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Amino Acid Composition of Ten Fish Species from Hel River, North-East India

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In this study, amino acid composition of 10 fish species *viz. Barilius bendelisis*, *Chagunius chagunio*, *Garra gotyla*, *Labeo pangusia*, *Neolissochilus hexagonolepis*, *Raiamas bola*, *Tor putitora*, *Cyprinion semiplotum*, *Barilius barna* and *Psilorhynchus nudithoracicus* from Hel river of North-East India are reported. A total of eleven different amino acids were detected in all the 10 fish species. *C. chagunio* showed the maximum total essential amino acid content (43.88 %) followed by *L. pangusia* (42.79 %) and *P. nudithoracicus* (42.05 %) and the lowest being in *G. gotyla* (26.45 %). Threonine, valine and lysine are the three essential amino acids found in all the 10 fish species with varying amounts. Therefore, the fish species selected for this study can be good sources of both essential and non-essential amino acids for the human diet.

Keywords: Amino acids, fish, Hel river, North-East India.

INTRODUCTION

Fishes are indispensible source of high quality animal proteins and one of the most significant nutritional qualities of protein is the composition of amino acid. Amino acids, the building blocks of body proteins, are mostly obtained from proteins in diet and responsible for growth, repair and maintenance of cell [1]. Proteins are the most abundant macromolecules found in biological systems. They are present in diverse forms such as structural elements, enzymes, hormones, antibodies, receptors, signalling molecules *etc*. with specific biological functions and play vital roles in human nutrition [2-4]. Thus, a sufficient amount of dietary protein is required to maintain cellular integrity and function, health and reproduction. Dietary protein's main purpose is to provide amino acids for the biosynthesis of proteins and all essential amino acids should be provided to the tissues in a proper amount for optimal protein synthesis. Fish proteins contain all the essential amino acids as needed for human nutrition and consequently, increase the overall quality of protein in a diet [1]. Amino acids can lessen blood cholesterol and antimutagenicity [5]. Amino acids like glutamic acid, aspartic acid and glycine are known to perform a role in the process of wound healing [6] and some other amino acids like methionine, histidine, tyrosine, lysine and tryptophan are considered to act as antioxidants [6,7].

North-East (NE) India consisting of eight states *viz*. Assam, Meghalaya, Tripura, Arunachal Pradesh, Manipur, Nagaland, Mizoram and Sikkim is known for its diverse ethnicity and bioresources. The Hel river stream of western Assam, North-East India, originates from Bhutan. It is known as Gangia river at Dotma, Kokrajhar and it meets the Brahmaputra river near Bagribari. In this study, we are reporting the amino acid composition of the 10 fish species from the Hel river of North-East India.

EXPERIMENTAL

Sample collection, identification and preparation: A total of 10 fish species *viz*., *Barilius bendelisis* (Hamilton, 1822), *Chagunius chagunio* (Hamilton, 1822), *Garra gotyla* (Gray, 1832), *Labeo pangusia* (Hamilton, 1822), *Neolissochilus hexagonolepis* (McClelland, 1839), *Raiamas bola* (Hamilton, 1822), *Tor putitora* (Hamilton, 1822), *Cyprinion semiplotum* (McClelland, 1839), *Barilius barna* (Hamilton, 1822) and *Psilorhynchus nudithoracicus* (Tilak and Husain, 1980) from Hel river were collected from Serfanguri, Kokrajhar. The species were identified at Zoological Survey of India, Shillong, Meghalaya (No. F. 4-2/2015-16/Tech./555, 4th August, 2015 and No. F. 4-2/2015-16/Tech./87, 5th February, 2016). After collection, the fish species were washed with running tap water, dried at 55 °C in a hot air oven for 2 days and boneless muscles were mashed to fine powder using a grinder. It was taken in an air tight plastic container and then stored at -20 °C in a deep freezer till further analyses.

Determination of amino acids: 20 mg of sample was magnetically stirred with 20 mL of 0.1 N HCl for 15 min, kept for 1 h, then 20 mL of 80 % methanol was added and again stirred for 15 min. It is incubated at -20 °C overnight, centrifuged at 4 °C at 13000 rpm for 15 min and the supernatant was dried by heating in a dry bath at 60 °C under nitrogen atmosphere. To this, 50 µL of PITC reagent was added and then incubated at 45 °C for 1 h and dried completely. To this dried sample, 200 µL of buffer A was added and 20 µL of it was injected into HPLC (Agilent 1200 series, Zorbax 300 SB-C18 column with 4.6 × 250 mm, 254 nm of wavelength). The sample was allowed to run for 82 min. The mobile phases used were buffer A (10 mM sodium acetate adjusted to pH 6.4 with 6 % acetic acid) and buffer B (buffer A + acetonitrile, 40:60). The identification and quantification of amino acids were done by comparing the retention times and peak areas of standards (mixture).

