

# Investigation of Nutritional Status of the Butter Catfish *Ompok bimaculatus*: An Important Freshwater Fish Species in the Diet of Common Bangladeshi People

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**Abstract:** Due to the availability throughout the all seasons and tastiness, butter catfish *Ompok bimaculatus* gained immense commercial importance as well as greater consumer preference in South-East Asian countries. It significantly contributes to the local fisheries production. In order to explore information on nutritional status of this commercially important fish species, the present study was carried out as such information is not available. Our study showed that this fish species is highly rich in polyunsaturated fatty acids. The percentage of total polyunsaturated fatty acids was found to be 40.92 followed by 26.54% of total monounsaturated fatty acids, indicating excellent nutritive value of this fish species. Amino acid analysis showed that the fish flesh contains essential amino acids like leucine, lysine, threonine, phenylalanine, valine and isoleucine in a significant amount. The ratio of essential to non-essential amino acid is 0.89 indicating its superior protein quality. The mineral profiling also showed that this species is rich in K, Ca, Na, Mg and Fe with other minerals. The present study showed that *Ompok bimaculatus* is a good source of lean meat and trace elements, especially Zn and Mn. Furthermore, exploration of vitamin contents showed that fat soluble vitamins like vitamin A, D, E and K are present in a considerable amount which is beneficial to health.

**Keywords:** PUFA (Polyunsaturated Fatty Acid), EAA (Essential Amino Acid), Nutrient Profile, Butter Catfish, AAS (Atomic Absorption Spectrophotometer)

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## 1. Introduction

Fish is an important dietary component, which covers about 60% of animal protein in the diet of common Bangladeshi people [1, 2]. It is a vital nutritional constituent as well as the cheapest sources of animal protein. Many regional studies have confirmed the importance of fish items in a Bangladeshi diet [3, 4]. Fish oil contains omega-3 polyunsaturated fatty acids (PUFAs), more specifically eicosapentaenoic acid (EPA; C20: 5) and docosahexaenoic acid (DHA; C22: 6) [5]. These fatty acids have great benefits for cardiac health, controlling blood glucose, reduction of arterial disease, lowering the risk of cancer. Fish oil also combat asthma, multiple sclerosis and systemic lupus erythematosus [6, 7]. These two fatty acids must be obtained from the diet as human body cannot synthesize thus called essential fatty acid. Oil from fish species prevents

cardio-vascular diseases (CVDs) [8]. In addition, fish oil plays vital role in remediation of many diseases and is a protective mean of various types of abnormalities such as diabetes mellitus, cancers, inflammation, hypertension, obesity, rheumatoid arthritis, osteoporosis and schizophrenia. Also, the fish goes to high protein, minerals and low lipid group. Fish contains lower caloric content per unit of protein compared to lipid and thus is an ideal source of animal protein for use in controlling diets [9]. Fish plays an important role in prevention of the chronic protein-calorie malnutrition diseases such as kwashiorkor and marasmus. Fish is also an important source of macro and micronutrients [2, 10]. Fish oil is an exceptional source of vitamins A, D, E and K. Vitamin A and D are essential for healthy skin and for the improvement of bones. Vitamin D plays an important

role in the body's use of calcium and mineral is vital for sound teeth and bones. Furthermore, fat fish in particular are the major source of vitamin D [11]. No studies regarding nutritional characterization of *Ompok bimaculatus* have been done so, it is very crucial to generate evidence on nutritional profile of this fish species as it can broaden our insight about information on precise nutrient abundance of particular species which will be helpful for the nutritionists and dieticians to design a proper 'dietary guidelines'. In the present study, oil and flesh were investigated from *Ompok bimaculatus* commonly known as Kani pabda. Several studies have been carried out on the effects of consumption of nutrient dense fresh water fishes [10, 12] and it is very important to make relationship between human nutrition and fisheries [2]. Due to physiological, geographical and ecological factors, these values vary widely. Therefore, in the present study, the assessment of different nutritional parameters such as amino acid, fatty acid, mineral and vitamin profiles in the selected fish species was taken into account to evaluate the nutritional status of *Ompok bimaculatus*.

