

Amino Acid Profile and Score of Some Smoke Dried Hill Stream Fishes from the Markets of Manipur, India

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Abstract: Fishes are known to be good sources of complete protein. The purpose is to study amino acids profile and protein digestibility corrected amino acid score of the smoke dried hill stream fish *Neolisochilus strachyi*, *Labeo pangusia*, *Semiplotus manipurensis*, *Schizothorax sp.* and *Ompok sp.* The highest level was glutamic acid (15.17 to 20.76g /100g) followed by aspartic acid (10.18 to 1.37g/100g protein). The lowest level was found in tryptophan (1.57 to 2.09g/100g). Total essential amino acid contents ranged from 54.43 to 55.08g/100g. Total sum of aromatic amino acid contents ranged from 14.44 to 24.88g/100g and sum of sulphur containing amino acids contents were from 4.15 to 5.64g/100g. Ratios of total essential amino acids of contents to total whole amino acid contents were from 1.64 to 1.98. Comparison of essential amino acids of the fishes with reference pattern shows fairly high level. Protein digestibility corrected amino acid score (PDCAAS) ranged from 119.93 to 88.97%. Essential amino acid contents were high enough even to complement other essential amino acid deficient diets or mixed diets. The present study shows that the fishes contains good levels of essential amino acids and have good amino acid scores though processing might leave some effect on the amino acids.

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1. Introduction

Hill stream fishes are important among the fishes of Manipur from the view points of their taste and unique ecological habitat. Manipur, a beautiful hilly state situated at the north east part of India bordering Myanmar, is endowed with various fish resources, wild and cultured, both in plain and hill regions. Hill stream fish forms an important part of diet of the people of the state. Knowledge of their tissue composition is essential for their utilization as food fish (Fagbenro et al., 2005).

Fishes are rich sources of essential nutrients required for supplementing both infant and adult diets (Abdullahi et al., 2001). Fish proteins contain all the essential amino acids and considered to be complete protein (Devdas, 1994, Gopakumar, 1997). Importance of fish as a source of high quality balanced and easily digestible protein is well understood. Fish is a good source of high quality protein that is not high in saturated fats (AHA, 2012). Eating food that contains saturated fats raises the level of cholesterol in blood. Many foods high in saturated fats are also high in cholesterol. Fish are rich in essential amino acids, the building block of protein. Essential amino acids are those that cannot be made by the body and must be supplied in the diet. Consumption of fish is a source of good supply of essential omega-3 fatty acids (Marichamy et al, 2009). Besides, fish contains a good level of mineral elements and vitamins. These essential nutrients keep our heart and brain healthy in addition to their other functions. Digestibility is an

important factor for determining adsorption of the essential amino acids and nitrogen by the consumers.

Smoking is an important method for preserving the hill stream fishes in the north east India. Smoke dried fishes are consumed favorably at any time when there is shortage of fresh fish or there is a lack of consuming desire due to excessive continuous eating of the fresh fish. Today smoking process is a traditional method of considerable economic importance worldwide (Huda et al., 2010). The processing and preservation of fish were of utmost importance since fish is highly susceptible to quick physical deterioration, to prevent economic losses (Okenta and Ekelemu, 2005). Productions of smoked fish are reported from the various part of the world viz., Japan, South Africa, Phillipine, UK and Norway (Burt, 1988), France (Espe et al., 2004), Poland (Koloziejaska et al., 2002, Usydu et al., 2009), Turkey and Nigeria (Yanar, 2007), Indonesia (Huda et al., 2010). The production of a sufficient supply of good quality protein sources to meet human requirement is a major challenge for the future as population growth continue throughout the world (Reeds et al., 2000).

The nutritive value of a protein depends upon its capacity to provide nitrogen and amino acids in adequate amounts to meet the requirements of an organism. This directly reflects two factors, the amino acid content and the digestibility of the protein which limits the bioavailability of the amino acids in food proteins. Consequently, both amino acid composition and digestibility measurements are considered

necessary to accurately predict the protein quality of foods for human diets. For some time the use of an amino acid score on its own has been advocated as an alternative to the protein efficiency ratio (PER). However, when there is poor bioavailability the quality of proteins will not be adequately assessed. This has led to the adoption of the protein digestibility-corrected amino acid score method, (FAO/WHO, 1971). The amount of protein that has to be consumed, as part of an otherwise nutritionally adequate diet, to achieve the desired structure and function is identified as the requirement. There is a need for accurate evaluation of quality of the protein sources as it is to be designed for the human demands, specially for women, children and adults according to their different requirements.