RESULTS AND DISCUSSION

In this study, amino acids were identified by comparing the retention times and peak areas of the individual fish sample with that of acidic and basic amino acid mixture standard. The amino acid profiles of 10 fish species expressed in percentage of total area of amino acids are presented Table-1. It is observed that the fish species contain various amino acids both essential and non-essential in varying amounts. *B. bendelisis* showed the highest anserine content (7.31 %) and lowest tryptophan content (0.61 %), while anserine was found to be lowest in *C. chagunio* (0.30 %) and 1-methyl histidine was found to be the maximum in *C. chagunio* (7.15 %). The highest taurine content (18.08 %) and lowest histidine content (0.75 %) was detected in *G. gotyla*. The highest percentage of amino acid detected in *L. pangusia* was asparagine (10.53 %) and threonine was found to be the lowest (0.41 %). The amount of amino acid content of fishes varies based on species, size, age, food resources, reproductive conditions and environmental conditions [6,8]. The highest content of amino acids observed in *N. hexagonolepis, R. bola*, *T. putitora*, *C. semiplotum*, *B. barna* and *P. nudithoracicus* were phosphoserine (13.22 %), tryptophan (11.48 %), ornithine (9.71 %), glycine (9.01 %), 1-methyl histidine (11.24 %), and valine (8.64 %), respectively. Glycine was detected in all other fish species except *L. pangusia*, *N. hexagonolepis* and *T. putitora* (Table-1). Glycine helps in metabolic regulation, prevents tissue injury, increases anti-antioxidant activity, promotes protein synthesis and wound healing, and improves immunity and treatment of metabolic disorders in obesity, diabetes, cardiovascular disease, ischemia-reperfusion injuries, cancer, and various inflammatory diseases [2,3,9]. It is seen from Table-1 that 11 different amino acids were detected in all the 10 fish species and these were phosphoserine (2.82 % in F-10 to 13.22 % in F-5), aspartic acid (0.55 % in F-7 to 6.43 % in F-1), glutamic acid (1.42 % in F-8 to 5.15 % in F-9), amino adipic acid (0.68 % in F-2 to 6.25 % in F-7), serine (1.97 % in F-7 to 13.63 % in F-3), taurine (1.96 % in F-1 to 18.08 % in F-3), 1-methyl histidine (1.57 % in F-1 to 11.24 % in F-9), ornithine (2.85 % in F-9 to 9.71 % in F-7), threonine (0.41 % in F-4 to 7.34 % in F-7), valine (0.82 % in F-3 to 10.35 % in F-4), and lysine (0.55 % in F-8 to 10.52 % in F-3). Therefore, all of the 10 fish species of this study can serve as good natural supplements for all these eleven number of amino acids. Amino acids are the important components for healing processes and deficiency of these components hinder the recovery process. The amino acid composition in fish is similar to that in man and therefore, people can gain the essential and non-essential amino acids in abundance and maintain proper balance by eating fish [10].