## 2. Materials and Methods

### 2.1. Sample Collection and Preparation

Kani pabda fish was collected from the Padma River in Rajshahi, Bangladesh. Then the fish was cleaned and bones were removed and the flesh stored in a refrigerator (at 4°C) for experimental purposes.

### 2.2. Extraction of Oil from Fish

The stored fish sample was sun-dried for about 72 hours and grinded by mechanical grinder. Oil was extracted by Soxhlet extraction apparatus using n-hexane. The extract was evaporated under reduced pressure in a rotary evaporator to obtain the oil and stored at 4°C for further analysis [13].

### 2.3. GC Mass Analysis of Fatty Acids

Fatty acid composition of extracted oil was investigated by Gas liquid chromatography [14]. Gas chromatography was carried out with a Gas Chromatograph GC-2025 series having AOC-20i Auto Injector (Shimadzu Co, Japan). Gas chromatographic condition included temperature 280°C, pressure 175.4 KPa, total flow 165 ml/min, purge flow 3 ml/min, column temperature 270°C. Fish oil was first saponified to prepare free fatty acid salts. Then, fatty acid salts were derivatized to produce the FAME (fatty acid methyl esters) according to the American Oil Chemists Society (AOCS) [15]. The FAME was extracted with hexane for analysis by GC. Each FAME (Fatty Acid Methyl Ester) in extract was identified by comparing retention times with those of known standard FAME (Lipid Standard Sigma chemical Co, St Louis, MO, USA). The area of fatty acids was measured with GC solution 2011. The results were expressed as relative percentage of fatty acids.

### 2.4. Investigation of Amino Acids from Flesh

#### 2.4.1. Acid Hydrolysis

The fish proteins were hydrolysed using 6N HCl at 110°C under nitrogen atmosphere for 24 h. Amino acids were explored by HPLC equipped with amino acid analyser (Shimadzu LC 10AS) [16]. The column and detector used were C-18 polystyrene column and FL 6A fluorescence detector respectively with CR6A Chrom Pac recorder software. The analyzer was calibrated using amino acid standard solutions (Sigma-Aldrich, A2161-5ML) and the same solutions were used to determine the amount of amino acids in the samples.

#### 2.4.2. Alkali Hydrolysis

The sample was digested using 5% NaOH for 24 h followed by neutralization at pH 7.0 using 6N HCl for the tryptophan analysis. Spectrophotometric technique was used to measure tryptophan content at wavelength 530 nm as per Sastry and Tammuru [17].

### 2.5. AAS Analysis of Minerals

The trace elements Na, K, Ca, Fe, Mg, Mn, Zn and Hg were assessed by "AAS-680" (Atomic Absorption / Flame Emission Spectrophotometer, Shimadzu, Japan). The instrumentation also included a single hollow cathode lamp for each element with an air-acetylene and nitrous-oxide-acetylene [18]. Standard stock solutions of 100 ppm were prepared using analytical grade reagents of respective salts with deionized water and stored in clean polythene bottles. Then, standard solutions were made using the standard stock solution by suitable dilution using deionized water. Finally, fish sample was diluted to a known volume and analysed by Flame AAS. The sample was examined against standard solution of each element. A blank reagent was also continued and the absorption due to reagent was subtracted. Pyrex glass wares were used, cleaned by detergent, 1: 1 HNO<sub>3</sub> and deionized water prior to use to avoid any contamination. Reagents were also prepared with deionized water.

### 2.6. Exploration of Vitamins

Fish oil (about 150 mg) was refluxed with 25 ml methanol and 150% potassium hydroxide (KOH) in water bath for about 30 minutes. 50 ml petroleum ether was used to extract fat soluble vitamins. The petroleum ether layer was collected, concentrated and dissolved in 5 ml of acetonitrile. Fat soluble vitamins were examined by HPLC (Shimadzu LC 10AS) equipped with C18 RP column and UV detector. Acetonitrile (as solvent A) and methanol (as solvent B) were used as mobile phase and the flow rate was 1 ml/min. A simple linear gradient system from (solvent A/solvent B) 50/50 to 70/30 in 20 minutes was used. The injection volume was 20 µl. The fat soluble vitamins were recognized and quantified by comparing with the retention times and peak area of respective vitamins standards (Sigma-Aldrich) [19].

