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Determination of fatty acid composition in two loach species (*Botia lohachata*, *Botia geto*) by GC-MS

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Abstract
The aim of this study was to explore the fatty acids (FAs) composition of Indian loach freshwater fish (*Botia lohachata* and *Botia geto*) and compare the existing levels of essential fatty acids. The fatty acid composition was evaluated by Gas chromatography–Mass spectrometry (GC-MS). Fatty acid methyl esters (FAMEs) were identified comparing their retention time with the commercial analytical standard mixtures FAME Mix, C4-C24. The fatty acids profiles include odd-number, branched-chain, and even-number fatty acids as well as saturated components, the monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The major Saturated fatty acids SFAs were C14:0, C16:0 and C18:0. The C18:1n-9c was the prominent MUFAs. The essential fatty acid compositions showed prominence in C18:3n-3 and C18:2n-6. The branched chain fatty acids identified were C15:0, C16:0, C17:0, C18:2 and C20:0. The results of the study has shown that *Botia lohachata* is a good source of MUFAs while *Botia geto* is a good source of ω-6 EFAs and the high percent of branched and saturated FAs.

Keywords: Fatty acids, *Botia lohachata*, *Botia geto*, and Gas chromatography, mass spectrometry

1. Introduction
Fish is considered as a vital food source for humankind all over the place since time immemorial. Fishes are not only foremost macromolecule supplier but also they contain nutritionally valuable lipids and fatty acids. Fresh fish contains saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and long-chain unsaturated fatty acids that play vital role in human health. Polyunsaturated fatty acids (PUFAs) are essential due to their ability to prevent certain disorders and diseases like arterial sclerosis, thrombogenesis, high blood pressure, cancer, and skin diseases [1, 2, 3, and 4]. It has been estimated that around 60 per cent of the Indian population devour fish diet and the consumption pattern varies spatio-temporally and across the different social systems. The annual per capita consumption of fish for the entire population is estimated at 5-6 Kg whereas in fish eating population, it is found to be 8-9 Kg, which is considered as a poor 50 percent of the global rates. The consumption of fish diet is usually recommended as a method of preventing diseases and has significantly increased over decades in several European Countries [1]. Further these fishes have huge anti-microbial peptide in protecting against dreadful human pathogens [5].
Fish species have variation in their fatty acids composition and levels. The variation in fatty acids of fish has been correlated with diet consumed, reproductive cycle, temperature, season, and geographical location. Botia lohachata is a freshwater fish of the family Cobitidae and distributed only in Asian countries i.e., Bangladesh, India, Nepal and Pakistan. It mainly inhabits rivers and streams. It is used as food fish in Bangladesh and also used as aquarium fish. *Botia geto* is a small sized popular food on account of revitalizing qualities of its flesh and found in rivers, lakes, ponds, streams and wetlands of Assam. The people for its good flavour like this fish. The objective of this study was to investigate the comparative composition of fatty acids between *Botia lohachata* and *Botia geto* loach fishes, as there was no research work was carried out in these species on the FAs profile.

2. Materials and Methods

2.1 Sample collection and preparation

Botia lohachata and *Botia geto* species have been obtained from middle to lower Ganges and Brahmaputra river drainages in northern India, respectively (Figure 1 & Figure 2). The fishes were weighed, beheaded, filleted, and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their fleshes were removed from their backbones, minced, blended, and immediately extracted using chloroform-methanol mixture in the ratio of 2:1.

2.2 Lipid extraction

Lipid extractions were carried out on minced fish samples (100 g each) using the extraction method [6]. In this method, chloroform-methanol was used in the ratio of 2:1. A total of three repetitions were performed per sample. The solvent mixtures were concentrated *in vaccuo* using a rotary evaporator maintained at 35°C and the extracts were stored in respective sample vials.

The method [7] was used in the methylation of the extracted fish lipids. Methylene chloride (100 µl) and 1 ml 0.5M NaOH in methanol were added to fish tissues in a test-tube and heated in water bath at 90°C for 10 min. The test tube was removed from the water bath and allowed to cool before adding 1 ml 14% BF3 in methanol. Again, the test tube was heated in water bath at 90°C for 10 min, and then cooled to room temperature. One ml of distilled water and 200 µl hexane was added to all the test tubes and then fatty acid methyl ester was extracted by vigorous shaking for one minute. After centrifugation, the top layer which contains the fatty acid methyl ester was collected and transferred into a sample vial for analysis.

The fatty acids profile were determined using an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25 mm id x 0.25 mm DB wax). The starting temperature was 150°C maintained for 2 minutes at a heating rate of 10°C /minute. The total running time was 22 minutes. Helium was the carrier gas whereas the injection volume was 1µl.

