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Fatty Acids Composition of Someselected Indian Fishes

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Abstract: A comparative study on the fatty acids content of four Indian fishescatla (*Catlacatla*), rohu (*Labeorohita*), magur (*Clariusbatrachus*) and pangas (*Pangasianodonhypophthalmus*) were carried out in the present study. The proximate analysis revealed that the protein content of catla, rohu, magur and pangaswas 10.11%, 9.53%, 14.87% and 13.6%(% of wet weight), respectively. The total lipid content was generally high, ranging from 1.2 % to 7.9 % and crude ash ranged from 1.25% to 2.7%. Saturated fatty acids (SFA) were most abundant in catla(60.92%) while in rohu(52.28%),pangas (47.15%) and magur (39.85%) respectively. Magurwas rich in polyunsaturated fatty acids (PUFA)(25.56%) than pangas (23.37%), rohu (15.84%) and catla (12.5%). The omega 3 and 6 ratio (ω3/ω6) was ranging from 0.706 to 6.544 and catlashowed the highest ratio.The most abundant fatty acid in all fishes was C16:0, ranging from 32.2% to 38.23%. The other major fatty acids detected were C18:0, C18:1 and C18:3. This result clearly shows that the importance of fish nutrition in the human diet for combating the diseases like heart problems, cholesterol and many nervous related problems.

Key words: Fatty Acid Content · Rohu · Catla · Pangas · Magur

INTRODUCTION

Fish has been recognized as an excellent food source for human beings for centuries and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and minerals for the formation of functional and structural proteins [1]. Fish are a rich source of polyunsaturated fatty acids (PUFAs), namely, the n-3 and n-6 PUFAs, which are beneficial to human health. Fish meat and oils are good sources of unsaturated omega-3 fatty acids, eicosapentacenoic acid (EPA, 20:5n-3) and docasahexaenoic acid (DHA, 22:6n-3), as well as its precursor, alpha linolenicacid [2, 3]. Compared to beef and chicken, fish meat contains higher levels of n-3 PUFAs [4], which are known to be cardio-protective, anti-atherosclerotic, antithrombotic, and anti-arrythmitic and also play a role in reducing the cholesterol level [5], regulate prostaglandin synthesis and hence induce wound healing [6] and stabilizing the electrical activity of the heart cells [7]. Therefore, fish meat containing high n-3/n-6 ratios of PUFAs are the most beneficial in terms of human health[8]. PUFA composition may vary among species of fish, even among fresh water and marine fish.

Lipids are one of the most important components of fish muscle providing energy reserves and components of cell bio-membranes. The lipid is extracted from the flesh for the analysis of lipid composition. Fish flesh is an important part of the fish, which people eat and is considered the important source of protein. Fish lipids are dominated by saturated fatty acids like palmitic (C16:0) and myristic (C14:0) acids followed by stearic acid, whereas the monosaturated fatty acids (MUFA) are oleic and palmitoleic acids [9] and in Polyunsaturated fatty acids (PUFA) are EPA and DHA, which may reduce the risk of coronary heart diseases [8, 10]. Studying the lipid composition of these local fishes, some important data or information regarding the biochemistry of fish lipids and their nutritional properties can be obtained.

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The main objective of this study was to determine the fatty acids profile and composition (percentages) of four species *Catlacatla, Labeorohita, Clariusbatrachus*and *Pangasianodonhypophthalmus.* These species are selected for the study because of their economical importance and consumer demand in the Indian aquaculture. Therefore, detailed information about their fatty acids composition was important from nutritional point of view and was needed because it influenced the quality in frozen storage of some fish species. Thus; this study was carried out to evaluate the lipid content and fatty acid profile of commercially important fresh water fishes from Indian waters.