#### Statistical Analysis

All the experiments were carried out in triplicates and the result was presented as mean±S. E.

### 3. Results and Discussion

#### 3.1. Fatty Acids Profile

The fatty acid profiles of oil obtained by GC analysis are presented in Table 1. The percentage of the total saturated, monounsaturated and polyunsaturated fatty acids in oil were found to be 32.40%, 26.54% and 40.92% respectively. The important polyunsaturated fatty acids EPA and DHA were found to be  $5.75 \pm 0.51\%$  and  $4.80 \pm 0.49\%$ , respectively. These values were comparatively lower than the values of Salmon fish oil (7.7% EPA and 14.5% DHA) [20]. Among the monounsaturated fatty acids, oleic acid (20.23%) was present in higher amount in oil. The most significant saturated fatty acid was palmitic acid (21.22%).

Table 1. Fatty Acid Compositions (%).

Fatty acid compositions	% of fatty acids
Saturated fatty acids (SFA)	
Myristic acid (C14: 0)	6.96±0.31%
Palmitic acid (C16: 0)	21.22±2.01%
Stearic acid (C18: 0)	2.91±0.24%
Arachidic acid (C20: 0)	0.52±0.09%
Tricosylic acid (C23: 0)	0.79±0.16%
∑ SFA	32.40±2.83%
Monounsaturated fatty acids (MUFA)	
Myristoleic acid (C14: 1)	0.74±0.16%
Palmitoleic acid (C16: 1)	1.89±0.71%
Oleic acid (C18: 1)	20.23±2.52%
Eicosenoic acid (C20: 1)	1.98±0.24%
Erucic acid (C22: 1)	1.70±0.34%
∑ MUFA	26.54±1.42%
Polyunsaturated fatty acids (PUFA)	
Linoleic acid (C18: 2)	6.90±0.95%
γ-Linolenic acid (C18: 3)	3.95±0.86%
α-Linolenic acid (C18: 3)	4.89±1.25%
cis-11, 14-Eicosadienoic acid (C20: 2)	2.45±0.73%
cis-11, 14, 17-Eicosatrienoic acid (C20: 3)	3.93±0.91%
cis-8, 11, 14-Eicosatrienoic acid (C20: 3)	2.42±0.36%
Arachidonic acid (C20: 4)	4.86±0.53%
cis-13, 16-Docosadienoic acid (C22: 2)	0.97±0.46%
EPA (C20: 5)	5.75±0.51%
DHA (C22: 6)	4.80±0.49%
∑ PUFA	40.92±5.36%

All experiments were carried out in triplicates and the result was presented as mean±S. E.

#### 3.2. Amino Acids Investigation

Dietary protein plays important role providing amino acids for the biosynthesis of the body proteins. It is very important to provide all essential amino acids to the tissues in an appropriate amount for optimal protein synthesis. Fish proteins comprise all the essential amino acids vital for human nutrition which expand the overall protein quality of a diet [21].

Amino acid compositions of *O. bimaculatus* presented in Table 2, reflect that glutamic acid (15.28%) is predominant followed by aspartic acid (12.11%) compared to other amino acids. All the data given in the table have been expressed in percentage of total area of amino acids. In case of EAA, the major amino acids are leucine (7.16%), lysine (6.81%),

threonine (6.03%) followed by phenylalanine (5.26%) and valine (5.11%) which have a close link with previous results found in *S. seenghala* [22]. The other amino acids like tyrosine, isoleucine, histidine, methionine and tryptophan were found to be 4.7%, 4.21%, 3.80%, 3.01% and 0.80%, respectively (e.g. Figure 1). Moreover, amino acids like glycine, serine, alanine, proline and arginine were found to be 7.53%, 6.81%, 4.87%, 3.18% and 2.72% respectively (e.g. Figure 2).