There is no work on amino acids and amino acid score of the smoke dried fishes though there are many work on nutritional value of the fishes of the state by Romharsha et al, 2014, Sarjubala and Sarojnalini, 2014, Hei and Sarojnalini, 2012, Vishwanath et al., 1998 etc. So the present work was adopted to study amino acid profile and protein digestibility corrected amino acid score (PDCAAS) of the smoke dried fishes.

2. Material and Methods

Collection of the smoke dried fishes *Neolissochilus stracheyi*, *Labeo pangusia*, *Semiplotus manipurensis*, *Schizothorax sp.* and *Ompok sp.* was done direct from markets of Manipur (Ukhrul, Churachandpur, Chandel and Tamenglong). Collection of fishes were done from March to May for three consecutive years.

Chemical Reagents

Chemicals (Merck and Sigma) were of analytical grades. For HPLC, solvents were of HPLC grade. Water was special from Millipore filter system (USA).

Amino acid Composition

The samples for the amino acid analysis were prepared according to the method of Ishida et al, 1981). Protein is hydrolyzed into their constituent amino acids by 6N hydrochloric acid. The amino acids are separated by an ion-exchange column in a HPLC (Shimadju). Estimation of Tryptophan was done following the method of Sastry and Tummuru, 1985.

Total Amino Acids and Amino Acid Scores

Total essential, total non-essential, total aromatic amino acid etc. were calculated by simple addition of the individual amino acid values. Protein digestibility corrected amino acids scores (PDCAAS) of the samples were calculated by multiplying the lowest amino acid ratio (mg of an essential amino acid in 1.0 g test protein/mg of the same amino acid in 1mg reference pattern for the eight essential amino acids plus tyrosine, cystine, and histidine) by true digestibility. Pre-school amino acid requirement

pattern of FAO, 1991 was used as reference pattern. The PDCAAS scores were calculated in percentage term (WHO, 2007).

3. Results

The number of amino acids was 16 in Table 1. The most dominant amino acid was glutamic acid. The highest level of glutamic acid was 20.76 g/100g in *Schizothorax sp.* and 19.42 g/100g in *S. manipurensis*. The second most dominant amino acid was aspartic acid. Aspartic acid also was higher (11.42 g/100g) in *Schizothorax sp.* and 11.12 g/100g in *Semiplotus manipurensis* than other fishes. Other dominant amino acids were leucine, lysine, alanine, valine. Alanine was found highest 8.32 g/100g in *Labeo pangusia*. Leucine was found highest (9.37 g/100g) in smoke-dried *Neolissochilus stracheyi*. Lysine was higher (12.25 g/100g) in *Schizothorax sp.*, 9.93 g/100g in *Ompok sp.* and 9.08g/100g in *Labeo pangusia*. Tryptophan was present in very low level as compared with other amino acids. Histidine as essential amino acid was significantly high in *Labeo pangusia*. In smoke-dried fishes, cysteine and arginine were not detected in the analysis by HPLC. Total amino acids contents of smoke-dried fishes are shown in Table 2. Total amino acid were 97.56, 106.18, 106.88, 108.38, 106.62 g/100g respectively in smoke-dried fishes *N. stracheyi*, *L. pangusia*, *Semiplotus manipurensis*, *Schizothorax sp.* and *Ompok sp.* Amount of total essential amino acids were 49.21 g/100g in smoke-dried *L. pangusia*, 52.26 in smoke-dried *Semiplotus manipurensis*, 56.72 g/100g in smoke-dried *Schizothorax sp.*, 52.7 g/100g in smoked dried *Ompok sp.*

Amounts of total non-essential amino acids in *N. stracheyi*, *L. pangusia*, *Semiplotus manipurensis*, *Schizothorax sp.* and *Ompok sp.* ranged 45.09 to 54.62 g/100g in smoke-dried fish samples. Total aromatic amino acid content was found highest (61.09 g/100g) in *L. pangusia*. Total aromatic amino acid amounts in *N. stracheyi*, *L. pangusia*, *S. manipurensis*, *Schizothorax sp.* and *Ompok sp.* were 15.51, 24.39, 14.51, 15.23, 17.09 g/100g in smoke-dried samples.

Comparison of essential amino acid profiles of the smoked dried fishes (Table 3) was done with the essential amino acid pattern of FAO, 1991. Total values of all the essential amino acids are also compared. The smoke-dried fishes have greater values of essential amino acid than the pattern of FAO, 1991, indicating high nutritive potentials of the fishes.

