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Fatty Acids Composition and Heavy Metals in Marine Fish Samples from the South-Eastern Part of Bangladesh

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Abstract: This study investigated the fatty acid compositions, moisture content, ash content, and heavy metals in ten marine fish samples namely, *Lates calcarifer*, *Tenualosa toli*, *Pampus chinensis*, *Lapturacanthus savala*, *Harpodon nehereus*, *Johnius angentatus*, *Awaous guamensis*, *Setipinna phasa*, *Sillogenopsis panigus*, *Sardinella longiceps* collected from Kuakata sea beach, Bangladesh (south-eastern part). Fatty acid compositions were determined by saponification and esterification followed by identification and quantitation by the gas chromatograph equipped with a flame ionization detector (GC-FID). Ash, fat and moisture contents of the fish samples were in the range of 3.25-6.27%, 0.89-6.08% and 71.47-90.16%, respectively. The amount of saturated fatty acids (SFA) varied in the range of 55.53-89.04% whereas unsaturated fatty acids (USFA) were of 16.45-44.48%. Lauric (3.93-15.19%), palmitic (40.27-67.34%) and stearic acids (5.18-22.88%) were predominant among the individual SFA compositions, whereas oleic (0.91-34.26%), linoleic (0.69-9.95%) and palmitoleic acids (7.66-19.05%) were predominant among USFA in these species. Samples were freeze-dried, digested and finally analyzed by atomic absorption spectroscopy (AAS) for the presence of Lead (Pb), Copper (Cu), Zinc (Zn), Manganese (Mn) and the range of these metals were 0.5-1.9, 2.4-30.4, 3.2-10.5, 0.3-8.5 mg/kg, respectively.
***Keywords*:** benefits of fishes, marine fish, fatty acid, lipid content, bioaccumulation, bio-magnification, toxicity

1. Introduction

Fish oils are considered as one of the important materials for determining nutrition value of food. It provides both beneficial effects in reducing coronary heart diseases and substance necessary for human body [1]. Omega-3 fatty acids are found in fish especially oily fish which helps lowering the blood pressure, lowering heart rate and improving other cardiovascular risk factors [2]. Being an excellent source of Omega-3 fatty acid [3, 4], fish consumption reduces the risk of death from heart disease which is the leading cause of death in both men and women [2]. Fish intake has also been linked to a lower risk of stroke, depression and mental health with age. For pregnant women, fish intake is important because it supplies Docosahexaenoic acid (DHA), a specific omega-3 fatty acid that is beneficial for the brain development of infants [5]. The numerous health benefit provided by fish consumption may be compromised by the presence of toxic metals such as lead, cadmium, manganese, zinc, copper, calcium, magnesium, arsenic and mercury, which can have harmful effects on the human body [6]. Fish tissues that accumulate heavy metals may catalyse reactions and generate reactive oxygen species (ROS) leading to environmental oxidative stress [7, 8]. Heavy metal pollution occurs as a result of excessive industrialization, unplanned urbanization, use of excess fertilizers and pesticides (insecticides, herbicides and fungicides) in the field of agriculture, discharge of effluents from textile, leather, chemical and pharmaceutical wastes in the rivers [9]. As fish is one of the bio-indicator to assess the toxicity in water bodies, the bioaccumulation and biomagnification of these toxic compounds depend on the percentage of fat content in the fish. This study was designed to investigate the fatty acids composition and heavy metals in 10 marine water fish samples *i.e.*, Koral (*Lates calcarifer*), Chandana ilish (*Tenualosa toil*), Rupchanda (*Pampus chinensis*), Churi (*Lapturacanthus savala*), Loitta (*Harpodon nehereus*), Poa (*Johnius angentatus*), Baila (*Awaous guamensis*), Phasa (*Setipinna phasa*), Tular dandi (*Sillogenopsis panigus*), Chotpoti (*Sardinella longiceps*) collected from Kuakata fish markets and fishermen close to the beach [10].

2. Materials and methods

2.1 Sample preparation

Ten different species of marine fish samples (n = 10) were purchased from Kuakata fish markets and fishermen close

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to the beach. All fish samples were wrapped and stored in zipper seal bag in a freezer at a temperature below -20°C until analysis.