Table 2. Amino Acid Composition (% of total area) of *O. bimaculatus*.

Amino acids	% of total area
Essential(EAA)	
Leu	7.16
Lys	6.81
Thr	6.03
Phe	5.26
Val	5.11
Tyr	4.7
Iso	4.21
His	3.80
Met	3.01
Trp	0.80
Non-essential(NEAA)	
Glu	15.28
Asp	12.11
Gly	7.53
Ser	6.81
Ala	4.87
Pro	3.18
Arg	2.72
EAA/NEAA	0.89

All experiments were carried out in triplicates and the result was presented as mean±S. E.

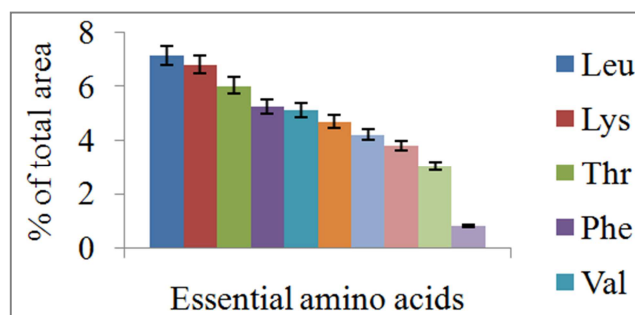


Figure 1. Essential amino acid composition of *O. bimaculatus*.

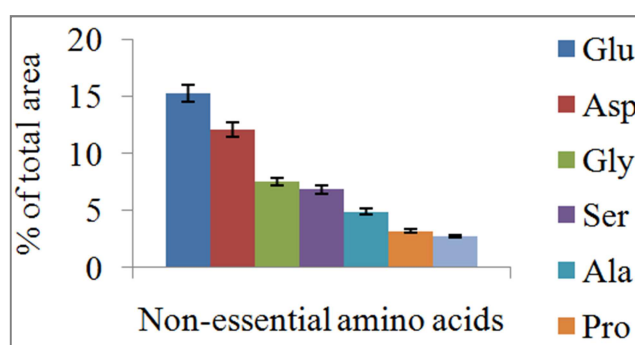


Figure 2. Non-essential amino acid composition of *O. bimaculatus*.

EAA to NEAA ratio is an index to define the quality of the protein [23]. Optimal EAA to NEAA ratio has been reported in gilthead sea bream (*Sparus aurata*) which is 0.71 and signify a high quality protein; on the other hand a very high ratio was recorded in squid roe (0.93) and a low value was reported in sea urchin roe (0.65) [24]. We calculated the EAA to NEAA ratio of *O. bimaculatus* to be 0.89. The higher value represents excellent protein quality.

### 3.3. Estimation of Minerals

The mineral content of *O. bimaculatus* is presented in Table 3. The obtained data has also been assimilated with other significant fish species. The macro-minerals sodium, potassium and calcium contents were found to be  $1032.41 \pm 15.12$ ,  $13428.02 \pm 52.36$  and  $3152.30 \pm 40.98$  (mg/kg of wet flesh), respectively (Figure 3). These values are closely related to the *S. aor* [13].

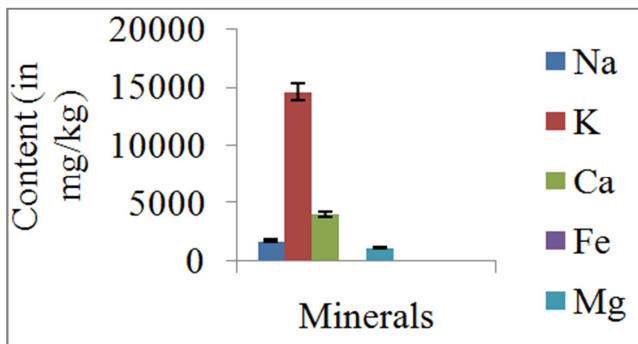


Figure 3. Minerals content of *O. bimaculatus*.