3. Results and Discussion

3.1 Composition of FAs in the selected loaches (*Botia lohachata and Botia geto*)

A total of 23 FAs were identified in the two *Botia lohachata and Botia geto* loach species. The unsaturated FAs were moderately high compared to SFAs. The most dominant SFAs were palmitic acid (16:0), stearic (octadecanoic) acid (18:0) and myristic acid (14:0). Nine of the sixteen unsaturated FAs were PUFAs and 7 were MUFAs. Among the nine types of PUFAs, the *ω*6 PUFAs were relatively more abundant, followed by *ω*3 PUFAs. Three omega 9 PUFA was recorded in both selected loaches. The dominant *ω*6 PUFAs were linoleic acid (C: 18:2n-6), eicosadenoic acid (C20:2n-6), eicosatetraenoic acids (20:3n-6). *Botia lohachata* had comparatively the highest number of FAs principally in MUFAs particularly in oleic acid 43.26% while *Botia geto* was 27.96% (Table 1). *Botia geto* had comparatively the highest number of FAs predominantly SFAs palmitic acid 27.59% whereas *Botia lohachata* was 19.70%.

Dominant fatty acids of the two loach species (*Botia lohachata and Botia geto*) were palmitic acid (16:0), stearic acid(18:0), behenic acid(22:0), palmitoleic acid(16:n-7), oleic acid(18:1-9), linoleic acid(18:2n-6), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid(22:6n-3). These fatty acids are preferred substrates for mitochondrial β-oxidation and the Krebs cycle to generate metabolic energy in fish [8, 9, and 10]. This study found 23 types of FAs with different saturation levels in the two commercial fish species. Out of the 23 FAs, the saturated SFAs were 7 (30%) and unsaturated ones were 16 (69%) which included 9 PUFAs and 7 MUFAs. These results are reasonably similar to those obtained by [11]. In their study, they obtained 22 FAs of different saturation levels comparable to the present results. The dominant unsaturated FAs were eicosatetraenoic, docosahexaenoic acid and oleic acid. The existence of more unsaturated FAs than saturated FAs in the fish samples is similar to [12] who obtained more categories of unsaturated FAs (53.91%) than saturated FAs (46.24%) in R. argentea. The dominant unsaturated FAs in this study are similar to those obtained by [13, 14, 15, and 16].

The higher amount of unsaturated FAs compared to saturated FAs as observed in this study could be due to their habitual occurrence. The MUFAs and SFAs can be synthesized de novo. De novo fatty acid synthesis increases when diets are rich in carbohydrate and protein is efficient use for energy provision in fish [17]. The existing interspecies variability in the composition of fatty acid of fish lipids (and of the specific PUFAs in particular) is usually interpreted by the existence of a large number of external factors (environment, culturing method, tropic effects) and internal factors (fish species, feeding regime and digestion, life cycle, stage, quantitative and qualitative characteristics of lipids triglycerols, phospholipids and their topographical origin dorsal and ventral part of muscle tissue) [14]. Freshwater species are known to contain appreciable load of unsaturated FAs.

3.2 Types and levels of PUFAs found in the *Botia lohachata* and *Botia geto*

Totally four number of *ω*3 PUFAs were detected in the fish sample. The dominant linolenic acid (4.48%) was found in *Botia geto*. A slight change in the levels of Eicosapentaenoic acid (EPA) was observed in the two freshwater species. Similarly, the quantity of *ω*6 PUFAs also showed variation among the two fish species. High levels of Eicosatetraenoic acid (2.58%) and Linoleic acid (4.48%) were found in *Botia geto* on compared to *Botia lohachata* which has (1.51%) of Eicosatetraenoic acid and (4.30%) of Linoleic acid. Docosapentaenoic acid (DPA) levels was not significantly detected among the selected loach species.

*ω*3 PUFAs can be divided into three main classes such as Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and linoleic acid. EPA and DHA are found mainly in fish oils while alpha- linoleic acid is usually derived from plant sources. *ω*3 PUFAs helps in the prevention of cardiovascular diseases by reducing the emergence of irregular heart rhythms that may lead to sudden fatal condition. In addition, *ω*3 PUFAs prevent asthma, hypertension, diabetes, cancer and kidney failure [18]. EPA possess a defensive effect to thrombosis and inflammatory diseases especially atherosclerosis due to its high antioxidant capacity [19].

Botia lohachata and *Botia geto* has considerable amount of UFA than SFA. The quantity of *ω*3 PUFAs was found to be high in *Botia geto* fish. *ω*3 PUFAs which are primarily DHA and EPA was found in higher amount in *Botia geto* and in appreciable amounts in *Botia lohachata* fish. These results can further enhance to prefer these fish varieties as they are considered as an unsaturated low fat diet with anti-

inflammatory properties. These results would be helpful for taking these fish as food as they contain highly unsaturated low-fat diet and help to prevent diseases. The fatty acid composition of the studied fish groups *Botia lohachata* and *Botia geto* indicate that these fish groups have considerable nutritional capacity. However, the fish groups gradually becoming vulnerable and critical one due to indiscriminate fishing and habitat loss. A management programme for these species conservation should be undertaken.