MATERIALS AND METHODS

Sample Collection: Four of the freshwater fishes were selected;catla (*Catlacatla*), rohu (*Labeorohita*), magur (*Clariusbatrachus*) andpangas (*Pangasianodonhypophthalamus*). Four samples of similar body weight and length for all analyzed fish species were collected from four bunglows fish market located at Mumbai, India. Prior to analysis, about 25 g of fish muscle tissue was separated for the determination of fatty acid composition and as well as for other tests.

Chemicals: All solvents used (analytical and GC MS grade) were obtained from Fisher Scientific (Mumbai, India). Anhydrous sodium sulphate, iodine and sodium hydroxide were purchased from Merck India. Trichloroacetic acid (TCA), linoleic acidand other chemicals were purchased from Sigma Chemicals (India). All other materials otherwise specified, were of reagent grade or the highest available grade from usual commercial sources.

Proximate Composition

Moisture: Moisture content in the fish sample was determined by using automatic moisture analyzer (IR 120, Denver, moisture analyzer). Briefly, 1 g of the sample (Fish muscle) was taken and cut into small pieces and spread on the clean plate. The sample was heated at an initial temperature of 100°C and a final temperature of 170°C until a stable weight was achieved. Moisture percentage was obtained from the weight loss due to heating.

Ash: The ash content of the samples was determined by following AOAC (11) method. 5 g of the sample (fish muscle) was taken in a previously ignited and weighed silica crucible. It was then transferred to muffle furnace (Phoenix CEM Corporation, USA) and the temperature was raised to 600°C and kept for 6 hours until white ash was obtained. Weight was taken after cooling and the percentage of ash was calculated from the weight difference.

Protein: Crude protein content was determined by using Automatic Microkjeldahl unit (Kel Plus-Classic DX (VA), Pelican Equipments) were followed by AOAC [11] method. Briefly, 0.5 gof sample (Fish muscle) was taken and digested with 1.6 g of digestion mixture (K_2SO_4 and $CuSO_4$ in 5:1 ratio) in 20 ml of conc. H_2SO_4 The digestion was carried out in the digestion unit till the solution become clear or light green colour. Digested sample was diluted to 250 ml and then 5 ml of the digested solution was taken for automatic distillation in the Microkjeldahl unit. The total program time was 9 min. and the liberated NH_3 was collected in a conical flask containing Boric acid and mixed indicator (Bromo cresol green and methyl red). The amount of NH_3 liberated was determined by titrating with 0.1 NH_2SO_4. Crude protein content was calculated by multiplying the total Nitrogen content with conversion factor of 6.25 and expressed as percentage.

Crude Fat: The crude fat content of muscle was determined by Soxhlet extraction method [11]. About 1g of sample was taken in a Whatman thimble. The thimble was plugged with cotton loosely and placed in Soxhlet extraction unit. Petroleum ether AR grade was used as solvent. Extraction was continued for 16 hours. After the extraction, the pre-weighed receiver flask containing the extracted fat was dried initially on a water bath at 98°C±5°C. After complete drying, the receiver flask was cooled in desiccators and then the weight was obtained. The difference in the initial and final weight of receiver flask was determined as fat content of samples was calculated.

Extraction of Total Lipids: Total lipids were extracted from muscle tissue according to Folch *et al*. [12]. Briefly, 5 g of fish muscle tissue of each fish was taken after they have been partially thawed. A chloroform/methanol solvent mixture (2:1 v/v) was added to fish muscle tissue sample in the ratio solvent: tissue of 20:1. The samples were homogenized 3 times for 10 min. with 3000 x g - 4000 x g,cooling of the sample for 1 hour at 4°C was done after each homogenization step. 4 ml of 0.034% $MgCl_2$ was added to the extracts for each 1 g of tissue.

The chloroform/methanol extracts were incubated overnight at 4°C, allowing the separation of the organic (containing the extracts of total lipids) and aqueous layers completely. Upper (aqueous) layer was removed and lower (organic) layer was rinsed with mixture of chloroform: methanol (2:1) and placed in glass tubes. Evaporation of the lower phase furnished the total lipid fraction. The solvent was removed in rotary evaporator under vacuum at 40°C. These extracts, representing the total lipids, were weighted and dissolved once again in a small volume (1-2 ml) of chloroform/methanol (2:1). The resulting extract of total lipids was stored at 4°C for further analysis. The total lipid was expressed as g/100 g tissue.