The potassium content of *O. bimaculatus* is about two times higher compared to *P. stigma* (male 7190.0 mg kg<sup>-1</sup>, female 6550.0 mg kg<sup>-1</sup>), a nutrient dense small indigenous fish [25]. Also in case of calcium content, *O. bimaculatus* has higher value compared to the *L. rohita* (862.8 mg kg<sup>-1</sup>) [26]. Calcium is essential for normal functioning of muscle, nervous system and strong bones (formation and mineralization). Calcium deficiency leads to the development of rickets in young children and is responsible for osteomalacia (softening of bones) in adults and aged people. So, *O. bimaculatus* can be a great supplementary source of calcium (e.g. Figure 3).

The other minerals such as magnesium, manganese, iron and zinc were found to be  $865.02 \pm 5.39$ ,  $4.52 \pm 1.02$ ,  $60.75 \pm 2.63$  and  $18.36 \pm 1.58$  (mg/kg of wet flesh), respectively. Zinc is required for growth, development as well as the proper functioning of immune system and healthy skin. It also has a role in cell division, cell growth, wound healing and metabolism. Deficiency of Zn is associated with skin problems, poor growth and loss of hair with other problems. Compared to the major carps *C. cirrhosus* ( $15.0 \pm 0.1$  mg kg<sup>-1</sup>) [27] and *L. rohita* ( $0.84 \pm 0.22$  mg kg<sup>-1</sup>) [28], *O. bimaculatus* contains higher level of Zn.

Moreover, iron is crucial for synthesis of hemoglobin in red blood cells which transports oxygen throughout the body. Iron deficiency is related with anemia, impaired brain function as well as poor learning ability and poor behavior in infants. Its

deficiency is also associated with increased risk of infection [29]. Iron content of *O. bimaculatus* is very high compared to the murrel *C. punctatus* (20.0 mg kg<sup>-1</sup>) known for its richness in micronutrients [30]. The high iron content makes this species nutritionally significant.

Mercury is a heavy metal which causes mercury toxicity [31]. We found mercury content in *O. bimaculatus* to be  $0.01 \pm 0.01$  mg kg<sup>-1</sup> which is well within the safety limits, as the prescribed permissible level according to WHO of mercury in fish flesh (0.50 mg kg<sup>-1</sup>) [32]. We found arsenic content in *O. bimaculatus* to be  $0.02 \pm 0.01$  mg/kg, which is much below the prescribed permissible level according to the Joint FAO/WHO Expert Committee [33] of arsenic in fish flesh (0.1 mg/kg) [34].

Table 3. Mineral Content of *O. bimaculatus*.

Minerals	Content (in mg/kg)
Sodium (Na)	1032.41±15.12
Potassium (K)	13428.02±52.36
Calcium (Ca)	3152.30±40.98
Iron (Fe)	60.75±2.63
Magnesium (Mg)	865.02±5.39
Manganese (Mn)	4.52±1.02
Zinc (Zn)	18.36±1.58
Arsenic (As)	0.02±0.01
Mercury (Hg)	0.01±0.01

All experiments were carried out in triplicates and the result was presented as mean±S. E.

### 3.4. Assessment of Vitamins

Estimation of vitamin content revealed that *O. bimaculatus* is a good source of fat soluble vitamins like A, D, E and K. Vitamin A is required for normal vision as well as for bone development and growth. Its retinoic acid regulates gene expression in the development of epithelial tissue [35].

Table 4. Fat-soluble vitamin profiles of *O. bimaculatus*.

Vitamins	Content (µg/kg)
A	1460.23
D	13108.39
E	7021.57
K	12156.12

All experiments were carried out in triplicates and the result was presented as mean±S. E.

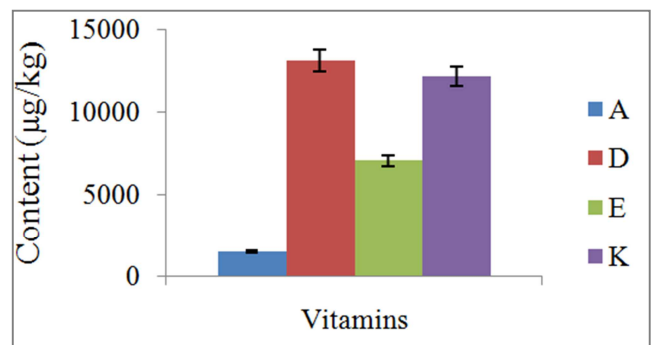


Figure 4. Vitamins content of *O. bimaculatus*.