4. Conclusion

Loach fish fillet is considered as nutritionally rich diet for the end users. Chemical and fatty acid compositions were totally varied between the selected species. This study demonstrated that *Botia lohachata* have regular pattern of FAs composition and a better source of MUFAs while *Botia geto* is a good source of ω-6 EFAs and contains highest percent of branched and saturated FAs. The fatty acids profiling undertaken on these two fresh water fish species shall be the inceptive study which provide valuable information to ecologists, environmentalists, nutritionists, food technologists and other biologists.

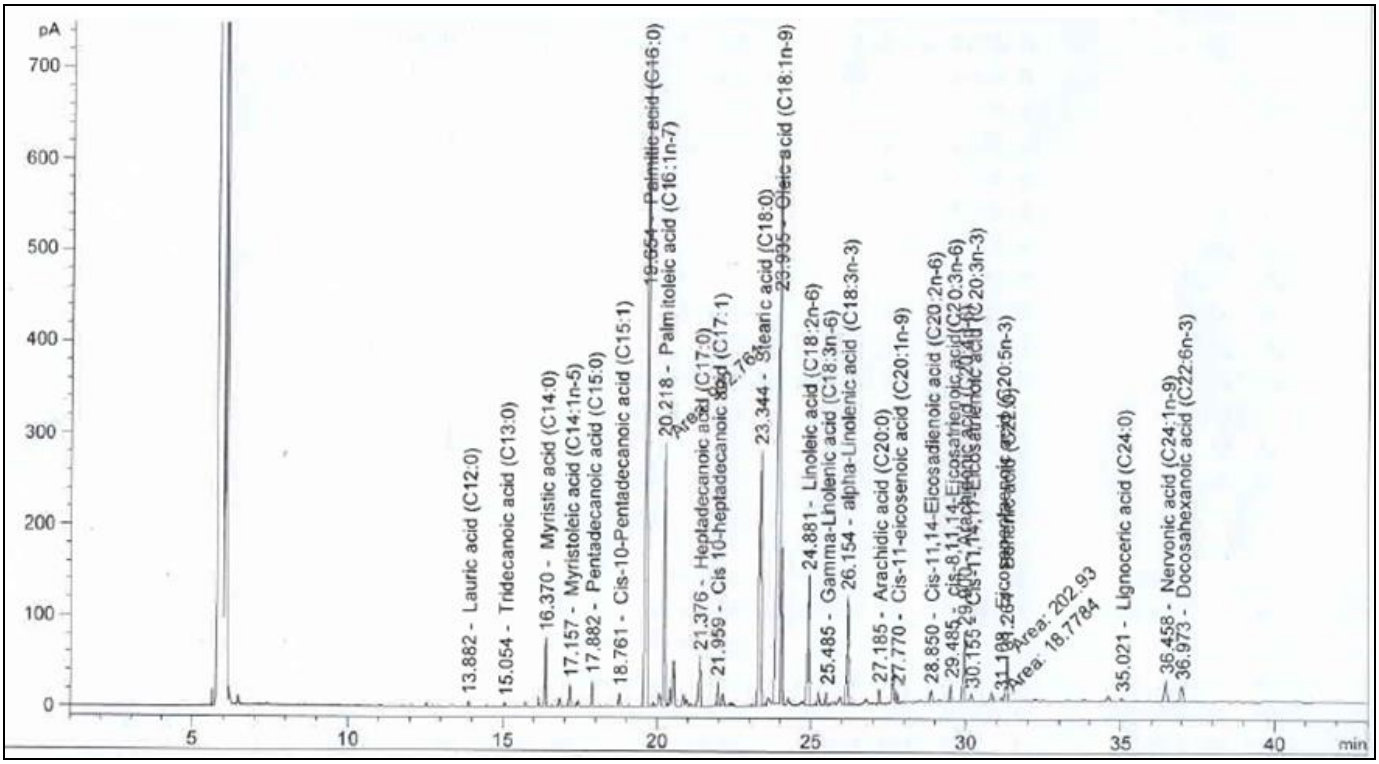

Fig 1: Gas Chromatogram of 22 fatty acids *Botia lohachata*. Each peak is labeled with its corresponding fatty acid.

