ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
Est. 1984
ISSN: 0970-020 X
CODEN: OJCHEG
2022, Vol. 38, No.(6):
Pg. 1453-1459

Amino acid Profiling of Some Fresh Water Fishes of Manipur

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http://dx.doi.org/10.13005/ojc/380617

(Received: September 17, 2022; Accepted: November 14, 2022)

ABSTRACT

Using a Liquid Chromatography-Mass Spectrometry (LC-MS) technique, we were able to determine the amino acid profiles of a few different types of fresh water fish. The fish were taken from various bodies of water in Manipur. Lysine, phenylalanine and isoleucine were discovered to be the most abundant essential amino acids, whereas glutamic acid, aspartic acid and serine were found to be the most abundant non-essential amino acids. *Mystus ngasep* had the highest values for lysine (6.220 g/100 g), glutamic acid (14. 326 g/100 g), and aspartic acid (9.166 g/100 g), followed by *Systomus clavatus* (4.390 g/100 g) and *Tor tor* (4.121 g/100 g) for phenylalanine and serine. There were statistically significant differences ($p<0.05$) between the fishes' amino acid profiles. Quantity of amino acids was used to determine quality of amino acids as well. From these findings, we can draw the conclusion that fish are a valuable resource for providing humans with essential amino acids and high-quality protein.

Keywords: Amino acid profiles, Fresh water fishes, Good quality protein, Human health.

INTRODUCTION

Manipur's fresh water fishes are an important part of the state's fish resources because the majority of the state is made up of hill regions, each of which contains a number of upland rivers, streams, high altitude natural lakes, reservoirs and a sizable population of indigenous and exotic, cultivable and non-cultivable fishes. It is well known that fish is a nutritious food since it contains many necessary nutrients, including fat-soluble vitamins, fatty acids, protein and vital amino acids. For low-income families in rural locations and developing countries, fish may be the only economical and readily available source of animal protein. Due to its high protein-to-fat ratio and plenty of essential vitamins and minerals, eating fish as a whole food is safer and healthier than eating meat or other animal products. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two essential n-3 polyunsaturated fatty acids and both are found in abundance in fish. Large rural populations in emerging and under-developed countries rely heavily on fish for their livelihood and food security. It's common knowledge that fish is a great way to get protein without sacrificing quality. Since the dawn of time, it has also been an indispensable part of the cuisine of the people of Manipur. Fish is a staple diet in Manipur because it is inexpensive and provides a wide range of essential elements. The resource is also significantly more plentiful in Manipur. The majority of people's daily calories came from fish in many different cultures[1-2].

Humans have consumed 146.3 million tons of the world's total 167.2 million tons of fish[3]. Reportedly, between 40 and 70 percent of the protein consumed by the West African population comes from fish[4]. Furthermore, it has been stated that fish accounts for as much as 60% of the total animal protein requirement in the diet of Ghanaians[5]. Particularly in rural regions, where infants, pregnant women and nursing mothers are at risk of malnutrition, fish eating has been shown to have favourable health effects and provide relief from malnutrition.

Muscles from fish are highly sought after because they contain 15–25% of the total protein. As a result of their shorter muscle fibres and reduced connective tissue, fish proteins have been shown to be more easily digested than those of terrestrial animals[6-8] by a number of researchers. Weanling male albino rat *In-vitro* and *In-vivo* digestibility studies also provided conclusive evidence[9].

Malnutrition and vitamin and mineral deficits are two health problems that can be avoided by eating fish[10]. In addition, studies have linked eating fish to positive health outcomes, such as reduced inflammation, improved growth and development, faster tissue repair and wound healing and even antibacterial and antioxidant effects[11,13]. Several studies have highlighted the good health consequences of eating fish, particularly the lower risk of cardiovascular disease and inflammatory disorders like arthritis[14-16].