The fat soluble vitamins found in *O. bimaculatus* were presented in Table 4 (e.g. Figure 4). The vitamin A content of *O. bimaculatus* were found to be 1460.23 ( $\mu\text{g}/\text{kg}$ ) which is higher than the value found in *A. testudineus* (281.7  $\mu\text{g}/\text{kg}$ ) [36]. Vitamin A from fish species is more readily available for the body than that of plant sources [37]. Furthermore, *O. bimaculatus* is also richer in vitamin D (13108.39  $\mu\text{g}/\text{kg}$ ), essential for bone development. Previous studies also suggest that vitamin D has anticancer properties [38]. Vitamin D deficiency plays major role in the development of rickets and osteomalacia. In children, vitamin D deficiency causes soft bones and skeletal deformities [39]. In adults, vitamin D deficiency can lead to osteomalacia, resulting weak bones [40]. The vitamin E content of *O. bimaculatus* (7021.57  $\mu\text{g}/\text{kg}$ ) is also higher than the result obtained from *A. testudineus* (6363.63  $\mu\text{g}/\text{kg}$ ) [41]. Vitamin E is a vital nutrient crucial to maintain immunity, normal resistance of red blood corpuscles to haemolysis, and also plays critical roles permeability of capillaries and heart muscle [41]. Antioxidant activity of vitamin E protects biological membranes, lipoproteins and lipid stores against oxidation as well as protects unsaturated fatty acids against free radical mediated oxidation [42].

From many investigations, it is clear that vitamin K is responsible for the complete biosynthesis of many proteins in the human body that are fundamentals for blood coagulation. Blood coagulation is seriously impaired and uncontrolled bleeding occurs in the absent of vitamin K. Low levels of vitamin K also weaken bones and stimulate calcification of arteries and other soft tissues. Our investigation shows *O. bimaculatus* contains significant amount of vitamin K (12156.12  $\mu\text{g}/\text{kg}$ ) which is closely related to the *S. seenghala* [22].

#### 4. Conclusion

Due to tastiness and fewer numbers of intramuscular bones, *O. bimaculatus* is an extremely favored fish species as food. Furthermore, *O. bimaculatus* with high protein, minerals and vitamins content can be an important source of nutrition. Also fatty acid investigation shows that *O. bimaculatus* could be used as a tremendous source of good quality fatty acids, especially PUFA.

Nowadays, malnutrition is an alarming concern in developing and under-developed countries. Being one of the inexpensive sources of animal proteins, fish can play a significant role to prevent the diseases associated with protein-calorie malnutrition [43]. From our nutritional characterization, we can conclude that this work will provide valuable information about to make a proper balanced diet by nutritionist and dietitians. The evidence on nutrient outline of *O. bimaculatus* from our study indicates if this species is brought into aquaculture, it can play a vital role in the prevention of protein-calorie malnutrition in the underprivileged population, including fisherman community.