Fig 2: Botia lohachata

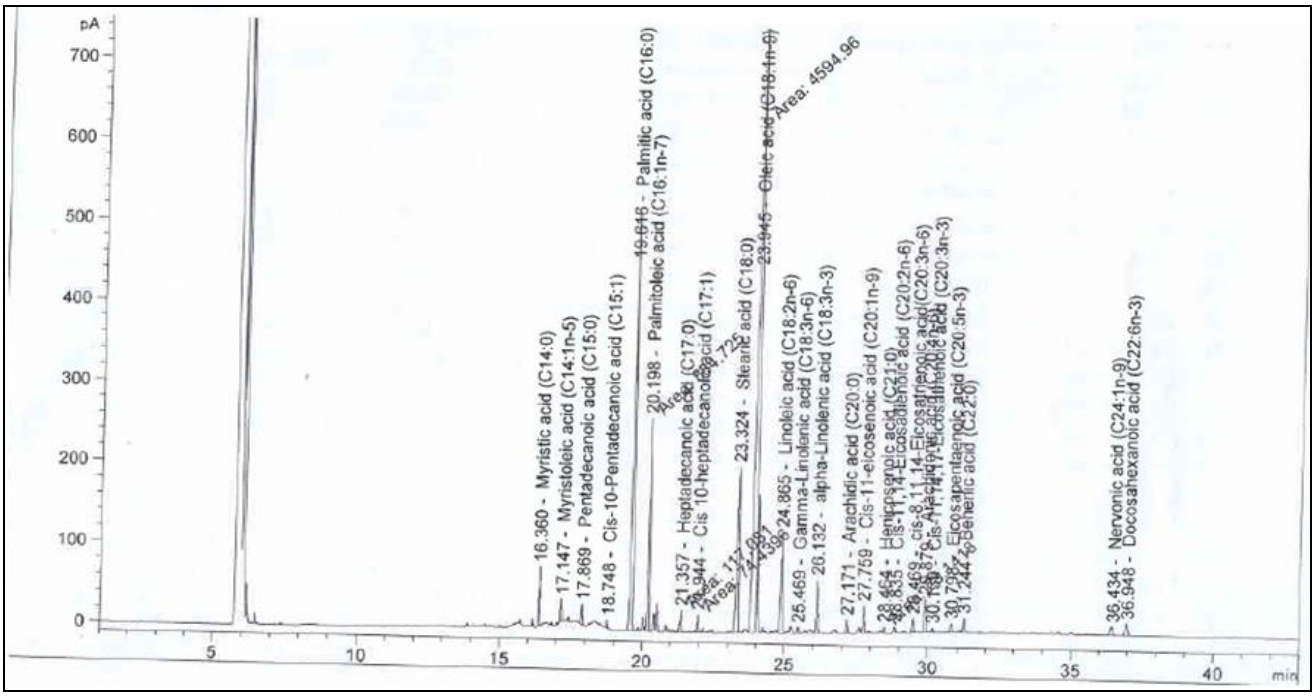

Fig 3: Gas Chromatogram of 22 fatty acids *Botia geto*. Each peak is labeled with its corresponding fatty acid.

Fig 4: Botia geto

Table 1: Fatty acid composition in *Botia lohachata* and *Botia geto* obtained by GS-MS analysis

Sl. No	Parameters	*Botia lohachata*	*Botia geto*
	I. Fatty Acid Profile:	Area (%)	Area (%)
1	Mono Unsaturated Fat	56.19	43.18
2	Poly Unsaturated Fat	9.63	10.34
3	Saturated Fat	34.18	46.48
	II. Saturated Fatty Acids		
1	Myristic Acid	1.74	1.61
2	Pentadecanoic Acid	0.65	0.69
3	Palmitic Acid	19.70	27.59
4	Heptadecanoic Acid	1.10	2.38
5	Stearic Acid	9.49	11.54
6	Arachidic Acid	0.59	0.54
7	Behenic acid	0.66	1.73
	III. Mono Unsaturated Fatty Acids		

1	Myristoleic Acid	0.71	0.55
2	Cis-10-Pentadecenoic Acid	0.31	0.41
3	Palmitoleic Acid	8.33	8.47
4	Cis-10-Heptadecenoic Acid	0.70	0.79
5	Oleic Acid	43.26	27.96
6	Cis-11-Eicosenoic Acid	1.20	0.42
7	Nervonic Acid	0.62	1.22
	IV. Poly Unsaturated Fatty Acids		
1	Linoleic Acid	4.30	4.48
2	Gamma-Linolenic Acid	0.23	0.46
3	Linolenic Acid	2.25	3.79
4	Cis-11-14-Eicosadienoic Acid	0.27	0.44
5	Cis-8,11,14-Eicosatrienoic Acid	0.60	0.60
6	Cis-11,14,17-Eicosatrienoic Acid	0.20	0.31
7	Cis-5,8,11,14-Eicosatetraenoic Acid	1.51	2.58
8	Cis-5,8,11,14,17-Eicosapentanoic Acid	0.42	0.16
9	Cis-4,7,10,13,16,19-Docosahexanoic Acid	0.89	0.89

5. Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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