The human body is unable to produce what are known as "*Essential amino acids*", yet those amino acids are readily available in fish. Most enzymes, and many hormones, as well as other types of human tissue, are synthesized using amino acids. Therefore, fish is an excellent food choice for obtaining human health-critical amino acids. The review has highlighted the many ways in which fish contribute to human well-being and reaffirmed the importance of the nutritional components in fish for cognitive and reproductive health. As such, the purpose of this study was to assess the quality of a variety of fish species found in Manipur's fresh water and create an amino acid profile for each.

MATERIALS AND METHODS

Collection of samples

Chanda nama, Gagata dolichonema, Trichogaster fasciata, Pethia manipurensis, Systomus clavatus, *Tor tor* and *Mystus ngasep* are just some of the fish species that were caught in three distinct areas of Manipur (Barak River in Tamenglong District, Iril River in Imphal-East District and Loktak Lake in Bishnupur District). Manipur University's Fishery Research Laboratory in the Department of Zoology received the samples thanks to a carefully maintained cold chain. They were washed under the faucet many times and then dried in the blotting paper to remove excess moisture. Amino acid profiles were prepared by collecting tens of each small fish species (*Chanda nama, Gagata dolichonema, Mystus ngasep, Pethia manipurensis*, and *Trichogaster fasciata*) and six numbers of each larger fish (*Tor tor* and *Systomus clavatus*) and homogenizing them separately in a mortar and pestle.

Table 1: Collection sites and length-weight of the selected fish species

Sl. No	Sites	Fish species	Local name	No. of samples	TL* (cm)	Weight (g)
1	Loktak Lake	*Chanda nama*	Ngamhai	10	5.50-5.70	2.48-3.55
2	Iril River	*Gagata dolichonema*	Ngarang	10	7.90-9.80	5.31-7.00
3	Loktak Lake	*Mystus ngasep*	Ngasep	10	11.50-11.80	9.13-9.26
4	Loktak Lake	*Pethia manipurensis*	Ngakha	10	4.40-4.50	0.89-0.99
5	Barak River	*Systomus clavatus*	Nung-nga	6	6-18.8	6.4-31
6	Barak River	*Tor tor*	Ngara	6	23-39	125-785
7	Loktak Lake	*Trichogaster fasciata*	Ngapemma	10	7.50-7.90	6.73-7.26

*TL: Total lengths of the samples, only similar sizes of the samples were used for the analysis.

Amino acids profiling

Amino acid profiles were calculated using the same methodology as described by Ishida *et al.,* 1981[17]. All chemicals used in the experiment were 99% HPLC-grade standards. Hydrolysis of 100mg of sample is performed with 10mL of 6N HCl at 110° C in anaerobic conditions for 24 hours. In order to remove any remaining hydrolysis byproducts, we filtered the samples using Whatman no. 42 filter paper. After bringing the filtrate to a known volume of 0.05M HCl, it was flash evaporated three times with double-distilled water to eliminate the hydrochloric acid. The samples were stored in a deep freezer at -20°C before detection. The amino acids were detected by using tandem mass spectrometry (LC-MS with UPLC), WATER-ACQUITY-PE SCIEX API-3000. The mobile phases consists of two solutions, 50mM ammonium acetate and formic acid as mobile phase A and acetonitrile as mobile phase B. 10µL of the digested samples were injected into the column. The filter used was a 0.2µm nylon syringe filter. The temperature of the column was maintained at 35°C elsius, and the column's dimensions were 4.6m by 150mm. 0.3 mL/min was the flow rate, and the detecting wavelength was 254nm. As a result of analyzing the retention duration and peak area %, the amino acid profiles could be identified and measured.

Estimation of quality of the amino acids

Several important metrics, such as total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) were calculated based on the amount of amino acids.

Data analysis

Triplicate data were averaged and then standard deviations were calculated. IBM SPSS Statistics 21 was utilized to conduct statistical analyses on the collected data. One-way analysis of variance (ANOVA) with Turkey's test was used to examine the data. When comparing means and standard deviations, a p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fish is well recognized as a superb food choice due to its high protein content. Various studies have emphasized the health-promoting aspects of ingesting fish proteins. Proteins are crucial for a number of bodily processes, including development and maturation, maintenance and repair of damaged tissue, immunity from infection via antibodies, and regulation of metabolic processes via enzymes and hormones[18]. Oyeniye *et al.,* 2020[19] reported that a good amount of protein content in flesh fish was in the range of 15 to 26%. Bomi Ryu *et al.,* 2021[6] also reported that the desirable protein of fish muscle contained 15 to 25% of the total protein.

