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AMINO ACID COMPOSITION OF SNAKEHEAD FISH (*CHANNA STRIATUS*) OF VARIOUS SIZES OBTAINED AT DIFFERENT TIMES OF THE YEAR

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Snakehead fish (Channa striatus) or Haruan is one of the favourite fresh water fish in the Asia-Pacific countries. The fish has been traditionally used to heal wounds. The amino acid composition of wild type Haruan was analyzed in this study. The most abundant amino acid in Haruan was glutamic acid, followed by aspartic acid, lysine, arginine, leucine, alanine, valine, threonine and glycine, in a decreasing order. The Haruan caught during rainy season was found to contain higher amount of total amino acids. The essential amino acids made up 56% of its total amino acids content. Furthermore, each of the essential amino acids (except lysine) was found in higher quantity compared to other types of fishes. Haruan was found significantly rich in arginine, an important constituent in the process of wound healing. The amino acid composition of Haruan indicates that the fish is an excellent source of dietary protein for human.

Keywords: *Haruan (Channa striatus), Amino acid composition, Wound healing, Dietary protein*

INTRODUCTION

Haruan or snakehead fish is an obligatory air-breather and predaceous fish that resides in swamps, slow-flowing streams and in crevices near riverbanks in Southern China. In taxonomy, it belongs to the family *Channidae* (Qasim 1966), and its scientific name is *Channa striatus.*

Haruan is consumed for its dietary proteins. Nevertheless, it is also consumed as a remedy for healing of wound. Haruan has been reported to enhance dermal wound healing, to reduce post-operative pain and discomfort (Mat Jais *et al.* 1994), and in the treatment of skin conditions such as eczema. The studies on determination of tensile strength of healed wounds treated with Haruan have shown the efficacy of the fish in wound healing (Baie and Sheikh 2000a, 2000b). Its effectiveness in wound healing has led to the commercial production of Haruan creams and Haruan tablets, which are available in countries like Malaysia, Singapore and Indonesia. Recently, Haruan is cultured commercially in Thailand, Philippines and India.

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Haruan is available throughout the year in Malaysia. However, the smaller sizes Haruan are preferred as they are thought to provide better effect on healing of wounds as compared to the bigger sizes fishes. The amino acids that have been reported as part of the constituent of wound healing are arginine (Bowman 1980; Shearer *et al.* 1997; Selamnia *et al*. 1998), glycine (Heimann 1982), lysine, proline, glucosamine (D'Ambrosio *et al*. 2004), D-glucoronic acid (Sato *et al.* 1986) and carnosine (Fitzpatrick and Fisher 1982). Carnosine is a dipeptide of alanine and histidine. The objective of this study was to analyse the amino acids content of different sizes of Haruan caught in different months of the year.

METHODS AND MATERIALS

Amino acid analysis kit (AccQ tag) was purchased from Waters (Rydalmere BC NSW, Australia). All other chemicals were purchased from Sigma (St Louise, MO, USA).

Preparation of Haruan's muscle tissue

Three batches of Haruan caught in November 2002 (B1), January 2003 (B2) and April 2003 (B3), respectively were used in this study. The fishes from each batch were further subdivided according to their lengths. All fishes were washed, beheaded, sliced and covered with ice to ensure freshness of the fish tissues. The fish's muscle tissue was then sliced into smaller pieces and placed in sterile universal bottles and kept at –20°C prior to freeze-drying.

Acid hydrolysis

All glassware used in the sample preparation and analysis were washed with 6N HCl, rinsed with Milli Q water and dried in the oven. Moist muscle tissues were freeze-dried in a vacuum freeze dryer (CHRIS). Dried samples were homogenised into powder form. Sufficient sample containing approximately 40 mg protein was weighed into a hydrolysis tube, and 5 mL 6N HCl was added to it. The tube was then flushed for 30 seconds with nitrogen gas and immediately sealed with a Telfon-lined cap. The tube was placed in an electric oven for 24 hours at 110°C for sample hydrolysis. The tube was then cooled to room temperature and 400 μL of internal standard solution, α-aminobutyric acid 2.5 μmol/mL

(AABA), was added. The content of the tube were quantitatively transferred to a 100 mL clean volumetric flask. After thorough mixing, 1 mL of dilute sample was filtered, and 10 µL filtrate was placed in a derivative tube.