Preparation of Fatty Acid Methyl Esters (FAME): Total fatty acid content and fatty acid composition were determined simultaneously in the fish oil samples. Fatty acid analysis was performed in triplicate consisted two consecutive steps, preparation of fatty acid methyl ester (FAME) and chromatographic analysis. The AOAC[13]method was followed to esterify the lipid extract. FAME was prepared from the lipids extracted from samples by heating with the methanolicNaOH first and then with BF3 Methanol for esterification. 5 ml n-heptane was added to recover the methyl esters in organic phase. Saturated NaCl solution was added to the mixture and the aqueous and organic layers were separated using a separating funnel. The upper n-heptane phase was pipetted out and stored in 10 ml glass vials and store under sub-zero temperature till GC-MS analysis is done.

Fatty Acid Methyl Ester (FAME) Analysis by Gas Chromatography Mass Spectrometer (GC MS): The gas chromatograph analysis of methylated fatty acids was performed on a Shimadzu Qp2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25ìm film thickness) capillary column (Cromlab S.A). One microliter of sample was injected into the capillary column. Helium was used as the carrier gas. Injector and detector temperatures were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50°C for 2 minutes and then to increase at a rate of 10°C per min to a final temperature of 230°C. Fatty acid methyl esters were separated at constant pressure (23.1 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. Besides that, the fatty acid methyl esters were identified also by comparing with their retention times with those of the commercial fatty acid methyl ester standards (GC 18-91) shown in Table 1.

Statistical Analysis: Data presented as mean ± standard error of the mean (SEM), and significant differences between the means were determined by using SPSS (version 16).

RESULTS AND DISCUSSION

This experiment was performed to study the percentage of moisture, total lipids and fatty acid profile in different fresh water fish of India. The data recorded through physical and chemical analyses were subjected to a statistical analysis which showed significant variations in moisture contents, total lipids content and the deposition of fatty acids in the muscle of these four fresh water fishes. The proximate composition of the selected Indian fishes shows that the differences in all the contents analyzed were shown in the Table 2.

Table 1: Details of characteristic ions used in the study for identification of fatty acids

Class of fatty acid	Characteristic ions (m/z)	Fragmentation
SAFA	74	Formation of McLafferty rearrangement.
MUFA	M-74	Formation of stable and abundant ion M-74after the loss of McLafferty ion.
PUFA	91	Formation of cyclic tropylium ions in fatty acids with four or more double bonds.
n-6	150	Cleavage of tail from n-6 terminal in metylene interrupted polyenoic bonds.
n-3	108	Cleavage of tail from n-6 terminal in metylene interrupted polyenoic fatty acids.

Table 2: Proximate Composition (%) of Different Selected Freshwater FishesIn India

Nutrients	Catla	Rohu	Magur	Pangas	Significant difference
Moisture	77.5 ±6.5	75.46 ±6.0	73.49±5.9	74.57±6.0	NS
Protein	10.11±0.80	9.53±0.72	14.87±1.19	13.60 ±0.98	S
Lipid	1.2±0.08	2.9 ±0.21•	7.90±0.63	4.98±0.38	S
Ash	2.7 ±0.22	2.20 ±0.17	3.74±0.28	1.25 ±0.28	S

NS=Non-Significant, S= Significant

Table 3: Fatty acid composition (% of total lipid extract) of meat from some selected freshwater fishes in India

