thr; (d) Try (Bingham 1977).

Table 1: Amino acid composition (g/100g) of *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii*

Amino Acid (g/100g cp)	C F	S F	T F	Mean	SD	CV%
Glycine	4.50	4.30	4.05	4.28	0.23	5.37
Alanine	6.29	5.93	6.38	6.20	0.24	3.87
Serine	4.39	4.08	4.03	4.17	0.20	4.80
Proline	3.03	3.25	3.17	3.15	0.11	3.49
Valine	4.66	4.65	4.66	4.66	0.01	0.21
Threonine	4.37	4.39	4.13	4.30	0.14	3.26
Isoleucine	4.66	4.33	4.22	4.40	0.23	5.23
Leucine	9.75	9.40	9.28	9.48	0.24	2.53
Aspartic acid	10.4	10.7	10.6	10.6	0.15	1.42
Lysine	9.91	9.72	10.3	9.98	0.30	3.01
Methionine	2.95	3.06	2.82	2.94	0.12	4.08
Glutamic acid	14.6	14.5	14.3	14.5	0.15	1.03
Phenylalanine	3.99	3.91	3.81	3.90	0.09	2.31
Histidine	3.02	3.37	3.02	3.14	0.20	6.37
Arginine	5.50	6.55	6.45	6.17	0.58	9.40
Tyrosine	3.55	2.39	2.76	2.90	0.59	20.3
Cystine	1.54	1.21	1.40	1.38	0.17	12.3

CF= Cat fish, SF= Snake fish, TP= Tilapia fish SD = Standard deviation, CV% = Coefficient of variation percent,

Table 2: Various classes of amino acid (g/100g cp) in *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii*

Amino Acid	CF	SF	TF	Means	SD	CV%
TAA	97.1	95.7	95.4	96.1	0.91	0.95
TNEAA	48.3	46.4	46.7	47.1	1.02	2.17
%TNEAA	49.7	48.5	49.0	49.1	0.60	1.22
TEAA						
With His	48.8	49.3	48.7	48.9	0.32	0.65
No His	45.8	45.9	45.7	45.8	0.10	0.22
%TEAA						
With His	50.3	51.5	51.0	50.9	0.60	1.18
No His	46.9	48.0	47.9	47.6	0.61	1.28
TNAA	53.7	50.9	50.7	51.8	1.68	3.24
%TNAA	55.3	53.2	53.1	53.9	1.24	2.30
TAAA	25.0	25.2	24.9	25.0	0.15	0.60
%TAAA	25.7	26.3	26.1	26.0	0.31	1.24
TBAA	18.4	19.6	19.8	19.3	0.76	3.94
%TBAA	18.9	20.5	20.8	20.1	1.02	5.07
TArAA	7.54	6.30	6.57	6.80	0.65	9.56
%TArAA	7.77	6.58	6.89	7.08	0.62	8.76
TSAA	4.49	4.27	4.22	4.33	0.14	3.23
%TSAA	4.62	4.46	4.42	4.50	0.11	2.44
Leu/Ile ratio	2.09	2.17	2.20	2.15	0.06	2.79
Lue-Ile (diff)	5.09	5.07	5.06	5.07	0.02	0.39
P-PER	3.59	3.55	3.46	3.53	0.07	1.98
PI	5.72	5.68	6.04	5.81	0.20	3.44

Table 3: Amino Acid pattern of *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii* based on whole hen's amino acid