Derivatization of amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)

A clean syringe was used to deliver 10 µL filtrate of acid hydrolysis to the bottom of a clean 6 × 50 mm sample tube. A 70 µL of AccQ•Fluor Borate Buffer (Reagent 1) was then added to the sample tube by using a micropipette. The sample tube was vortexed briefly prior to adding of 20 µL of reconstituted AccQ•Fluor Reagent to the sample tube. After vortex for several seconds, the sample tube was let to stand for 1 minute at room temperature. A 9-inch Pasteur pipette was used to transfer the content of the sample tube to the bottom of an auto-sampler vial limited volume insert (LVI). The LVI was capped with a silicone-lined septum. Reaction requires only 1 minute, after which samples were heated for 10 minutes at 55°C.

Preparation of internal standard and calibration standard

The internal standard, AABA stock solution was used to prepare the calibration standard. To prepare a 50 µmol/mL internal standard stock solution, 0.258 g AABA was added to 50 mL 0.1N HCl. Prior to the preparation of the calibration standard with an internal standard, 1 mL of 50 µmol/mL internal standard stock solution was transferred to a clean 20 mL volumetric flask. The solution was then topped up to 20 mL with 0.1 N HCl until the final concentration of the internal standard solution was 2.5 µmol/mL. The calibration standard consisted of 1:1 (v/v) mixture of Pierce H amino acid standard (which contained 2.5 µmol/mL of each amino acid standard, except for 1.25 µmol/mL of cystine) and a 2.5 µmol/mL of AABA. Typically, a calibration standard with an internal standard was prepared by combined 80 µL 2.5 µmol/mL AABA with 80 µL Pierce H and then was topped up with 840 µL Milli-Q water in a 1000 µL clean auto-sampler vial. A volume of 10 µL calibration standard (contains of 2nmol of each standard amino acid components) was transferred from the 1:1 (v/v) mixture of Pierce H amino acid standard,

and 2.5 µmol/mL of AABA was placed in a derivatisation tube and carried through the same derivatisation procedure as mentioned earlier.

HPLC analysis of amino acids

The Waters AccQ•Tag amino acid analysis method requires a fluorescence detector. The excitation wavelength was 285 nm, the emission wavelength was 354 nm, filter and gain set were 1.5 second and 10, respectively. Eluent A and Eluent B were acetate-phosphate buffer and 60% acetonitrile: water, respectively. The column temperature was set at 37°C. The Column (Waters AccQ•Tag) was first conditioned with Eluent B at 1 mL/min flow rate for 5 minutes. This was followed by equilibrating the column in 100% AccQ•Tag Eluent A for 9 minutes at the same flow rate. Consistent period of the equilibration was kept for all the analysis. A blank (Milli-Q water) run was carried out before each analysis to determine baseline performance. The gradient shown in Table 1 was used in the process of analysing amino acids using HPLC. The total time between injections to end of the analysis was 50 minutes.

Table 1: Gradient table for amino acids analysis using HPLC

Time (min)	Flow rate (mL/min)	%A	%B
Initial	1.0	100.0	0.0
0.50	1.0	98.0	2.0
15.0	1.0	90.0	10.0
19.0	1.0	87.0	13.0
32.0	1.0	65.0	35.0
33.0	1.0	65.0	35.0
34.0	1.0	0.0	100.0
37.0	1.0	0.0	100.0
38.0	1.0	100.0	0.0
50.0	1.0	100.0	0.0

Statistical analysis

Each amino acid analysis was performed in triplicate. Results are presented as means ± the standard error of the mean (S.E.M). Analysis of variance (ANOVA) followed by Tukey's (Zar 1996) multiple comparisons of means procedure was used to evaluate differences between means in groups where appropriate. Differences where $p \leq 0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

Wild type Haruan was used in this study. Only the muscle tissue of Haruan was subjected for analysis as it is the main part of the fish that is consumed. All the fishes were caught at approximately the same location in the northern part of Malaysia. Three batches of fishes BI, B2 and B3 were caught in November, January and April, respectively. It is a dry period in November (30°), extremely dry in January (33°), while it is rainy in April (28°). The fish length was used as a guide to the age of the fishes; the length of the fish increases with age. Fish lengths were measured from head to tail and they were grouped into 16, 23, 24, 25, 28, 29, 30 and 38 cm.