2.2 Extraction of fat

Fish samples (10.0 g quantitatively) were blended and homogenized, muscle tissue was taken into a pre-cleaned and dried mortar with 10.0 g silica sand and 30.0 g anhydrous sodium sulphate (Na_2SO_4). The mixture was grinded and more anhydrous sodium sulphate was added to make the sample float freely. This powder was then taken in a 250 mL ground joint conical flask. Then 60 mL ethyl acetate was added and it was extracted by shaking for 3 minutes. This extraction process was repeated twice by using 20 mL ethyl acetate per time. The extract was evaporated to dryness and the weight of the fat was recorded on the basis of the fresh weight (Table 1). The solvent was exchanged from ethyl acetate to n-hexane and dried up to a constant weight [11].

2.3 Saponification of fats and conversion into methyl esters

Approximately 50-100 mg of fish oil extracted from the fish sample was taken in a pear-shaped flask and 5.0 mL of 0.5 M methanolic-NaOH solution was added to it. The mixture was ultrasonicated for 1 minute and then refluxed on boiling water at about 96°C for 30 minutes. The mixture was evaporated with a rotavapor to dryness and 2.0 mL water was added to it. The mixture was shaken vigorously and then extracted with n-hexane. The organic layer was collected. The hexane part was made free from water by adding anhydrous sodium sulphate (Na_2SO_4). The solution was filtered, evaporated to dryness and 2 mL of borontrifluoride-methanol (BF_3-MeOH) complex was added. The mixture was refluxed on a boiling water bath for 20 minutes. The mixture was then evaporated to nearly dryness and finally 2.0 mL of n-hexane was added and filtered through pasture pipette containing cotton filter and anhydrous sodium sulphate on the cotton filter. The filtrate was concentrated to 1.0 mL and was analysed by GC-FID to find out the fatty acids composition of fish oil [12].

2.4 GC-FID analysis of methyl ester of fatty acids

Gas chromatograph with FID detector (Shimadzu GC-2025) was used for identification and quantification of fatty acids. Separations were performed on WCOT quartz capillary (DB-5) column (30 m in length and 0.25 mm in diameter). The temperature program in the oven was as followed: 120°C for 1 min (hold) then increased by 7°C/min to 280°C and again hold for 6 min. N_2 was used as carrier gas with a column flow rate of 2 mL/min. Total run time was 28.0 minutes. Injection volume was 1.0 µL with splitless/split mode and split ratio was 1:80. Injector and detector temperatures were 275°C and 285°C, respectively. Air and hydrogen gases were used as fuel for FID.

2.5 Identification and quantification

A mixture of methyl esters of twelve fatty acids standard was used as the reference. The identification of fatty acids was done by comparing retention times of the samples with that of the corresponding fatty acid standards in the chromatograms. Quantification was carried out by accounting the areas of individual fatty acids and the results were expressed in terms of the relative percentages. The amount of individual fatty acids present in the fish extracts were calculated by using the following formula [12]:

$$\text{Amount of individual fatty acids (\%)} = \frac{\text{Peak area of each fatty acids}}{\text{Total peak area}} \times 100$$

2.6 Heavy metals analysis

Fish samples were grinded homogenously by blender and dried completely by freeze drier. Dried samples (3 g) were placed in the muffle furnace consecutively at 700°C for 4 hours. After removing the samples from furnace, they were cooled at room temperature and conc. HNO_3 and deionised water were added to the sample to dissolve it. The digested solution after filtration was analysed by atomic absorption spectrophotometer (AAS) [3].

3. Results

Ten marine fish samples (n = 10) were collected from Kuakata sea beach and the flesh were extracted by established method [11]. After extraction, the extract was analysed for the composition of fatty acid in the fish oil and total fat content was determined by the solid phase dispersion method. The range of fat content in 10 marine fish species was 0.89-6.08%. Chandana ilish was found to have the highest fat content (6.08%) and Tular dandi had the least amount of fat (0.89%). Lipid content in the fish sample varied with the moisture content (Table 1).