Amino Acid	CF	SF	TF	Mean	SD	CV%
Glycine	1.50	1.43	1.35	1.43	0.08	5.59
Alanine	1.16	1.10	1.18	1.15	0.04	3.48
Serine	0.56	0.52	0.51	0.53	0.03	5.66
Proline	0.08	0.86	0.83	0.83	0.03	3.71
Valine	0.62	0.62	0.62	0.62	0.0	0.0
Threonine	0.86	0.86	0.81	0.84	0.03	3.57
Isoluecine	0.83	0.77	0.75	0.78	0.04	5.13
Leucine	1.17	1.13	1.12	1.14	0.03	2.63
Aspartic acid	0.97	1.0	0.99	0.99	0.02	2.02
Lysine	1.60	1.57	1.66	1.61	0.05	3.11
Methonine	0.92	0.96	0.88	0.92	0.04	4.35
Glutamic acid	1.22	1.21	1.91	1.21	0.02	1.65
Phenylatanine	0.78	0.77	0.75	0.77	0.02	2.60
Histidine	1.26	1.40	1.26	1.31	0.08	6.11
Arginine	0.90	1.07	1.06	1.01	0.10	9.90
Tyrosine	0.89	0.60	0.69	0.73	0.15	20.5
Cystine	0.86	0.67	0.78	0.77	0.10	12.99
Try	-	-	- -	-		-

CF= Cat fish, SF= Snake fish, TP= Tilapia fish, SD = Standard Deviation, CV% = Coefficient of Variation percent,
= not determined

Table 4: Amino Acid scores of Clarias gariepinus, Channa striatus and Tilapia zillii based on amino acid scoring pattern

Amino Acid	CF	SF	TF	Mean	SD	CV%
Isoleuccine	1.17	1.08	1.06	1.10	0.06	5.45
Leucine	1.39	1.34	1.33	1.35	0.03	2.22
Lysine	1.80	1.77	1.87	1.81	0.05	2.76
Met + Cys	1.28	1.22	1.21	1.24	0.04	3.23
Phe + Try	1.26	1.05	1.10	1.14	0.11	9.65
Thry	1.09	1.10	1.03	1.07	0.04	3.74
Try	-	-	-	-	-	-
Val	0.93	0.93	0.93	0.93	0.0	0.0
Total	1.31	1.31	1.30	1.31	0.01	0.76

SD = Standard Deviation, CV% = Coefficient of variation percent,
= not deviation

Table 5: Amino Acid scores of Clarias gariepinus, Channa striatus and Tilapia zillii based on suggested amino acid requirements of pre-school children (2 -5 years)

Amino Acid	CF	SF	TF	Mean	SD	CV%
Isoleucine	1.66	1.55	1.51	1.57	0.08	5.10
Leucine	1.48	1.42	1.41	1.44	0.04	2.78
Lysine	1.71	1.68	1.78	1.72	0.05	2.91
Met + Cys	1.80	1.71	1.69	1.73	0.06	3.47
Phe + Tyr	1.20	1.0	1.04	1.08	0.11	10.2
Thr	1.29	1.29	1.21	1.26	0.05	3.97
Try	-	-	-	-	-	-
Val	1.33	1.33	1.33	1.33	0.0	0.0
His	1.59	1.77	1.59	1.65	0.10	6.06
Total	1.49	1.51	1.48	1.49	0.02	1.34

CF= Cat fish, SF= Snake fish, TP= Tilapia fish, SD= Standard Deviation, CV% = Coefficient of Variation percent,
- = Not determined

These are the essential amino acids that must be corrected for to bring all other EAAs to the expected standard requirement.

Conclusion

Investigations into the amino acid concentration of *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii* showed that the samples are very rich in acidic amino acids (aspartic and glutamic acids). The concentration of total non essential amino acid was fairly above that of total essential amino acids. Generally, the essential amino acids (EAAs) were much concentrated in the samples.The concentrations of leucine were generally much higher than those of isoleucine. Dietary excess of leucine could be counteracted by increasing the intake of niacin or tryptophan or by supplementation with isoleucine (FAO, 1995). The P-PER values in the samples were considerably high and would therefore be good complements in foods with relatively low values.

The amino acid scores (AAS) based on whole hen's egg, based on provisional essential amino acid scoring pattern and based on requirement of pre-school children (2 – 5 years old) in the samples showed valine as being limiting, although val is not actually one those that were considered limiting amino acids i.e. Lys, Met + Cys, Thr and Try.

Generally, the fish samples; *Clarias gariepinus*, *Channa striatus* and *Tilapia zillii* are good sources of amino acids and would be useful as food supplements and in food fortification.

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