The method used in this study only allowed the analysis of 17 amino acids. These amino acids were arginine, lysine, valine, threonine, leucine, tyrosine, histidine, isoleucine, phenylalanine, methionine, cysteine, glycine, proline, alanine, glutamic acid, aspartic acid and serine. Upon acid hydrolysis, asparagines and glutamine will be hydrolyzed to aspartic acid and glutamic acid, respectively. Thus, the content of aspartic acid represents the total content of asparagines and aspartic acid, and the same applies to glutamic acid. Tryptophan is destroyed upon acid hydrolysis, thus was not measured in this study.

Tables 2, 3 and 4 show the amino acids contents of different lengths of Haruan obtained from B1, B2 and B3, respectively. The amino acid content is presented as gram amino acid/100 gram total amino acids. In B1 (Table 2), there was no significant variation ($p>0.05$) in the amino acids contents amongst the 8 different fish lengths tested.

The major components of the amino acids were glutamic acid followed by aspartic acid, lysine, arginine, leucine, alanine, valine, threonine and glycine. In B2 (Table 3), the contents of arginine and lysine were significantly higher in Haruan with length of 23 cm or longer as compared to Haruan of 16 cm. Furthermore, the content of certain essential amino acids such as alanine and aspartic acid were significantly reduced when the fish length increased from 16 cm to 23 cm or longer. In B3 (Table 4), the content of arginine was significantly higher, parallel with the increase of fish length from 25 cm to 28 cm.

On the other hand, histidine was significantly higher in fish of 23 cm but lower in 29 cm fish. Both glycine and proline contents in all the three batches of Haruan increased with advancing fish length although the increase was not significant ($p>0.05$). The rationale to the increase of both

of these amino acids in fish is perhaps a consequence of increased amounts of connective tissue such as collagen, which indicates progressive growth of the smaller sizes fish while glycine is apparently important in collagen formation (Fietzek *et al*. 1972).

Table 2: Amino acid composition of Haruan's muscle tissue for fishes with different lengths from Batch B1

Amino acid	Amino acid composition (g amino acid/100 g total amino acids)							
	16 (n=3)	**23 (n=3)**	**24 (n=3)**	**25 (n=3)**	**28 (n=3)**	**29 (n=3)**	**30 (n=3)**	**38 (n=3)**
Arginine	8.713[a]	8.915[a]	8.389[a]	8.422[a]	9.040[a]	9.177[a]	8.530[a]	8.686[a]
Lysine	8.612[a]	8.609[a]	8.776[a]	8.785[a]	8.616[a]	8.810[a]	9.132[a]	9.062[a]
Valine	5.136[a]	4.791[a]	4.665[a]	4.687[a]	4.752[a]	5.083[a]	4.900[a]	5.119[a]
Threonine	5.449[a]	5.445[a]	5.434[a]	5.444[a]	5.187[a]	5.228[a]	5.153[a]	5.156[a]
Leucine	8.871[a]	8.826[a]	8.570[a]	8.488[a]	8.563[a]	8.663[a]	8.554[a]	8.353[a]
Tyrosine	4.220[a]	4.260[a]	4.136[a]	4.307[a]	4.132[a]	4.077[a]	4.190[a]	4.020[a]
Histidine	2.948[a]	3.060[a]	2.767[a]	2.808[a]	2.755[a]	2.755[a]	2.601[a]	2.486[a]
Isoleucine	5.226[a]	4.651[a]	4.494[a]	4.490[a]	4.557[a]	5.164[a]	4.770[a]	4.889[a]
Phenylalanine	5.088[a]	4.984[a]	4.822[a]	4.677[a]	4.884[a]	4.958[a]	4.638[a]	4.704[a]
Methionine	3.923[a]	3.778[a]	3.734[a]	3.674[a]	3.672[a]	3.142[a]	3.493[a]	3.441[a]
Cysteine	0.871[a]	1.017[a]	1.197[a]	1.641[a]	1.293[a]	1.045[a]	1.390[a]	1.173[a]
Glycine	4.550[a]	4.858[a]	4.902[a]	4.963[a]	5.197[a]	5.030[a]	4.724[a]	5.967[a]
Proline	3.596[a]	3.575[a]	3.815[a]	3.812[a]	4.015[a]	4.077[a]	3.623[a]	3.651[a]
Alanine	5.777[a]	5.999[a]	5.835[a]	5.783[a]	6.044[a]	5.931[a]	5.814[a]	5.823[a]
Glutamic acid	13.483[a]	13.468[a]	14.135[a]	14.002[a]	13.415[a]	13.485[a]	14.568[a]	13.838[a]
Aspartic Acid	8.420[a]	8.533[a]	9.373[a]	8.987[a]	8.919[a]	8.362[a]	9.220[a]	8.845[a]
Serine	5.120[a]	5.234[a]	4.959[a]	5.032[a]	4.961[a]	5.017[a]	4.703[a]	4.812[a]