Table 1. Percentage of lipid, moisture and ash content in marine fish samples

Sample Name		Lipid content (%)	Moisture content (%)	Ash content (%)
Local names	Commercial names			
Baila	Pacific river goby (*Awaous guamensis*)	0.93	75.90	5.59
Churi	Small-head ribbonfish (*Lepturacanthus savala*)	1.40	79.42	6.17
Loitta	Bombay duck fish (*Harpodon nehereus*)	1.31	78.21	3.62
Poa	Silver croaker (*Johnius argentatus*)	1.00	72.89	4.06
Rupchanda	Chinese pomfret (*Pampus chinensis*)	1.22	71.47	3.54
Koral	Asian sea bass (*Lates calcarifer*)	1.27	90.16	4.08
Phasa	Gangetic hairfin anchovy (*Setipinna phasa*)	1.02	80.89	4.76
Tular dandi	Flathead sillago (*Silloginopsis panijus*)	0.89	80.52	6.27
Chandana ilish	Chinese herring (*Tenualosa toli*)	6.08	76.95	5.32
Chotpoti	Indian oil Sardine (*Sardinella longiceps*)	1.85	75.63	3.25

Note: Parentheses indicates scientific name

Again, the other two parameters *i.e.* moisture content at 105°C and ash content at 700°C were also studied in collected 10 marine fish samples according to standard AOAC (1990) method (Table 1). It was found that the moisture content was more than 70% of the total body weight and ranged from 71.47-90.16%. The ash content was below 6.27% and ranged from 3.25-6.27%. Ash content in the fish samples varied depending on the moisture content.

Table 2. Relative percentage of fatty acids composition (%) in marine fish samples

Sample name (Local Name)	Fatty acids composition (%) in marine fish samples							
	Saturated fatty acids					Unsaturated fatty acids		
	Lauric ($C_{11}H_{23}COOH$)	Palmitic ($C_{15}H_{31}COOH$)	Stearic ($C_{17}H_{35}COOH$)	Arachidic ($C_{19}H_{39}COOH$)	Behenic ($C_{21}H_{43}COOH$)	Palmitoleic ($C_{15}H_{29}COOH$)	Oleic ($C_{17}H_{33}COOH$)	Linoleic ($C_{17}H_{31}COOH$)
Koral	3.93	49.75	22.88	6.99	-	6.57	9.88	-
Phasa	-	46.09	21.90	-	-	-	-	-
Tular dandi	8.01	44.45	14.41	8.01	-	16.91	1.39	7.01
Chandana ilish	15.19	40.27	9.94	5.82	-	19.05	1.42	8.30
Chotpoti	11.50	44.64	10.86	5.63	-	16.51	0.91	9.95
Baila	4.63	43.29	5.18	2.43	-	10.22	34.26	-
Churi	5.45	48.43	16.35	1.24	-	-	20.88	-
Loitta	8.09	63.10	12.40	2.10	2.46	7.66	3.77	8.02
Poa	6.73	42.40	9.78	2.73	-	12.55	13.02	0.69
Rupchanda	-	67.34	21.70	-	-	-	-	-

Note: "-" indicates below detection limit

Fatty acid composition was analysed with twelve different standards by GC-FID. Eight different fatty acids were found among the selected fish samples. Among saturated fatty acids (SFA), lauric acid (3.93-15.19%), palmitic acid (40.27-67.34%), stearic acid (5.18-22.88%) and arachidic acid (1.24-8.01%) were found in significant amount in the fish samples. Palmitic acid was found to be predominant among all of the fish species ranged within 40.27-67.34%. Unsaturated fatty acids *i.e.*, palmitoleic acid, oleic acid and linoleic acid were found in the range of 6.57-19.05%, 0.91-34.26% and 0.69-9.95%, respectively.

In this study, concentration of lead (Pb), copper (Cu), zinc (Zn), and manganese (Mn) were determined in 10 species of fish samples using atomic absorption spectrophotometry (AAS). Concentration of Pb, Cu, Zn and Mn in these fish samples were 0.5-1.9, 2.4-30.4, 3.2-10.5 and 0.3-8.5 mg/kg, respectively (Table 3).