Protein digestibility corrected amino acid scores (PDCAAS) are shown in Table 4 for the smoked fishes. Higher PDCAAS were in smoked *N. Stracheyi* (111.26%), *L. pangusia* (116.20%), *S. manipurensis* (113.65%), *Schizothorax sp* (119.93%). Lowest PDCAAS was in smoked *Ompok sp.*

Table 1. Amino acid composition of the smoked fishes (g/100g protein).

Samples	<i>Neolissochilus stracheyi</i>	<i>Labeo pangusia</i>	<i>Semiplotus manipurensis</i>	<i>Schizothorax sp.</i>	<i>Ompok sp.</i>
Aspartic acid	10.18	11.36	10.66	11.37	10.24
Threonine	5.65	5.65	5.91	5.81	6.38
Serine	4.61	2.38	5.31	5.43	4.80
Glutamic acid	15.17	15.27	19.42	20.76	18.21
Proline	4.94	1.08	5.91	3.72	5.72
Glycine	3.01	2.91	3.01	3.01	3.11
Alanine	7.59	8.32	7.61	8.17	7.46
Cysteine	-	-	-	-	-
Valine	6.13	6.56	6.41	5.60	6.57
Methionine	3.34	4.67	5.29	3.22	3.99
Isoleucine	5.22	6.32	6.41	5.51	5.62
Leucine	9.37	8.20	8.10	8.30	7.50
Tyrosine	2.85	3.77	2.70	3.20	4.38
Phenyl alanine	5.04	6.76	5.89	5.76	6.46
Histidine	6.05	11.76	4.02	4.73	4.56
Lysine	6.84	9.08	8.44	12.25	9.93
Arginine	-	-	-	-	-
Tryptophan	1.57	2.09	1.79	1.54	1.65

Table 2. Total amino acids of the smoked fishes (g/100g protein).

Samples	<i>Neolissochilus stracheyi</i>	<i>Labeo pangusia</i>	<i>Semiplotus manipurensis</i>	<i>Schizothorax sp.</i>	<i>Ompok sp.</i>
Total amino acid	97.56	106.18	106.88	108.38	106.62
Total essential amino acid	49.21	61.09	52.26	52.72	52.7
Total non- essential amino acid	48.35	45.09	54.62	54.62	53.92
Total aromatic amino acid	15.51	24.39	14.51	15.23	17.09
Total sulphur amino acid	3.34	4.67	5.29	3.22	3.99
Ratio of TEAA / TNEAA Essential amino acid / total amino acid	1.98	1.64	1.94	1.93	1.89

Note: TEAA- Total Essential Amino acid, TNEAA-Total non- essential amino acid

Table 3. Essential amino acid composition in the protein of the smoked fish (g/100g protein) with reference to FAO/WHO pattern, 1991.

Samples	FAO/WHO 1991	<i>N. stracheyi</i>	<i>L. pangusia</i>	<i>S. manipurensis</i>	<i>Schizothorax sp.</i>	<i>Ompok sp.</i>
Histidine	1.9	6.35	11.79	4.30	4.3	4.65
Threonine	3.4	5.65	5.65	5.91	5.81	6.38
Isoleucine	2.8	5.22	6.32	6.41	5.51	5.62
Leucine	6.6	9.37	8.20	8.10	8.30	7.50
Lysine	5.8	6.84	9.08	8.44	12.25	9.93
Met+Cys	2.5	3.34	4.67	5.29	3.22	3.99
Phen+Tyr	6.3	5.04	6.76	5.89	5.76	6.46
Tryp	1.1	1.57	2.09	1.79	1.54	1.65
Valine	3.5	6.13	6.56	6.41	5.60	6.57
Total Essential	32.0	49.21	61.09	52.26	52.72	52.7

Note: Met - methionine, cys - cysteine, phen - phenylalanine, tyr - tyrosine

Table 4. Protein digestibility corrected amino acid score (PDCAAS) in the smoked fish (%).