(i) Means in the same row with unlike superscript differ significantly ($p \leq 0.05$)

Table 3: Amino acid composition of Haruan's muscle tissue for fishes with different lengths from Batch B2

Amino acid	Amino acid composition (g amino acid/100 g total amino acids)							
	16 (n=3)	**23 (n=3)**	**24 (n=3)**	**25 (n=3)**	**28 (n=3)**	**29 (n=3)**	**30 (n=3)**	**38 (n=3)**
Arginine	8.438[a]	9.748[b]	8.859[ab]	8.868[ab]	8.978[ab]	9.149[ab]	8.566[ab]	8.656[ab]
Lysine	7.545[a]	9.008[b]	8.643[ab]	8.425[ab]	8.387[ab]	8.405[ab]	8.821[b]	8.155[ab]
Valine	4.992[a]	5.051[a]	4.909[a]	4.777[a]	4.778[a]	5.097[a]	4.934[a]	5.198[a]
Threonine	4.950[a]	5.280[a]	5.340[a]	5.243[a]	5.207[a]	5.416[a]	5.020[a]	5.103[a]
Leucine	8.599[a]	8.773[a]	8.564[a]	8.692[a]	8.635[a]	8.771[a]	8.592[a]	8.427[a]
Tyrosine	4.015[a]	4.737[a]	4.439[a]	5.221[a]	5.168[a]	5.439[a]	5.038[a]	4.070[a]
Histidine	2.765[a]	2.913[a]	2.789[a]	2.827[a]	2.833[a]	2.886[a]	2.725[a]	2.572[a]
Isoleucine	4.984[a]	4.920[a]	4.799[a]	4.645[a]	4.607[a]	5.110[a]	4.928[a]	4.995[a]
Phenylalanine	4.730[a]	4.968[a]	4.862[a]	4.859 [a]	4.814[a]	5.044[a]	4.760[a]	4.698[a]
Methionine	3.495[a]	3.066[a]	3.487[a]	3.502[a]	3.376[a]	3.367[a]	3.301[a]	3.429[a]
Cysteine	0.968[a]	1.195[a]	1.083[a]	1.506[a]	1.175[a]	1.197[a]	0.822[a]	1.076[a]
Glycine	4.842[a]	4.972[a]	5.214[a]	5.202[a]	5.298 [a]	5.184[a]	4.926[a]	5.631[a]
Proline	3.661[a]	3.589[a]	3.673[a]	3.782[a]	3.969[a]	3.776[a]	3.862[a]	3.704[a]
Alanine	6.160[b]	5.866[ab]	5.868[ab]	5.685[a]	5.858[ab]	5.602[a]	5.840[ab]	5.952[ab]
Glutamic acid	14.845[a]	13.241[a]	13.893[a]	13.603[a]	13.456[a]	12.961[a]	14.187[a]	14.098[a]
Aspartic acid	10.457[b]	7.836[a]	8.625[a]	8.300[a]	8.578[a]	7.767[a]	8.965[ab]	9.388[ab]
Serine	4.554[a]	4.837[a]	4.955[a]	4.855[a]	4.883[a]	4.830[a]	4.713[a]	4.848[a]

