CHAPTER VI

Amino Acid Composition of Fish Species

VI.1. Materials and Methods

VI.1.1. Sample preparation

The sample of nine fish species was prepared as per the procedure mentioned in the **Section IV.1.1** (**Page no. 58**).

VI.1.2. Determination of amino acids

The fish sample of 20 mg was mixed with 20 mL of 0.1 N HCl and stirred magnetically for 15 min. It was then kept for 1 h and 20 mL of methanol (80 %) was added and stirred again for 15 min. Thereafter, it was incubated overnight at -20^{o}C, centrifuged at 13000 rpm at 4^{o}C for 15 min. The supernatant was then dried through heating over the dry bath at 60^{o}C under the nitrogen atmosphere. To the dry mass, PITC reagent of 50 µL was added, incubated for 1 h at 45^{o}C, and then it was completely dried. To the dry sample, the buffer A solution of 200 µL was added and then 20 µL of this was injected to High Performance Liquid Chromatography (HPLC) (Agilent 1200 series, Zorbax 300 SB, column C18 with 4.6 × 250 mm, wavelength of 254 nm), and allowed to run for 82 min. The mobile phases are buffer A (10 mM CH_3COONa adjusted to pH 6.4 with 6% CH_3COOH) and buffer B (40:60 of buffer A + acetonitrile). The amino acids were identified and quantified by comparing retention times and the peak areas of standards (acidic and basic amino acid mixture).

VI.2. Results and Discussion

The amino acid compositions of the nine fish species were investigated by HPLC (**Appendix Section, Fig. VI.A.1 – Fig. VI.A.10**) and the results are presented in **Table VI.1**. It is seen that the fishes contain different essential and non-essential amino acids in varying quantities. *B. bendelisis* exhibited the lowest amount of tryptophan (0.61 %) and the highest amount of anserine (7.31 %). The lowest anserine content was observed in *C. chagunio* (0.30 %), however, this species contained the maximum amount of 1-methyl histidine (7.15 %). *G. gotyla* contained the lowest amount of histidine (0.75 %) and the highest amount of taurine

(18.08 %). The highest level of asparagine (10.53 %) and the lowest level of threonine (0.41%) could be detected in *L. pangusia*. The composition of amino acids of the fish species varies based on their age, size, food resources, species, environmental conditions and reproductive conditions (Karuppasamy et al., 2013; Sarma et al., 2013). The highest amount of amino acids detected in *R. bola*, *N. hexagonolepis*, *T. putitora*, *C. semiplotum*, and *B. barna* were tryptophan (11.48 %), phosphoserine (13.22 %), ornithine (9.71 %), glycine (9.01 %), 1-methyl histidine (11.24 %), respectively. Glycine could be detected in all other fishes except *N. hexagonolepis*, *L. pangusia* and *T. putitora* (**Table VI.1**). Glycine helps in preventing tissue injury, metabolic regulation, increases anti-antioxidant activity, wound healing, promotes protein synthesis and improves immunity, and helps in the treatment of metabolic disorders in obesity, cardiovascular disease, diabetes, cancer, ischemia-reperfusion injuries, and various inflammatory diseases (Wang et al., 2013; Mahanty et al., 2014; Mohanty et al., 2014). It is be observed from the **Table VI.1** that a total of 11 amino acids could be noticed in all the 9 fishes, which were glutamic acid (1.42 % in FS-8 to 5.15 % in FS-9), phosphoserine (3.51 % in FS-7 to 13.22 % in FS-5), serine (1.97 % in FS-7 to 13.63 % in FS-3), 1-methyl histidine (1.57 % in FS-1 to 11.24 % in FS-9), threonine (0.41 % in FS-4 to 7.34 % in FS-7), aspartic acid (0.55 % in FS-7 to 6.43 % in FS-1), amino adipic acid (0.68 % in FS-2 to 6.25 % in FS-7), taurine (1.96 % in FS-1 to 18.08 % in FS-3), ornithine (2.85 % in FS-9 to 9.71 % in FS-7), lysine (0.55 % in FS-8 to 10.52 % in FS-3), and valine (0.82 % in FS-3 to 10.35 % in FS-4). Therefore, the nine fish species selected in this study can be considered as worthy natural supplements for these amino acids. Amino acids are important constituents for the healing processes of the body and their deficiency may hinder the processes of recovery. The amino acid composition in fish species is comparable to that of human being, and therefore by consumption of fish, the individuals can acquire the essential amino acids (EAA) and non-essential amino acids (NEAA) as per the requirement of the body system and can maintain the proper balance (Oluwaniyi et al., 2010).

Table VI.1. Amino acid composition (% of total area of amino acids) of nine fishes

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

FS = Fish species; FS-1 = *B. bendelisis*; FS-2 = *C. chagunio*; FS-3 = *G. gotyla*; FS-4 = *L. pangusia*; FS-5 = *N. hexagonolepis*; FS-6 = *R. bola*; FS-7 = *T. putitora*; FS-8 = *C. semiplotum*; FS-9 = *B. barna*; nd, not detected; EAA, Essential amino acid; TAA, Total amino acid; TEAA, Total essential amino acid.