Samples	Amino acid pattern (FAO, 1991)	<i>N. stracheyi</i>	<i>L. pangusia</i>	<i>Semiplotus manipurensis</i>	<i>Schizothorax sp.</i>	<i>Ompok sp.</i>
Digestibility (Hei and Sarojnalini, 2012)		94.34	93.53	92.53	95.37	78.29
Threonine	3.4	156.77	155.42	160.83	162.97	146.90
Isoleusine	2.8	175.87	165.51	211.83	187.67	157.13
Leucine	6.6	133.93	116.20	113.65	119.93	88.97
Lysine	5.8	111.26	146.42	134.64	201.42	134.03
Met+Cys	2.5	126.03	174.71	195.79	122.84	124.9
Phen + Tyr	6.3	112.91	156.39	126.16	135.63	134.71
Tryptophan	1.1	134.65	177.71	150.57	133.51	145.54
Valine	3.5	165.23	175.30	169.49	152.59	146.96
Histidine	1.9	300.00	578.90	195.77	237.42	187.89

Note: Met - methionine, cys - cysteine, phen - phenylalanine, tyr - tyrosine

4. Discussion

Amino acid profiles of the fishes (Table 1) shows different values for different fish species. Evaluation of protein quality aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. Evaluation of protein quality aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen.

The fish species show essential and non-essential amino acids in the composition of the protein. There is variation of amino acids contents in the different species. Presence of high amount of glutamic acid in the fishes contributes to the sweet taste of the fishes. High level presence of histidine in *L. pangusia* will contribute to better taste. Total amino acid, total essential amino acid and total non-essential amino acid, aromatic amino acids and sulphur containing amino acids show different values (Table2). Presence of all the essential amino acids in the fish protein makes them complete proteins. In the study of Indonesian smoked cat fishes by Huda et al. (2010), the most dominant amino acids was glutamic acids (8.11-9.18 g/100g protein) in *Macrones nemerus* and *Orypterus microneme*; lysine contents was 4.97 to 5.80 g/100g in the two fishes. In the study of Indian major carps, *Rohu*, *Catla*, and *Mrigal* by Sankar and Ramachandran (2002), the dominant acid was glutamic acids (15.63 to 20.06 g/100g protein) followed by aspartic acid (10.26 to 20.25g/100g protein); and important amino acid lysine was 3.01 to 8.20 g/100g. These are found similar in our present study. Kaya et al. (2010) reported an increase (P<0.05) in aspartic acid> isoleucine> methionine>hydroxyl-proline>valine, and a decrease (P<0.05) in glutamic acid> serine>threonine>leucine>tyrosine>histidine>lysine> praline in hot smoking of sturgeon. He also reported

that the changes in alanine, glycine and phenylalanine are insignificant (P>0.05).

Cysteine and arginine was not detected in the smoked dried fishes. This may be due to smoking and drying effect. Proctor and Lahiry (1956) also reported that cysteine was not detected in the dehydrated fishes. At higher temperatures protein suffers thermal degradation. Thus at 115°C losses of cystine/cysteine occur both at low (14%) (Bjarnarson and Carpenter, 1970) and high (70.80%) moisture content (Opstvedt et al., 1984). Proctor and Lahiry (1956) reported that there are no consistent significant differences in the content of amino acids between raw and dehydrated fish but valine, phenylalanine and tryptophan tend to be somewhat lower in the dehydrated compared with the raw fish.

Amino acid scores are high (100%) except *Ompok sp.* (87%). Essential amino acids are compared with amino acid pattern (FAO, 1991) in Table 3. Comparison of the essential amino acid contents of the fishes with the required amino acids pattern, FAO, 1991 indicates high amino acid score. Essential amino acids are present in the higher proportion in the present findings. The fresh fishes (Romharsha et al, 2014) shows higher values than the smoked fishes (Table 3).

The score for the protein in the fishes samples tested in the present studies (Table 4) varied from 88.9 to 121.13%. However there are opposing opinions claiming that the truncation of PDCAAS values to 100% is justifiable only in those situations in which the protein in question is used as the sole source of protein in human diet, and the truncated value should not be used for the evaluation of the nutritional significance of protein as part of a mixed diet. Actually the limiting amino acid, singly, does not reflect the nutritional value of the protein in a mixed diet, because there are examples wherein the

constituents of the one protein can be complemented by another of diet. For the best representation of results regarding the protein quality of the fishes, the PDCAAS scores are not rounded to 100% in Tables 16 and 17. Moreover these tables contain data not only regarding the limiting amino acids but also for all the 10 essential amino acids and histidine.