(i) Means in the same row with unlike superscript differ significantly ($p \leq 0.05$)
No significant difference among the values which are annotated with the same superscript 'a' in the same row.
No significant difference among the values which are annotated with the same superscript 'b' in the same row.
Values annotated with superscript 'ab' mean there are no significant difference either to 'a' or 'b' in the same row.
Significant difference only refers to the values which are annotated with different superscript in the same row.

Table 4: Amino acid composition of Haruan's muscle tissue for fishes with different lengths from Batch B3

Amino acid	Amino acid composition (g amino acid / 100 g total amino acids)							
	16 (n=3)	23 (n=3)	24 (n=3)	25 (n=3)	28 (n=3)	29 (n=3)	30 (n=3)	38 (n=3)
Arginine	8.378^{a}	9.004^{ab}	8.913^{ab}	8.431^{a}	9.176^{b}	8.413^{a}	8.563^{ab}	8.525^{a}
Lysine	9.367^{a}	8.852^{a}	8.826^{a}	9.359^{a}	8.682^{a}	9.107^{a}	8.986^{a}	9.038^{a}
Valine	5.137^{a}	4.981^{a}	5.028^{a}	5.014^{a}	5.284^{a}	5.174^{a}	5.198^{a}	5.204^{a}
Threonine	4.823^{a}	5.347^{a}	5.079^{a}	5.001^{a}	5.162^{a}	4.932^{a}	4.901^{a}	5.064^{a}
Leucine	8.650^{a}	8.833^{a}	8.629^{a}	8.373^{a}	8.493^{a}	8.308^{a}	8.280^{a}	8.355^{a}
Tyrosine	3.853^{a}	4.215^{a}	4.201^{a}	4.167^{a}	4.214^{a}	3.950^{a}	4.065^{a}	4.136^{a}
Histidine	2.816^{ab}	3.015^{b}	2.938^{ab}	2.813^{ab}	2.928^{ab}	2.713^{a}	2.812^{ab}	2.822^{ab}
Isoleucine	5.056^{a}	4.901^{a}	4.948^{a}	4.883^{a}	5.264^{a}	5.017^{a}	5.135^{a}	5.055^{a}
Phenylalanine	4.780^{a}	4.983^{a}	4.868^{a}	4.644^{a}	4.861^{a}	4.559^{a}	4.583^{a}	4.597^{a}
Methionine	3.191^{a}	3.411^{a}	3.667^{a}	3.314^{a}	3.471^{a}	3.243^{a}	3.241^{a}	3.005^{a}
Cysteine	1.140^{a}	0.486^{a}	1.031^{a}	1.231^{a}	0.828^{a}	0.830^{a}	0.989^{a}	0.902^{a}
Glycine	4.539^{a}	4.821^{ab}	4.902^{ab}	4.854^{ab}	5.068^{b}	4.765^{ab}	4.756^{ab}	4.811^{ab}
Proline	3.355^{a}	3.721^{a}	3.582^{a}	3.600^{a}	3.666^{a}	3.619^{a}	3.635^{a}	3.763^{a}
Alanine	6.024^{a}	5.881^{a}	5.748 ^{a}	5.810^{a}	5.747^{a}	5.997^{a}	5.938^{a}	5.820^{a}
Glutamic acid	14.444^{ab}	13.585^{ab}	13.858^{ab}	14.465^{ab}	13.557^{a}	14.446^{ab}	14.568^{b}	14.300^{ab}
Aspartic acid	9.881^{a}	9.030^{a}	9.097^{a}	9.492^{a}	8.982^{a}	10.133^{a}	9.872^{a}	10.083^{a}
Serine	4.566^{a}	4.932^{a}	4.685^{a}	4.548^{a}	4.618^{a}	4.794^{a}	4.478^{a}	4.518^{a}

(i) Mean ± S.E.M. (n=3)

(ii) Means in the same row with unlike superscript differ significantly ($p \leq 0.05$)
No significant difference among the values which are annotated with the same superscript 'a' in the same row.
No significant difference among the values which are annotated with the same superscript 'b' in the same row.
Values annotated with superscript 'ab' mean there are no significant difference either to 'a' or 'b' in the same row.
Significant difference only refers to the values which are annotated with different superscript in the same row.