Fatty acid	Catla	Rohu	Magur	Pangas
C12:0	0.24	ND	ND	0.38
C13:0	0.15	ND	0.01	0.06
C14:0	4.17	2.5	1.65	10.15
C15:0	1.73	1.03	0.3	0.63
C16:0	38.23	32.39	32.2	34.93
C17:0	2.3	1.28	0.32	0.47
C18:0	13.22	14.15	5.09	ND
C19:0	0.43	0.62	0.08	0.09
C20:0	0.45	0.31	0.2	0.44
Σ Saturates	60.92	52.28	39.85	47.15
C16:1,n-7	ND	0.67	3.51	2.27
C16:1,n-9	ND	ND	0.44	ND
C18:1n-9	14.13	27.18	27.8	29.43
C20:1,n-9	1.13	1.7	ND	1.77
C22:1,n-9	ND	ND	ND	ND
Σmonounsaturates	15.26	29.55	31.75	33.47
C18:2 n-6	1.58	4.65	14.98	11.71
C20:2,n-7	0.31	ND	ND	0.49
C18:3 n-3	3.04	2.84	4.72	2.58
C20:3,n-7	0.27	0.45	ND	0.79
C20:4,n-6	ND	2.87	ND	0.98
C20:3,n-3	ND	ND	ND	0.41
C20:5 n-3	1.9	1.29	2.1	1.93
C22:6 n-3	5.4	3.74	3.76	4.48
Σpolyunsaturates	12.5	15.84	25.56	23.37
n3/n6	6.544	1.046	0.706	0.740

ND: not detected

The moisture content was found to be high in catla and low in magur. However, there was no significant difference in moisture content amongthe species taken in this study. Magur produces the highest oil content which is 7.90 g/100 g followed by pangas (4.98 g/100 g), rohu (2.9 g/100 g) and catla (1.2g/100 g). Lipids are generally regarded as the most important constituent used to evaluate the quality of fish meat [14, 15]. The lipid content in the muscle showed an increasing trend with decreasing moisture contents of the fish. The difference in lipid content may be attributed to difference in their feeding habits.The protein content was also found to be high in magur and low inrohu.The maximum and minimum ash contents were found in magur and pangas respectively. However, the significance difference was found in protein, lipid and ash contents among species. Literature reports also reveal that the lipid contents in the fish body is also affected by environmental and nutritional conditions[16].

Fatty Acid Composition of Selected Indian Freshwater Fish Oils: The mass spectrums and structure of fatty acid methyl esters were obtained by using the mass spectrometer (MS). In this fatty acid analysis study, fish oils give a total of 10 to 20 fatty acid peaks with a slight variation of fatty acid concentration (%). Detailed fatty acid compositions and the ratios of ω-3 to ω-6 are listed in Table 1, which is based on GC chromatogram obtained from GC MS analysis. In addition, Table 3 also shows the ratios of ω-3 to ω-6 in oils extracted from the fish studied. The ratio of ω-3 to ω-6 are useful to determine that the fish gives out a better index in comparing relative nutritional value of fish oils in different species. The chromatograms also showed that all the local fish oils are made up of long chain fatty acid, with a minimum carbon chain length of 12 to 22carbons which is considered as typical characteristic of fish oil.

In this study the PUFA content in fresh water fishes was lesser (12.5-23.56%) than the saturated fatty acids (39.85-60.92%) and mono-unsaturated fatty acids (MUFA) (15.26-33.47%). It was observed that the fatty acid composition of total lipids varied greatly in the muscle of all four types species. The differences in fatty acid composition may be influenced by environmental and nutritional conditions [16]. Other researchers have also shown that freshwater fish have lower content of PUFAs [17] because fresh water fishes feed largely on