The variation of total essential amino acids (TEAA) of 10 fish species are shown in Fig. 1. *C. chagunio* showed the maximum total essential amino acid content (43.88 %) followed by *L. pangusia* (42.79 %) and *P. nudithoracicus* (42.05 %) and the lowest total essential amino acid was observed in *G. gotyla* (26.45 %). Therefore, the fish species with high content of total essential amino acid can be used for natural supplementation of essential amino acids (EAA). Threonine, histidine, arginine, valine, methionine, isoleucine, leucine, tryptophan, phenylalanine and lysine are the 10 essential amino acids. In this study, threonine (0.41 to 7.34 %), valine (0.82 to 10.35 %) and lysine (0.55 to 10.52 %) are the three essential amino acids found in all the ten fish species in varying amounts (Table-1). While the threonine, valine, and lysine content in five cold-water fish species reported by Sarma *et al.* [6] varied from 0.382 to 1.033 %, 0.528 to 0.855 % and 0.537 to 2.031 %, respectively which are comparatively below the levels to that of the report presented in this study. Therefore, all the fish species of this investigation can serve as a good natural supplement for threonine, valine and lysine. Threonine is an essential amino acid used for treating several nervous system complaints including spinal spasticity, multiple sclerosis, familial spastic paraparesis, and amyotrophic lateral sclerosis [2,3]. Valine involves in many metabolic pathways and is considered to be essential one for protein synthesis and optimal growth [11]. Lysine is also essential for optimal growth of the body, used for treatment of cold sores, and lack of this leads to immunodeficiency [2,3]. Histidine and phenylalanine were found in the 9 fish species in varying amounts (Table-1), while histidine was not detected in *N. hexagonolepis* and phenylalanine was not detected in *B. barna*. Histidine is a precursor of histamine. It is required for various roles in protein interaction, growth and repair of tissue, maintenance of myelin sheaths, and is also needed in removing heavy metals from the body [2,3,12]. Glycine, one of the major components of human skin collagen together with other amino acids like alanine, proline, serine, isoleucine, and phenylalanine, form a polypeptide that promotes regrowth and tissue healing [3]. However, in the present study, arginine was seen only in two fish species *viz*. *G. gotyla* (1.14 %) and *C. semiplotum* (3.36 %) and it was not detected in other fish species. Arginine plays significant roles in cell division, wound healing, ammonia removal, immune function, hormone release, neurotransmission, blood clotting, and maintenance of blood pressure [2]. Methionine was not detected only in *G. gotyla* and *N. hexagonolepis*, isoleucine was not found in *G. gotyla* and *T. putitora*, and tryptophan was not detected only in *L. pangusia* and *C. semiplotum*. While leucine was found in 5 fish species *viz*. *B. bendelisis* (4.46 %), *C. chagunio* (6.09 %), *L. pangusia* (9.88 %), *B. barna* (5.23 %) and *P. nudithoracicus* (6.33 %) and it was not detected

TABLE-1
AMINO ACID PROFILES (% OF TOTAL AREA OF AMINO ACIDS) OF 10 FISH SPECIES

Amino acids	Fish species									
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10
Phosphoserine	6.61	4.35	8.73	9.44	13.22	10.86	3.51	3.11	4.12	2.82
Aspartic acid	6.43	5.16	3.93	2.51	4.58	1.70	0.55	1.76	1.41	5.25
Glutamic acid	4.78	3.30	4.83	2.60	3.60	2.11	3.56	1.42	5.15	4.58
Amino adipic acid	4.96	0.68	2.40	2.26	2.66	0.69	6.25	3.13	3.91	2.63
Hydroxyproline	nd	nd	1.46	0.64	1.76	0.85	1.38	nd	nd	nd
Phosphoenolamine	6.82	2.61	1.05	nd	4.20	1.78	3.99	2.99	5.34	6.74
Serine	5.42	2.94	13.63	4.57	10.09	2.71	1.97	2.12	6.99	4.31
Glycine	6.88	6.45	2.88	nd	nd	4.25	nd	9.01	4.86	5.92
Asparagine	nd	nd	11.40	10.53	2.80	4.67	7.86	nd	3.71	nd
Taurine	1.96	3.70	18.08	3.76	5.45	6.52	4.33	3.47	1.55	2.26
Alanine	3.73	2.70	nd	3.60	2.20	1.88	6.28	0.78	4.01	3.42
β-Amino butyric acid	nd	nd	nd	1.43	nd	nd	2.77	nd	nd	nd
Carnosine	2.52	3.85	nd	2.34	nd	nd	2.28	5.32	2.65	3.24
Proline	2.05	1.57	nd	nd	2.01	nd	nd	nd	1.72	4.36
3-Methyl histidine	1.63	nd	nd	1.05	nd	nd	nd	1.80	1.89	1.09
1-Methyl histidine	1.57	7.15	1.79	4.56	3.04	10.17	2.66	5.11	11.24	5.34
Anserine	7.31	0.30	nd	0.74	5.82	11.08	nd	nd	nd	nd
Tyrosine	nd	4.30	nd	1.32	nd	nd	nd	4.53	1.74	nd
Cystathionine	1.99	nd	nd	1.43	2.95	nd	1.47	4.80	2.22	1.54
Cysteine	nd	2.91	nd	nd	nd	nd	nd	1.57	nd	nd
Hydroxylysine	nd	nd	nd	nd	nd	nd	7.78	7.77	nd	nd
Ornithine	3.10	4.14	3.34	4.43	4.63	7.15	9.71	5.58	2.85	4.41
Essential amino acid										
Threonine	3.92	5.98	0.77	0.41	3.95	4.78	7.34	1.33	4.29	0.51
Histidine	4.08	6.31	0.75	4.33	nd	2.10	3.46	3.27	4.49	3.43
Arginine	nd	nd	1.14	nd	nd	nd	nd	3.36	nd	nd
Valine	5.53	2.58	0.82	10.35	5.94	5.26	8.10	7.64	3.72	8.64
Methionine	3.94	5.35	nd	5.66	nd	2.96	3.29	8.45	7.01	3.61
Isoleucine	0.74	1.94	nd	1.20	2.37	0.36	nd	3.04	1.48	0.65
Leucine	4.46	6.09	nd	9.88	nd	nd	nd	nd	5.23	6.33
Tryptophan	0.61	5.02	10.43	nd	7.74	11.48	2.57	nd	2.07	5.55
Phenylalanine	5.58	6.86	2.02	6.27	5.30	4.53	6.25	8.07	nd	5.91
Lysine	3.38	3.75	10.52	4.69	5.67	2.10	2.62	0.55	6.35	7.42
Total amino acid	100	99.99	99.97	100	99.98	99.99	99.98	99.98	100	99.96
Total essential amino acid	32.24	43.88	26.45	42.79	30.97	33.57	33.63	35.71	34.64	42.05