The crude protein content of the fish samples ranged from a high of 26.24% for *Tor tor* to a low of 5.99% for *Systomus clavatus*. High levels of protein were also discovered in the fish samples; specifically, 20.10% in the *Gagata dolichonema*, 17.16% in the *Mystus ngasep*, 15.17% in the *Chanda nama*, 12.45% in the *Pethia manipurensis*, 11.86% in the *Trichogaster fasciata*, and 5.99% in the *Systomus clavatus*. All of the fish tested had significantly higher protein content than the average (p<0.05). This finding demonstrates that these fish varieties are valuable food resources, particularly because they contain high levels of protein and essential amino acids. However, factors like as diet, adaptability, temperature, age, sex, breeding season, size and genetic features may account for the observed variability in the protein values found[20-21].

Amino acid profiling

The amino acid profiles of the fish samples are shown in Table 2. Concentrations of total essential amino acids in the fish samples ranged from 2.608 to 16.883 g/100 g of crude protein (cp), while concentrations of non-essential amino acids ranged from 2.308 to 33.815 g/100 g of cp. The three most common necessary amino acids were lysine, phenylalanine and isoleucine; the three most common non-essential amino acids were glutamic acid, aspartic acid and serine. The amino acid levels of lysine, phenylalanine and isoleucine were also measured, with values ranging from 0.478 to 6.220 g/100 g cp, 0.430 to 4.390 g/100 g cp and 0.300 to 4.902 g/100 g cp. *Mystus ngasep* (6.220 g/100 g cp), *Chanda nama* (3.416 g/100 g cp) and *Trichogaster fasciata* (2.340 g/100 g cp) were discovered to have the highest concentrations of lysine. *Systomus clavatus* (4.39 g/100 g cp), *Tor tor* (4.121 g/100 g cp) and *Gagata dolichonema* (4.049 g/100 g) were the top three fish species for phenylalanine content. *Systomus clavatus* (4.561 g/100 g cp) and *Tor tor* (4.902 g/100 g cp) were found to have the greatest isoleucine content". Lysine is a crucial amino acid for human development and growth, and a lack of it has been linked to impaired immunity[22]. Patients with immuno-deficiencies may benefit from consuming the fish species investigated. Because of its function in the manufacture of other amino acids, phenylalanine is an essential component of proteins in the human body. Dopamine and nor-epinephrine can't be made without it, and it also aids in the conversion of tyrosine. Isoleucine's immune-boosting properties were also highlighted by Gu C *et al.,* 2019[23].

The glutamic acid was the most common amino acid that wasn't required for survival in this research. *Mystus ngasep* (14.326 g/100 g cp) had the highest concentration of glutamic acid, followed by *Trichogaster fasciata* (6.510 g/100 g cp) and *Tor tor* (6.013 g/100 g cp). This amino acid is necessary for the elimination of peroxides and polyglutamate folate cofactors[24], as it is a cofactor in the transamination processes necessary for the formation of glutathione. Aspartic acid and serine, two more non-essential amino acids, were also identified in high concentrations. The highest concentration of aspartic acid was observed in *Mystus ngasep* (9.166 g/100 g cp), followed by *Trichogaster fasciata* (5.373 g/100 g cp) and *Chanda nama* (1.036 g/100 g cp). Cancer cells may be inhibited by aspartic and glutamic acids[25]. The most serine was found in *Systomus clavatus* (5.713 g/100 g cp) and the least in *Tor tor* (3.257 g/100 g cp). Additional abundance was seen for the non-essential amino acids arginine and tyrosine. Cysteine (Sulfur-containing amino acids), tyrosine (aromatic amino acids), histidine and arginine were all recognized as essential by Imura & Okada, 1998[26] for newborns and developing children.