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#### References

- [1] Belton B., Karim M., Thilsted S., Jahan K. M., Collis W. and Phillips M. 2011. Review of aquaculture and fish consumption in Bangladesh. Studies and Reviews 2011-53. The World Fish Center, Penang, p. 76.
- [2] Roos N., Wahab M. A., Hossain M. A. R. and Thilsted S. H. 2007. Linking human nutrition and fisheries: incorporating micronutrient dense, small indigenous fish species in carp poly culture production in Bangladesh. Food and Nutrition Bulletin, 28 (2), pp. S280–S293 (Suppl).
- [3] Minkin S. F., Rahman M. M. and Halder S. 1997. Fish biodiversity, human nutrition and environmental restoration in Bangladesh. In: Open water Fisheries of Bangladesh (Tsai, C. & Ali, M. Y., eds.), pp. 75–88. The University Press Limited, Dhaka, Bangladesh.
- [4] Hels O., Hassan N., Tetens H. and Thilsted S. H. 2002. Food consumption, energy and nutrient intake and nutritional status in rural Bangladesh: changes from 1981–82 to 1995–96. Eur. J. Clin. Nutr. 57: 586–594
- [5] Osman H., Suriah A. R. and Law E. C. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. J. Food Chem., 73: 55-60.
- [6] Iso H., Rexrode K. M. and Stampfer M. J. 2001. Intake of fish and omega-3 fatty acids and risk of stroke in women. JAMA285: 304-312.
- [7] Nagakura T., Matsuda S., Shichijyo H. and Hata K. 2000. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. EurRespir J, 16: 861-865.
- [8] Breslow J. S. 2006. n-3 fatty acids and cardiovascular disease. Am J Clin Nutr 83(6 Suppl): 1477S–1482S.
- [9] Silva J. J. and Chamul R. S. 2000. Composition of marine and fresh water finfish and shell fish species and their products. In: RE Martin, EP Carter, EJ Flick and LM Davis (Eds.), Marine and fresh water products handbook, Lancaster, Pennsylvania, USA, 31-46 pp.
- [10] Kongsbak K., Thilsted S. H., Wahed M. A. 2008. Effect of consumption of the nutrient-dense, fresh water small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: a randomized, controlled study of efficacy. Br J Nutr 99: 581–597.
- [11] Bechtel, P. J. and Oliveira, A. C. M. 2006. Characterization of liver lipid and protein from cold-water fish species. J. Food Sci., 7: 480-485. doi: 10.1111/j.17503841.2006.00076.x.
- [12] Thilsted S. H., Roos N. and Hassan N. 1997. The role of small indigenous fish species in food and nutrition security in Bangladesh. Naga, the ICLARM Quarterly, 20(3-4), 82-84; 102.
- [13] Alam S. M. D., Uddin M. S. and Hasan S. 2015. Extraction, biochemical analysis and characterization of oil and lecithin from two selected fish species. International J. of Biosciences (IJB), vol. 7, No. 6, p. 92-102.