F-1 = *B. bendelisis*; F-2 = *C. chagunio*; F-3 = *G. gotyla*; F-4 = *L. pangusia*; F-5 = *N. hexagonolepis*; F-6 = *R. bola*; F-7 = *T. putitora*; F-8 = *C. semiplotum*; F-9 = *B. barna*; F-10 = *P. nudithoracicus*. nd = not detected.

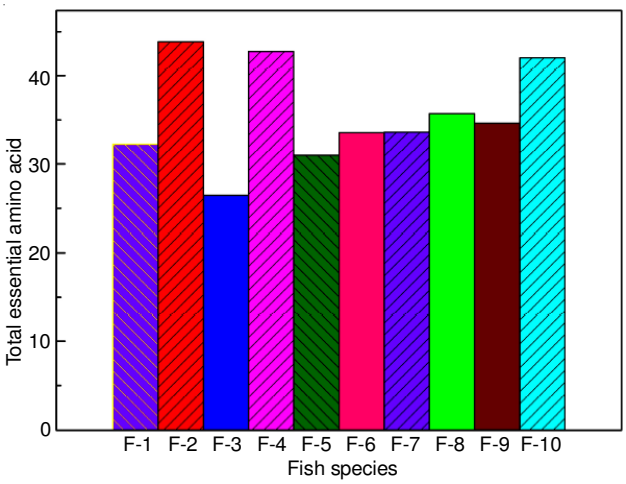

Fig. 1. Variation of total essential amino acid (% of total area of amino acids) of 10 fish species

in other fish species. Methionine is used for treating liver disorders, improving wound healing, and treating depression, alcoholism, allergies, asthma, copper poisoning, radiation side effects, schizophrenia, drug withdrawal, and Parkinson's disease [2,3,13]. Tryptophan is the precursor of serotonin, melatonin, tryptamine, and kynurenine and it plays an important role in the functioning of neurotransmitters [14]. Tryptophan supplement is used in treatment of pain, insomnia, depression, seasonal affective disorder, bulimia, premenstrual dysphoric disorder, hyperactivity disorder, and chronic fatigue [2,14]. Leucine can stimulate muscle protein synthesis and has key therapeutic roles in burn, trauma and sepsis [2,3,15]. Kim *et al.* [15] reported that leucine has strong cytotoxic activity against cancer cells. Therefore, it can be summarized that all these ten fish species selected for the present study have a good source of essential amino acids for the human diet.

Conclusion

This investigation revealed that the fish species from Hel river of North-East India have variable amino acid composition. *C. chagunio* exhibited the maximum total essential amino acid content followed by *L. pangusia* and *P. nudithoracicus* and the lowest total essential amino acid was observed in *G. gotyla*. It can be summarized that these fish species can be good sources of both essential and non-essential amino acids, and can serve as a good natural supplement for essential amino

acids which can contribute to the growth and proper maintenance of human health.

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