In the present study, total essential amino acids (TEAA) varied among the different fish species. The highest amount of TEAA was detected in *C. chagunio* (43.88 %) followed by *L. pangusia* (42.79 %), and the lowest TEAA content was detected in *G. gotyla* (26.45 %). Therefore, the fishes of this study with high levels of TEAA could be suggested for natural supplementation of EAA. Threonine, arginine, histidine, valine, isoleucine, methionine, leucine, phenylalanine, tryptophan, and lysine are the ten EAA. In this study, valine (0.82 % to 10.35 %), threonine (0.41 % to 7.34 %) and lysine (0.55 % to 10.52 %) are the three EAA that were detected in all the nine fishes in varying amounts (**Table VI.1**). Sarma et al. (2013) reported the amounts of valine, threonine, and lysine in five cold-water fishes that varied from 0.528 % to 0.855 %, 0.382 % to 1.033 %, and 0.537 % to 2.031%, respectively, and these concentrations are comparatively lower in comparison to the results of our study. Joshi et al. (2017) also reported the valine, threonine and lysine contents in the 7 species of Indian Himalayan Snow trout that ranged from 3.33 to 6.27 g/100 g, 4.57 to 7.49 g/100 g, and 1.98 to 3.94 g/100 g, respectively. Similarly, Mohanty et al. (2014b) reported the threonine, valine and lysine content in 27 food fishes of India which varied from 0.3 to 7.9 g/100 g protein, 0.05 to 8.6 g/100 g, and 0.09 to 16.1 g/100 g protein, respectively. ElShehawy et al. (2016) also reported the threonine, valine and lysine content in the important fish species of Saudi Arabia which ranged from 2.66 to 3.49 g/100 g protein, 3.23 to 4.37 g/100 g crude protein and 4.47 to 6.28 g/100 g crude protein, respectively. Threonine (EAA) is used for the treatment of several nervous system disorders including multiple sclerosis, familial spastic paraparesis, spinal spasticity and amyotrophic lateral sclerosis (Mahanty et al., 2014; Mohanty et al., 2014). Valine works for several metabolic pathways and it is considered to be an important one for the synthesis of protein and optimal growth (Babitha and Murugaian, 2015). Lysine also is a very important one for the optimal growth of the body, for the treatment of cold sores, and the lack of this can lead to Immuno-deficiency (Mahanty et al., 2014; Mohanty et al., 2014). Phenylalanine and histidine were detected in the eight fishes in varying quantities (**Table VI.1**), while phenylalanine was not observed in *B. barna* and histidine was not observed in *N. hexagonolepis*. Histidine is needed for several roles in the growth and repair of tissue, maintenance of myelin sheaths, protein interaction and is also required for the removal of heavy metals from the body (Liao et al., 2013; Mahanty et al., 2014; Mohanty et al., 2014). Glycine which is one of the main constituents of human skin collagen along with other amino acids like proline, phenylalanine, alanine, serine, and isoleucine, form a polypeptide that can promote tissue healing and regrowth (Mohanty et al., 2014). In this study, arginine was detected only in the two fish species *viz. C. semiplotum*

(3.36 %) and *G. gotyla* (1.14 %). Arginine plays very important roles in wound healing, cell division, ammonia removal, hormone release, immune function, blood clotting, neurotransmission, and control of blood pressure (Mohanty et al., 2014). Methionine could not be detected only in *N. hexagonolepis* and *G. gotyla*, isoleucine was not observed found in *T. putitora* and *G. gotyla*, and tryptophan was not found in *C. semiplotum* and *L. pangusia*. Leucine was detected only in four fish species *viz. B. barna* (5.23 %), *B. bendelisis* (4.46 %), *L. pangusia* (9.88 %) and *C. chagunio* (6.09 %). Methionine can be used for the treatment of liver disorders, in improving wound healing, and used for treating depression, allergies, alcoholism, copper poisoning, asthma, radiation side effects, drug withdrawal, schizophrenia, and Parkinson's disease (Mischoulon and Fava, 2002; Mahanty et al., 2014; Mohanty et al., 2014). Tryptophan is the precursor of tryptamine, melatonin, serotonin, and kynurenine and it can play a significant role in neurotransmitters functioning (Richard et al., 2009). The supplement of tryptophan is used in the treatment of pain, depression, insomnia, seasonal affective disorder, premenstrual dysphoric disorder, bulimia, chronic fatigue and hyperactivity disorder (Richard et al., 2009; Mohanty et al., 2014). Leucine has important therapeutic roles in trauma, burn, and sepsis (Mahanty et al., 2014; Mohanty et al., 2014). Kim et al. (1999) stated that leucine has a strong cytotoxic activity against cancer cells.

Conclusions

In this study, the TEAA and other amino acids were found to vary among the nine fish species. The highest amount of TEAA was detected in *C. chagunio* (43.88 %) followed by *L. pangusia* (42.79 %), and the lowest TEAA content was observed in *G. gotyla* (26.45 %). Valine (0.82 % to 10.35 %), threonine (0.41 % to 7.34 %) and lysine (0.55 % to 10.52 %) were the three EAA that were detected in all the nine fishes in varying amounts. Other EAAs like histidine, methionine, phenylalanine, isoleucine, etc. were also detected in some of the fish species in varying quantities. Therefore, the nine fish species of this study have good sources of both EAA and NEAA, and these could be suggested for the natural supplementation of EAA for proper maintenance and the growth of human health.