vegetation and plant materials. It was observed that the C12:0 saturated fatty acids in muscle of all four species were negligible but C14:0 has significant amount (1.65-10.15%). Long carbon chain saturated fatty acids like C20:0, C22:0 were found negligible in all species. Saturated fatty acid C16:0, observed as a major constituent of muscle lipid of all species which is present in range of 32.2-38.23%. Catla has highest amount of C16:0 (38.23%) followed by pangas (34.93%). The finding of the present experiment contradicts the results declared by Mohamed and Al-Sabahi [18] who reported a higher concentration of C16:0 in the muscle tissue of *Latesniloticus, Bagrusbayad, Oreochromisniloticus, Synodontisschall*and *Tetraodonlineatus*. Our findings are also in closeagreement with the results reported by Swapna *et al*. [19] and Ho and Paul [20] for the saturated fatty acids in the fresh water fish flesh. In mono-unsaturated fatty acids, C18:1 fatty acid was observed in the highest concentration which was ranged from 14.13%, 27.18%, 27.8% and 29.43% in the muscle lipids of catla, rohu, magur and pangas, respectively. Our findings regarding the C18:1 fatty acid is almost comparable with the concentration reported by Swapna *et al*. [19] for the same fatty acid in the muscle of Indian fresh water fishes. These similar results were obtained by Ackman *et al*. [21].

Majority of PUFAs, in fish oils ranged between C18 to C22 in chain length and are the omega-3 type. However, the fresh water fishes usually contained higher amounts of omega-6 fatty acids than the marine fish oils [22]. This was found to be the same in Indian freshwater fishes where the freshwater fishes are rich in 18:2 (n-6) whose range is 1.58 to 14.98 % but low in PUFAs content. This result is supported by Muhamad and Mohamed [23]. However, the MUFAs appeared to be the major fatty acid in freshwater fishes.The local freshwater fishes such as the catla, rohu, magur and pangas contained higher proportions of saturated fatty acids and C18 PUFAs but lower levels of C20 and C22. The major monounsaturated fatty acid observed in these four local freshwater fishes was 18:1. Similar results were also obtained by Ackman *et al*. [21].

In present study, linoleic acid (18:2, n-6) range is 1.58 to 14.98 % which is similar to result obtained by Sharma *et al*. [2] and Swapna *et al*. [19]. This result is supported by Muhamad and Mohamed [23]. The recent study regarding the nutritional properties of these species suggested that, they have a higher nutritional value due to the unsaturated fatty acids and comparative low saturated fatty acid content. The palmitic, stearic and myristic are the major types of saturated fatty acids but the content of these saturated fatty acids are considered low when compared to other animal fats. The values of eicosapentaeonoic acid (EPA) and docosahexaenoic acid (DHA) of local fishes studied are showed in Table 3. The maximum amount of polyunsaturated fatty acid (PUFA) was estimated to be 25.56% in the muscle of magurfollowed by 23.37%, 15.84%, and 12.5% in pangas, rohu and catla respectively. The result obtained in this study is similar to result reported by Ho and Paul [20]. PUFA, linolenic acid (C18:3, n-3) was found in the muscle lipids of above species between 2.58- 4.72%. It was found that magur has highest amount (4.72%) of C18:3 (n-3) in muscle lipid. Overall, the total PUFA were found in comparable concentrations with those reported by Swapna *et al*. [19]. These polyunsaturated fatty acids even present in low concentrations, are an important group of lipid metabolism and are bases for the formation of arachidonic acid C20:4 (n-6) and eicosapentaenoic acid 20:5 (n-3), precursors for eicosanoic acid and the relative levels of these two fatty acids have profound effect on the formation of very active substances metabolically [24]. Polyunsaturated fatty acids (PUFA n-6) which dominate structural lipids and, like monoenes, are highly affected by factors such as lipid contents, growth levels and body weight. Particularly as precursor fatty acids to eicosanoids and arachidonic acid, PUFA (n- 6) are metabolized from adipose tissues [24].

CONCLUSION

The nutritional value of fish and fish oil has attracted a lot of public attention. It is believed to have many protective effects to various chronic diseases such as arteriosclerosis, coronary heart diseases (CHD) and many other diseases.From the present study clearly shows that all the selected Indian fishes for this study has favorable amount of polyunsaturated fatty acids which are very much known to prevent all sorts of diseases. This signifies that the Indian fishes has a good oil value and is suitable for applications in pharmaceutical and food industries and further the Indian fishes have the potential to be commercialized.

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