Table 3. Heavy metals (mg/kg) in marine fish samples

Sample Name	Pb	Cu	Zn	Mn
Koral	0.5	5.1	3.6	0.3
Phasa	1.9	30.4	8.4	0.9
Chandana ilish	0.6	2.7	7.5	0.7
Tular dandi	0.6	2.8	3.2	0.4
Chotpoti	0.7	4.7	7.5	1.3
Baila	1.4	7.2	8.5	1.5
Churi	0.5	4.5	9.9	0.8
Loitta	0.6	2.4	6.8	8.5
Poa	0.9	7.2	10.5	0.4
Rupchanda	1.3	16.2	8.0	2.3

4. Discussion

The moisture content in body muscles of selected fishes ranges from 75% to 90%. Among them moisture content of Koral was observed to be highest (90%). Moisture content is related to keeping quality and storage stability of food. Microbial activity depends on moisture content of fish. Koral has high moisture content so as expected it is easily susceptible to microbial growth such as algae which is the leading cause of coral bleaching [13]. Ash content of the selected fishes ranges from 3.25-6.27%. Ash content was the highest in Tular dandi and lowest observed in Chotpoti. Higher ash content indicates the fish containing more inorganic substances [14]. A variation of moisture and ash content occur depending on the morphological and structural differences of tissues as well as age, size, collection site or seasonality [15]. Among the ten selected fishes, Chandana fish contain most lipid content 6.08%. Similar range of fat content was also observed in previous studies in fish samples collected from other parts of Bangladesh. It was observed that other nine fish samples contain lipid content lower than 2%. So they can be classified as lean fish [16].

Marine fish is identified as an important source of a large variety of fatty acids [17]. Saturated fatty acid was higher in all selected fish samples. Palmitic acid and stearic acid, two saturated acids were found to be present in all the samples. The highest percentage of palmitic acid was found to be 67.34% in Rupchanda and the lowest 40.27% in Chandana ilish. Behenic acid was only found in Loitta 2.46% (Table 2).

Three unsaturated fatty acids were traced in picked fish specimens. Among the specimens, five of them comprised all unsaturated fatty acids. But Rupchanda and Phasa didn't contain any of these. Palmitoleic acid and oleic acid were found in higher amount among unsaturated fat. The highest amount of palmitoleic acid was in Chandana ilish (19.05%) and oleic acid was in Baila (34.26%). The increased level of unsaturated fatty acid is beneficial for human consumption. The consumption of MUFAs is involved with lowering low-density lipoprotein (LDL) and increasing of high-density lipoprotein (HDP) cholesterol [12].

Table 4. Maximum and standard level of heavy metals (mg/kg) in fish species

Organization/Country	Pb	Cu	Zn	Mn	Reference
FAO/WHO limits	0.5	30	40	-	[18]
EU limits	0.1	10	-	-	[19]
FAO (1983)	0.5	30	30	-	[20]
European Community	0.2	-	-	-	[21]
Turkish guidelines	1	20	50	20	[22]
Kuakata	0.5-1.9	2.0-30.0	3.0-10.5	0.3-8.5	Present study

Note: "-" indicates data were not available in publications or variable was not studied

The present investigation detected considerable level of Pb and Cu in the muscle tissue of Kuakata fish samples. High concentration of this metals sometimes exceeded the permissible level, though Zn and Mn concentration was relatively low in collected fish specimens and below permissible level in all of the fishes (Table 4). Highest concentration of Zn and Mn was determined in Poa (10.50 mg/kg) and Loitta (8.5 mg/kg), respectively which are far less than permissible limit. Cu,

Zn and Mn are essential enzymatic co-factor of our body [23] but presence in excess amount above MRL value may cause health risks. Pb has no essential function in body. It can only cause severe illness [24]. Pb concentration was the highest in Phasa fish (1.9 mg/kg) which slightly exceeded all of the given standard MRL value set by different organizations [25]. More surprisingly, Cu content was also highest (30.0 mg/kg) in the same Phasa. Therefore, it could strongly suggest that Phasa sample was highly contaminated with Pb and Cu and pose risk for human uptake. The Pb content was high in almost all of the fishes even the lowest concentration was found 0.48 mg/kg found in Churi. The variation of metal contents may occur due to the differences in metabolism and feeding patterns of fish species [26]. The overall analysis was carried out according to AOAC (1990) method with certain modifications [3, 27]. However, the values obtained for metal analysis were below the permitted level set by FAO/WHO 2004 [27, 28].

5. Conclusion

The present study revealed that marine fish available in Kuakata sea beach is an efficient and balanced source of different types of fatty acids. In case of heavy metals, the content of Pb was above the permissible level in most of the fish samples. More study are required to monitor heavy metal content in Kuakata to protect biodiversity of marine aqua system and to ensure safe food for human consumption.

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