- [14] Stoffer W, Florence CHU, Ahrens E. 1959. Analysis of long chain fatty acids by Gas Liquid Chromatography. *Anal Chem.* 31: 307-308.
- [15] AOCS. 1998. Official method and recommended practices of the AOCS, American Oil Chemists' Society, Volume I & II. III.
- [16] Ishida Y., Fujita T. and Arai K. 1981. New detection and separation method for amino acid by high performance liquid chromatography. *J Chromatogr* 204: 143-148.
- [17] Sastry C. S. P. and Tammuru M. K. 1985. Spectrophotometric determination of tryptophan in protein. *J. Food Sci. Technol.* 22: 146-147.
- [18] Okorie I. A. 2010. Determination of potentially toxic elements (PTEs) and an assessment of environmental health risk from environmental matrices. A PhD Thesis, Northumbria University, Newcastle, North East of England.
- [19] Chatzimichalakis P. M., Samanidou V. F. and Papadoyannis J. N. 2004. Development of validated liquid chromatography method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction. *J. Chromatogr. B* 805: 289-296.
- [20] Bratu A., Mihalache M., Hanganu A., Chira N. A., Todașcă M. C. and Roșca S. 2013. Quantitative determination of fatty acids from fish oils using GC-MS method and <sup>1</sup>H-NMR Spectroscopy. *U. P. B. Sci. Bull., Series B, Vol. 75, Iss. 2, ISSN 1454-2331.*
- [21] Mohanty S. N. and Kaushik S. J. 1991. Whole body amino acid composition of Indian major carps and its significance. *Aquat Living Resour* 4: 61-64.
- [22] Mohanty B. P., Paria P., Das D., Ganguly S., Mitra P., Verma A., Sahoo S., Mahanty A., Aftabuddin M., Behera B. K., Sankar T. V. and Sharma A. P. 2012. Nutrient Profile of Giant River-Catfish *Sperata seenghala* (Sykes). *Natl. Acad. Sci. Lett.* 35(3): 155-161.
- [23] Swendseid M. E., Villalobos J. and Friedrich B. 1963. Ratios of essential-to-nonessential amino acids in plasma from rats fed different kinds and amounts of proteins and amino acids. *J. Nutr* 80: 99-102.
- [24] Pinto J. F., Nunes M. L. and Cardoso C. 2007. Feeding interruption and quality of cultured gilthead sea bream. *Food Chem.* 100: 1504-1510.
- [25] Musa A. S. M. 2009. Nutritional quality components of indigenous fresh water fish species, *Puntius stigma* in Bangladesh. *Bangladesh J Sci Indu Res* 44: 367-370.
- [26] Gopakumar K. 1997. Biochemical composition of Indian food fish. Central Institute of Fisheries Technology (ICAR), Cochin.
- [27] Roos N., Islam M. M., Thilsted S. H. 2003. Small fish is an important dietary source of vitamin A and calcium in rural Bangladesh. *J. Nutr* 133: 4021S-4026S.
- [28] Gulfranz M., Ahmad T., Afal H. 2001. Concentration levels of heavy and trace metals in the fish and relevant water from Rawaland Mangla lakes. *J. BiolSci* 1: 414-416
- [29] Tulchinsky T. H. and Varavikova E. A. 2009. Nutrition and food safety. In: *The new public health*, 2nd edn. Elsevier Academic Press, San Diego, p 310.
- [30] Gopakumar K. 1997. Biochemical composition of Indian food fish. Central Institute of Fisheries Technology (ICAR), Cochin.
- [31] World Health Organization. 1990. Environmental health criteria 101: methyl mercury. WHO, Geneva.
- [32] Agarwal R., Kumar R. and Behari J. R. 2007. Mercury and lead content in fish species from the river Gomti, Lucknow, India, as biomarkers of contamination. *Bull Environ Contam Toxicol* 78: 118-122.
- [33] FAO/WHO Expert committee of food additives (1983), 27th report, Technical Report Series No. 696, World Health Organization, Geneva.
- [34] De Gieter M., Leermakers M., Van Ryssen R., Noyen J., Goeyens L. and Baeyens W. 2002. Total and toxic arsenic levels in North Sea fish. *Arch Environ Contam Toxicol* 43: 406-417.
- [35] Roos N., Islam M. M., Thilsted S. H. 2003. Small fish is an important dietary source of vitamin A and calcium in rural Bangladesh. *J. Nutr* 133: 4021S-4026S.
- [36] Mohanty B. P., Sudheesan D., Sankar T. V., Das M. K. and Sharma A. P. 2011. Therapeutic value of fish. Central Inland Fisheries Research Institute, Kolkata, India. Bulletin No. 170. <http://www.cifri.ernet.in/ebulletins.html>.
- [37] Liu R. H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 78(suppl): 517S-520S.
- [38] Byers T. 2010. Anticancer vitamins du Jour--The ABCED's so far. *Am. J. Epidemiol. (Review)* 172 (1): 1-3.
- [39] Wharton B. and Bishop N. 2003. Rickets. *Lancet* 2003; 362: 1389-400.
- [40] Institute of Medicine, Food and Nutrition Board, Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press, 2010.
- [41] Halver J. E. 2002. The vitamins. In: Halver JE, Hardy RW (eds) *Fish nutrition*. Academic Press, San Diego, pp 61-141.
- [42] Hamre K., Berge R. K. and Lie O. 1998. Turnover of a-, c- and d- tocopherol and distribution in subcellular and lipoprotein fractions indicate presence of an hepatic tocopherol binding protein in Atlantic salmon (*Salmo salar L.*). *Fish Physiol Biochem* 8: 71-83.
- [43] Beveridge M. C. M., Thilsted S. H., Phillips M. J., Metian M., Troell M., and Hall S. J. 2013. Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *J Fish Biol.* 83(4): 1067-1084.