Table 2: Amino acid profiles of the selected fish species (g /100 g protein)

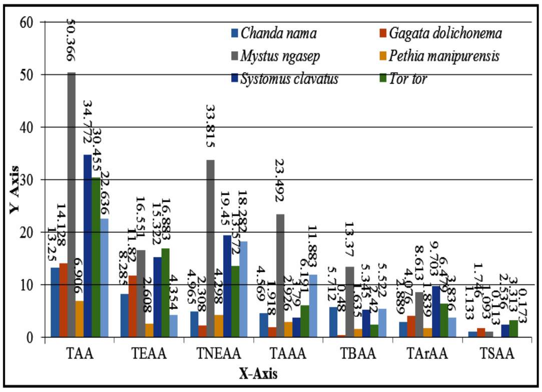
Fish samples	*Chanda nama*	*Gagata dolichonema*	*Mystus ngasep*	*Pethia manipurensis*	*Systomus clavatus*	*Tor tor*	*Trichogastor fasciata*
Essential Amino Acid (EAA) (g/100g)							
Histidine	2.156±0.011^{d}	0.002±0.000^{a}	2.040±0.010^{d}	0.166±0.152^{d}	0.026±0.002^{d}	0.021±0.000^{d}	0.056±0.005^{d}
Isoleucine	0.340±0.010^{a}	3.337±0.305^{d}	3.306±0.011^{d}	0.300±0.010^{a}	4.561±0.051^{d}	4.902±0.000^{d}	0.576±0.015^{d}
Lysine	3.416±0.011^{d}	0.478±0.099^{a}	6.220±0.200^{d}	1.353±0.152^{d}	0.659±0.006^{d}	0.612±0.000^{d}	2.340±0.010^{d}
Methionine	1.120±0.010^{d}	1.746±0.046^{d}	1.093±0.005^{d}	0.113±0.015^{a}	2.536±0.101^{d}	3.313±0.010^{d}	0.173±0.011^{a}
Phenylalanine	0.520±0.010^{d}	4.049±0.042^{d}	3.413±0.015^{d}	0.430±0.017^{a}	4.390±0.099^{d}	4.121±0.000^{d}	0.570±0.010^{d}
Threonine	0.500±0.010^{d}	0.355±0.040^{ad}	0.146±0.005^{d}	0.040±0.020^{a}	0.520±0.098^{d}	0.564±0.000^{d}	0.176±0.115^{d}
Valine	0.233±0.015^{a}	1.853±0.004^{d}	0.333±0.015^{ad}	0.206±0.005^{a}	2.630±0.249^{d}	3.350±0.004^{d}	0.463±0.011^{d}
∑EAA	8.285±0.077	11.82±0.536	16.551±0.251	2.608±0.371	15.322±0.606	16.883±0.014	4.354±0.177
Non-Essential Amino Acid (NEAA) (g/100g)							
Aspartic acid	1.036±0.005^{d}	N.D.	9.166±0.015^{d}	0.300±0.100^{d}	N.D.	0.178±0.015^{a}	5.373±0.015^{d}
Serine	0.030±0.017^{a}	0.365±0.022^{d}	2.053±0.005^{d}	0.013±0.005^{a}	5.713±0.190^{d}	3.257±0.051^{d}	0.063±0.005^{a}
Arginine	0.140±0.020^{a}	N.D.	5.110±0.010^{d}	0.116±0.005^{a}	4.660±0.072^{dd}	1.787±0.100^{d}	3.126±0.015^{d}
Tyrosine	0.213±0.011^{d}	0.025±0.016^{a}	3.160±0.010^{d}	1.243±0.135^{d}	5.287±0.168^{d}	2.337±0.047^{d}	3.210±0.010^{d}
Glutamic acid	3.533±0.005^{d}	1.918±0.063^{a}	14.326±0.005^{d}	2.626±0.020^{d}	3.790±0.192^{d}	6.013±0.008^{d}	6.510±0.010^{d}
Cysteine	0.013±0.005^{a}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
∑NEAA	4.965±0.063	2.308±0.101	33.815±0.045	4.298±0.265	19.45±0.622	13.572±0.221	18.282±0.055

Each value is expressed as mean ±SD (n=3). Values in the same row followed by different superscript letters are significantly different ($p<0.05$); N.D.: Not Detected.