In summary, there was no general pattern of correlation between the fish length and amino acid content for the three batches of Haruan suggesting that the fishes with length between 16 cm to 38 cm offer relatively similar value as regard to its dietary protein. However, from an economical point of view, consumption of bigger fish is advised.

When comparing between the different batches of Haruan, the contents of the amino acids in all the three batches of Haruan were almost similar except for lysine and aspartic acid, which were significantly higher ($p \leq 0.05$) in B3 as compared to B1 and B2. The most abundant amino acid in Haruan (in decreasing order) was glutamic acid, aspartic acid, lysine, arginine, leucine, alanine, valine, threonine and glycine. However, histidine and cysteine were present in much lower amount. The essential amino acids comprised of more than 50% of the total amino

acid content of Haruan; 56.56%, 56.74% and 56.41% of the total amino acids of B1, B2 and B3, respectively, are essential amino acids.

The total amino acid contents of B1, B2 and B3 were 65.431 ± 1.245, 64.031 ± 0.891, 71.126 ± 0.707 (g/100 g dry sample), respectively. The total amino acids contents from the three batches of Haruan showed that fishes that were caught during the dry season (B1 and B2) yielded relatively low protein as compared to the fishes caught during the rainy season (B3), whereby the average protein contents of B1, B2 and B3 fish were 364.2 ± 4.6 mg protein/g tissue 370.5 ± 5.2 mg protein/g tissue and 449.2 ± 9.7 mg protein/mg tissue, respectively. This can be explained by the increase in food supply during the rainy season as Haruan is a carnivore that feeds on smaller organisms (fishes).

Table 5 shows the mean amino acid contents from the three batches of Haruan and other types of fishes among the teleost family; Keli, Rainbow trout, Atlantic salmon (Wilson and Cowey 1985). Keli is a local air-breathing fish that shares the same habitat with Haruan. When compared to Keli, Haruan was found to contain significantly higher ($p \leq 0.05$) arginine, threonine and serine whilst the contents of valine, isoleucine, proline and aspartic acid in Haruan were significantly lower ($p \leq 0.05$). When compared to the published data of Rainbow trout and Atlantic salmon, the amino acid composition of Haruan was much higher in arginine, threonine, leucine, tyrosine, isoleucine, phenylanine and methionine but lower in glycine, proline, alanine and aspartic acid. The amino acids composition of Haruan that was reported by Mat Jais *et al.* (1994) showed some differences as compared to result of this study. The rationale for this may be due to the location where the Haruan that were used. In our study, the fish were caught around the northern region of peninsular Malaysia, whereas the Haruan that were used in the Mat Jais *et al.* (1994) were obtained from Kota Kinabalu, Sabah.

With regard to its uses as remedy for wound healing, Haruan is found to be rich in arginine. Other amino acids reported to be involved in wound healing (valine, histidine, glycine, proline and alanine) were present in relatively lower quantity compared to other fishes (Table 5). The quantity of arginine may be critical as it plays multiple roles in the process of wound healing. Arginine stimulates the release of hormones paramount to wound healing, such as growth hormone from the pituitary and insulin from the pancreas (Barbul 1990). In post-injury catabolic states, arginine has also been shown to decrease urinary nitrogen losses in order to promote positive nitrogen balance (Elsair 1978).