Protein digestibility corrected amino acid (PDCAAS) score of fresh fishes (Table 4) is high and record 100% (if truncated) and 97% and 88.9% in fresh and smoked *Ompok sp.* The un-truncated different calculated amino acid scores show the strength of essential amino acids than can complement other cereal based proteins that are deficient in essential amino acids. The smoked fishes show lower values in the essential amino acid contents because heating and smoking processes affects the proteins. Proctor and Lahiry (1956) also reported that cysteine was not detected in the dehydrated fishes.

The sulphur containing amino acid cysteine has been considered conditionally essential for premature and full term newborn infant following Snyderman's work demonstrating improved N-balance and growth when cysteine was provided (Snyderman, 1971). The sulfuric amino acid taurine is considered conditionally essential for the growth weight infant. Other functional roles of taurine include its role in the conjugation of bile acids, its putative neurotransmitter role and an antioxidant effect on cell membrane, the latter influence being particularly important (Gaul and Wright, 1986). Amino acids viz., valine, methionine, threonine, serine, isoleucine, tryptophan, lysine, histidine and arginine have dietary essentiality for growth and other physiological activities of the body.

Amino acids such as tryptophan, tyrosine, histidine, and arginine are used by the brain for the synthesis of various neurotransmitters (Betz et al., 1994) and neuromodulators. Tryptophan contributes to normal growth and protein synthesis and participates in numerous biochemical processes (Umezawa, 1989). Tryptophan is the rarest of the essential amino acids found in food and is the precursor of serotonin. The availability of tryptophan to the brain can alter behavioral factors such as alertness, level of depression, aggression and pain sensitivity (Lieberman et al., 1985).

Protein energy malnutrition is fairly common in both children and adult population of the world as a whole (Stephenson et al. 2000) and is associated with the death of about 6 million children each year (FAO, 2000). Unless amino acids are present in the diet in the right balance, protein utilization will be affected (Duffy et al. 1981). Protein deficiency has been shown to have adverse effects on immune system (Bistran, 1990) and kidney function (Benabe and Martinez-

Moldonads, 1998). Obviously it has adverse effects on all organs (Corish and Kennedy, 2000). All these fish are rich sources of lysine as compared to the reference amino acid score pattern, FAO, 1991. They will have capacity to complement plant proteins that are deficient in lysine. Other essential amino acids are present in more than adequate level to meet the amino acid requirement pattern. So the fish have capacity to complement plant protein sources that are different in any specific essential amino acids except *Ompok sp.*

In *Ompok sp.* the limiting amino acid is leucine. Amino acid score (PDCAAS) was 88.80 in the smoke-dried *Ompok sp.* However other essential amino acids are present in *Ompok* in levels more than the amino acids requirement pattern, FAO, 1991. Non-essential amino acids were present with abundant contents of glutamic acid and aspartic acids. So they are sources of very healthy protein that can be designed to meet the different needs of growing children, pregnant and lactating women, and adults. Glutamic acid is the major constituents but some marine fishes like grey mullet. Indian halibut and silver jaw fish show higher amounts of glutamic acid (Mukundan et al. 1986). Glutamic acid especially its monosodium salt is a known flavouring agent and hence its predominance in amino acid make up can be a reason for the preferred flavour of the fishes (Mukundan et al., 1986).

In protein quality evaluation, consideration should be (i) ability of the protein to satisfy nitrogen and amino acid requirement when used as the sole or principal protein source (ii) the capacity of the protein source to complement another protein to complement plant protein deficient in either lysine or the sulphur-containing amino acids (Reeds et al., 2000). These two aspects of protein quality have been considered in the amino acid scoring procedure of the present study. Now it is clear that the studied fishes are of high quality protein with protein digestibility corrected amino acid score (PDCAAS) 100% in the four fishes and 96-97 % and 88.80 % in the fresh and smoke dried *Ompok sp.* As they contain higher level of essential amino acids in reference with the amino acid requirement pattern, they have capacity to complement other protein deficient in essential amino acids. So the fishes as source of protein satisfy the two conditions. A healthful diet, therefore, should consist of a sufficient and balanced supply of both essential and nonessential amino acids in order to ensure high levels of protein production, FAO, 2002). The fishes *Neolissochilus stracheyi*, *Labeo pangusia*, *Semiplotus manipurensis*, *Schizothorax sp.* are better in protein quality with higher amino acid score, which is also clear in *in-vivo* digestibility tests (Hei and Sarojnalini, 2012).

5. Conclusion

The present study shows that the fishes contains good levels of all essential amino acids and have good amino acid scores though processing might leave some effect on the amino acids. So these fishes are very good sources of high quality protein for human nutrition.

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