According to Ozden and Erkan, 2008[27], lysine, glutamic acid and aspartic acid were found to be the most prevalent amino acids in the fish species tested (*Dicentrarchus labrax, Sparus aurata* and *Deentex dentex*). According to Adeyeye's, 2009[28], glutamic acid, aspartic acid and lysine were the three most common amino acids. Numerous studies have highlighted the significance of amino acids including glutamic acid, aspartic acid, alanine and glycine since they are the building blocks of flavour and aroma. The present study found the highest concentrations of the amino acids lysine, glutamic acid and aspartic acid. Some factors, including species, size, feeding behaviour, spawning, seasonal change, sexual maturity and nutrition, may account for the observed variance in amino acid concentration among the fish samples. As a result, these variables can alter fish chemical makeup.

Important steps in determining amino acid quality included calculating total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total aromatic amino acid (TArAA) and total sulphur amino acid (TSAA) (Fig. 1). The total essential amino acids value is the sum of all the essential amino acids; conversely, total non-essential amino acids value defines the sum of all the non-essential amino acids contain. The values are expressed in g/100 g of crude protein.

Fish species were analysed for their total amino acid (TAA) content and were found to have values ranging from 6.906 g/100 g (*Pethia manipurensis*) to 50.36 g/100 g (*Mystus ngasep*). The highest value for total acidic amino acids (TAAAs) was found in *M. ngasep*, with 23.492 g/100 g cp. TAAAs contain the two non-essential amino acids (viz.) aspartic acid and glutamic acid. The highest concentration of total basic amino acids (TBAAs) (which include arginine, lysine and histidine) was discovered in *M. ngasep* (13.37 g/100 g cp). The highest value for the three aromatic amino acids (phenylalanine, tyrosine and tryptophan) that make up total aromatic amino acids (TArAAs) was found in *Systomus clavatus* (9.703 g/100 g cp). In addition, the amount of total sulphur amino acids (TSAAs), which comprise methionine and cysteine, was determined, with *T. tor* having the highest value (3.313 g/100 g cp).



(X-axis) TAA: Total amino acid; TEAA: Total essential amino acid; TNEAA: Total non-essential amino acid; TAAA: Total acidic amino acid; TBAA: Total basic amino acid; TArAA: Total aromatic amino acid; TSAA: Total sulphur amino acid. (Y-axis) g/100 g cp

Fig. 1. Flowchart showing the different amino acids content among the fishes. Fish species were analysed for their total amino acid (TAA) content and were found to have values ranging from 6.906 g/100 g (*Pethia manipurensis*) to 50.36 g/100 g. (*Mystus ngasep*)

In Table 3 we can see the Pearson's correlation between the EAAs. Among the essential amino acids, this study found that histidine had the strongest positive correlation with lysine (0.863), and the strongest negative correlations with isoleucine (-0.269), methionine (-0.230), phenylalanine (-0.245), threonine (-0.040) and valine (-0.562). Aside from a positive correlation between isoleucine and the other essential amino acids, methionine (0.890), phenylalanine (0.976), threonine (0.549) and valine (0.862). Positive correlations were also identified between methionine and phenylalanine (0.828), threonine (0.839) and valine (0.798). Again, phenylalanine was discovered to have a positive correlation with both threonine (0.495) and valine (0.798), whereas threonine had a similar positive correlation with valine (0.726).