Table 5: Mean average amino acid content of fishes in B1, B2 and B3 Haruan, Keli, Rainbow troat, Atlantic salmon

Amino acid	Amino acid composition (g amino acid/100 g total amino acids)						
	Haruan B1 (n=16)	**Haruan B2 (n=16)**	**Haruan B3 (n=24)**	**Keli (n=6)**	**Rainbow trout***	**Atlantic Salmon***	**Haruan Mat Jais *et al.* (1994)**
Arginine	8.734^{b}	8.908^{b}	8.675^{b}	7.925^{a}	6.41	6.61	2.34
Lysine	8.800^{ab}	8.424^{a}	9.027^{b}	8.606^{ab}	8.49	9.28	5.89
Valine	4.892^{a}	4.967^{a}	5.128^{ab}	5.258^{b}	5.09	5.09	4.14
Threonine	5.311^{b}	5.195^{b}	5.039^{ab}	4.822^{a}	4.76	4.95	6.10
Leucine	8.611^{a}	8.632^{a}	8.490^{a}	8.536^{a}	7.59	7.72	10.51
Tyrosine	4.168^{a}	4.766^{b}	4.100^{a}	4.317^{ab}	3.38	3.50	3.36
Histidine	2.772^{a}	2.789^{a}	2.857^{a}	2.833^{a}	2.96	3.02	3.24
Isoleucine	4.779^{a}	4.874^{a}	5.032^{ab}	4.734^{b}	4.34	4.41	5.54
Phenylalanine	4.844^{ab}	4.842^{ab}	4.734^{a}	5.039^{b}	4.38	4.36	5.04
Methionine	3.607^{a}	3.378^{a}	3.318^{a}	3.567^{a}	2.88	1.83	5.99
Cysteine	1.203^{b}	1.128^{ab}	0.930^{ab}	0.835^{a}	0.80	0.95	3.82
Glycine	5.024^{a}	5.160^{a}	4.815^{a}	4.705^{a}	7.76	7.41	9.77
Proline	3.770^{ab}	3.752^{a}	3.618^{a}	3.965^{b}	4.89	4.64	9.17
Alanine	5.876^{a}	5.855^{a}	5.871^{a}	8.938^{b}	6.57	6.52	8.40
Glutamic acid	13.799^{a}	13.786^{a}	14.153^{a}	13.853^{a}	14.22	14.31	8.46
Aspartic acid	8.832^{ab}	8.739^{a}	9.571^{b}	8.447^{a}	9.94	9.92	4.19
Serine	4.980^{b}	4.809^{b}	4.642^{b}	3.645^{a}	4.66	4.61	4.01

(i) Mean ± S.E.M.

(ii) Means in the same row with unlike superscript differ significantly ($p \leq 0.05$)

No significant difference among the values which are annotated with the same superscript 'a' in the same row.

No significant difference among the values which are annotated with the same superscript 'b' in the same row.

Values annotated with superscript 'ab' mean there are no significant difference either to 'a' or 'b' in the same row.

Significant difference only refers to the values which are annotated with different superscript in the same row.

*Wilson and Cowey (1985)

Furthermore, arginine is a substrate for two enzymes integral to wound healing namely, nitric oxide synthetase (NOS) and arginase. Arginine is metabolised in wounds via arginase, an enzyme that is abundantly present in wound fluid (Albina *et al.* 1990).

Finally, during the formation of the connective tissue, arginine is utilised to produce hydroxyproline, which is important in collagen formation as it contains about 9.1% of the total amino acid residues in the collagen sequence (Devlin 1992). Nevertheless, wound healing process is complicated and not fully understood. Besides amino acids, the composition of fatty acids in Haruan was also recognised to stimulate healing of wounds (Baie and Sheikh 2000a, 2000b).

With regard to its nutritional value, Haruan provides all the essential amino acids needed by human. The quantities of these amino acids (except lysine) were present at higher amount than other types of fishes (Table 5). Furthermore, the ratio of essential amino acid to non-essential amino acid was 1:33 indicating that Haruan could provide high quality proteins or well-balanced protein deposition as the main source of amino acids is proteins.

CONCLUSION

The amino acid composition of Haruan's muscle tissue showed that it is a good source of dietary amino acid. All the essentially amino acids required by human can be obtained from Haruan. Furthermore, most of the essential amino acids are present in higher quantities compared to Keli, Rainbow trout or Atlantic salmon. Haruan is found rich in arginine, which is a critical amino acid in the process of wound healing. It also supplies physiological beneficial amino acids, such as histidine. Therefore, in view of the potential values of its amino acid contents, Haruan serves as a nutritious health food.

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