Table 4 displays the Pearson's correlation between the NEAAs. Except for serine (-0.140) and cysteine (-0.154), aspartic acid was shown to have a positive connection with the other three non-essential amino acids, arginine (0.632), tyrosine (0.290) and glutamic acid (0.913). Again, serine was found to have favourable correlations with arginine (0.645), tyrosine (0.778) and glutamic acid (0.150), whereas it had a negative correlation with cysteine (-0.325). Additionally, arginine was found to have a negative association with cysteine (-0.400) and a positive correlation with tyrosine (0.896) and glutamic acid (0.721). For all amino acids except cysteine, tyrosine was found to have a positive correlation with glutamic acid (0.394) except cysteine (-0.470). The association between glutamic acid and cysteine was found to be weakly negative (r = -0.471).

Table 3: Pearson's correlation among the Essential amino acids

	Histidine	Isoleucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Histidine	1						
Isoleucine	-0.269	1					
Lysine	0.863	-0.245	1				
Methionine	-0.230	0.890	-0.406	1			
Phenylalanine	-0.245	0.976	-0.229	0.828	1		
Threonine	-0.040	0.549	-0.388	0.839	0.495	1	
Valine	-0.562	0.862	-0.648	0.933	0.798	0.726	1

Table 4: Pearson's correlation among Non-essential amino acids

	Aspartate	Serine	Arginine	Tyrosine	Glutamate	Cysteine
Aspartate	1					
Serine	-0.140	1				
Arginine	0.632	0.645	1			
Tyrosine	0.290	0.778	0.896	1		
Glutamate	0.913	0.150	0.721	0.394	1	
Cysteine	-0.154	-0.325	-0.400	-0.470	-0.209	1

Table 5 displays the Pearson's correlation between different classes of amino acids. Total acidic amino acid (0.769), total basic amino acid (0.784), total aromatic amino acid (0.898) and total sulphur amino acid (0.370) were all found to have positive correlations with total amino acid content. Again, we discovered a significant positive correlation between the sum of essential amino acids and the sums of the other types of amino acids: non-essential (0.504), acidic (0.290), basic (0.345), aromatic (0.847) and sulphurous (0.828).

Table 5: Pearson's correlation among various groups of amino acids

	TAA	TEAA	TNEAA	TAAA	TBAA	TArAA	TSAA
TAA	1						
TEAA	0.765	1					
TNEAA	0.942	0.504	1				
TAAA	0.769	0.290	0.881	1			
TBAA	0.784	0.345	0.871	0.904	1		
TArAA	0.898	0.847	0.763	0.427	0.537	1	
TSAA	0.370	0.828	0.062	-0.228	-0.170	0.601	1

Positive results were seen in correlation analyses including the sums of acidic, basic, aromatic and sulphur amino acids ($r = 0.881$, 0.871, 0.763 and 0.062 respectively). Total acidic amino acid was found to have a positive correlation with total basic amino acid (0.904) and total aromatic amino acid (0.427), and a negative correlation with total sulphur amino acid (-0.228). Results showed a positive relationship ($r = 0.537$) between total basic amino acid and total sulphur amino acid, and a negative relationship between total basic amino acid and total sulphur amino acid (-0.170). Total aromatic amino acid was shown to have a positive connection with total sulphur amino acid ($r = 0.601$).

In this study, the amino acid content and quality of several freshwater fishes found in Manipur are described. As a result of their high protein and amino acid content, it's possible that the species of fish used in this study play an important role in consumer nutrition. There's a chance that this research can help raise awareness about the need of making healthy choices when purchasing and consuming fish. This information could also help us better understand the fish's nutrient consumption. In addition, the nutritional benefits of fish are highlighted in this study, particularly their use in resolving protein and amino acid shortages among the people of Manipur. Therefore, certain approaches incorporating capture, cultural fisheries and environmental initiatives are necessary for the conservation and sustainable development of such fresh water fishes. An improved risk and hazard assessment, as well as a more planned approach to protecting these species and other forms of biodiversity, are crucial.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Head of Department of Zoology, Manipur University, Canchipur for providing the university's lab space, as well as the staff at the Fishery Research Laboratory for their assistance during the course of this research.

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