Fatty Acid Composition of Hilsa (*Tenualosa ilisha*) Fish Muscle from Different Locations in Bangladesh

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ABSTRACT

Hilsa *(Tenualosa ilisha)* is a highly preferred food fish in Bangladesh and the best source of n-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoie acid (EPA) and docosahexaenoic acid (DHA). The present study was to investigate the variations of fatty acid composition of hilsa collected from Barisal, Patuakhali, Bhola, Cox's Bazar, Chandpur and Shariatpur fish markets in Bangladesh. Hilsa fish oils were obtained from fish muscle using the wet rendering method, followed by fatty acid composition by gas chromatography. Further, the acid value, iodine value, peroxide value and free fatty acid value were also investigated and the values found to be 4.04–6.45 mg KOH/g, 5.02–6.01 meq O_2/kg, 54.90–72.06 g I_2/100g and 2.03–2.72% respectively. In general, fatty acid compositions ranged from 44.61% to 47.94% saturated (SFA), 32.76% to 36.74% monounsaturated (MUFAs) and 16.34% to 22.04% polyunsaturated acids (PUFAs) in extracted oil of hilsa fish. Among them, the fatty acids myristic acid (C14:0, 7.34–9.56%), palmitic acid (C16:0, 28.93–31.86%), palmitoleic acid (C16:1, 10.15–14.10%), stearic acid (C18:0, 5.96–7.48%), oleic acid (C18:1n-9 cis, 14.36–21.87%), vaccenic acid (C18:1, 3.44–4.03%), linoleic acid (C18:2n-6, 1.17–1.90%), octadecatetraenoic acid (C18:4n-3, 0.92–1.35%), cis-5, 8, 11, 14, 17-eicosapentaenoic acid (EPA, C20: 5n-3, 4.76–8.61%) and cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA, C22: 6n-3, 1.45–2.64%) were maximum in extent. The extent of PUFAs n-3 (ranging from 12.04 to 17.68) were higher than PUFAs n-6 (ranging from 2.45 to 3.20). The results revealed that PUFAs n-3 was highly dominant in Barisal, Bhola and Cox's Bazar hilsa compared to Patuakhali, Chandpur and Shariatpur hilsa.

Keywords: Hilsa (*Tenualosa ilisha*), fatty acid composition, gas chromatography (GC), n-6/n-3 and EPA/DHA

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INTRODUCTION

The hilsa (*Tenualosa ilisha)* belongs to the family Clupeidae and has wide range of distribution including marine, estuarine and riverine environments in Bangladesh (Hossain *et al.*, 2014). Aquatic animal oils are good sources of essential fatty acids that are not synthesized in the human body (Pal *et al.*, 2011). Fatty acids in fish oil have a very distinctive character compared to fatty acids from other sources (Pal *et al.*, 2011; Hossain *et al.*, 2014; Rodrigues *et al.*, 2017). At the same time, hilsa are highly

nutritious and its oils contains the long chain of n-3 polyunsaturated fatty acids (PUFAs) which have a number of health benefits (Kinsella, 1988; Sidhu, 2003; Steffens, 2006; Fawole *et al.*, 2007; Pal *et al.*, 2011). However, different fishes of the same species show a big variation in fatty acid compositions. Based on the factors such as salinity, temperature, season, age, size, species habitat, life stage, and the type and abundance of food, especially whether a species is herbivorous, carnivorous or omnivorous (Gruger, 1967; Ackman, 1989; Pal *et al.*, 2011; Rodrigues *et al.*, 2017). Different studies show that freshwater fish carries lower proportions of n-3 PUFAs than marine fish (Rahman *et al.*, 1995). Consumption of these PUFAs seems to be significant in human nutrition, health and disease prevention. Especially, consumption of fatty acids acquired from marine fish oil like eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) is connected to the growth of brain, nervous tissue and visual function in the infants. Besides, they have the ability to reduce levels of cholesterol, very low-density lipoproteins (VLDL), triacylglycerol's, and low-density lipoproteins (LDL) in blood (Mahmud *et al.*, 2004; Kolanowski, 2005; Mnari *et al.*, 2007). Moreover, these fatty acids are found to be effective in decreasing the risk of diabetes, hypertension, coronary heart diseases, cardiac arrhythmias, stroke, rheumatoid arthritis, depression, cancer and photoreception system (Mahmud *et al.*, 2004). In spite of its wide-ranging significance in human nutrition, fatty acid composition of hilsa in different fish markets of Bangladesh have not been investigated. Therefore, the objective of this study was to investigate the quantity of fatty acids in hilsa fish, collected from some selected fish markets in Bangladesh, in terms of saturated fatty acids (SFA), unsaturated fatty acids (UFA) and PUFAs n-3 and n-6 fatty acids, in order to make nutritional database for health concerned consumers in Bangladesh as well as for the world population.

MATERIALS AND METHODS

Samples for Laboratory Analysis

Hilsa fishes were bought from six selected fish markets, namely Barisal, Bhola, Chandpur, Cox's Bazar, Shariatpur and Patuakhali. On arrival at the laboratory all fishes were 3 to 4 days post-captured and preserved in ice. Average weight of the fishes was 500–800 g. As a minimum, twenty specimens from each market was cleaned, filleted, minced and stored separately at 20°C prior to extraction. Fish oils were extracted by wet rendering methods (AOAC, 2000) and standard IUPAC method (IUPAC, 1979) was used for the measurement of acid value (AV), peroxide value (PV), iodine value (IV) and free fatty acid (FFA) measurement.

Preparation of Fatty Acid Aethyl Ester (FAME)

About 50 mg of the fat extracts was mixed with 2 ml of solvent (methanol/hexane, 4:1) and 200 µl of acetyl chloride was steadily added. The aliquot was heated at 100°C in a heating box for one hour. Following this, 5 ml of 6% K_2CO_3 and 2 ml of hexane were gradually added. After that, the solution was centrifuged at 3000 rpm for 15 min. Finally, the hexane layer was collected and dried with Na_2SO_4.

Fatty Acid Analysis

Fatty acid composition was determined by preparing methyl esters and analysing them by gas chromatography (AOCS, 1992). The gas chromatography (Model 14B SHIMADZU, Japan) with flame Ionization detector (GC-FID) was loaded with software class GC-10 (Version-2.0). The GC was prepared with flame ionization detector (FID) and capillary column, with dimension 15 m length and 0.25 mm ID. The functional condition was automated at oven temperature 150°C (hold time 5 min), 8°C /min-190°C (hold time 0 min), 2°C/min-200°C (hold time 10 min), injection port temperature 250°C and detector temperature 250°C. Fatty acid peaks were identified from standard fatty acid mixtures. Nitrogen was used as carrier gas, flow rate 20 ml/min and aliquots of 1 µl FAME (formed by esterification of fish oil samples) was injected and the peaks of fatty acids were documented for their particular holding time and areas by the data processor unit of GC.

Data Analyses

Experiments were performed at least three times, independently (n = 3). The data were presented as mean ± SD. Statistical analyses were conducted using XL-stat version 16 for the DMRT to understand the differences between the variables.

RESULTS AND DISCUSSION

Acid Value, Peroxide Value, Iodine Value and Free Fatty Acid of Hilsa Fish

Table 1 shows the acid value (AV), peroxide value (PV), iodine value (IV) and percentage of free fatty acid (FFA) content of hilsa fish oil. In fact, these parameters provide the quality index of the fish oil. Acid value is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat. Along with the specification of oils, acid value (AV) is a common parameter and it indicates the quantity of the free fatty acids (FFA) exist in the oil. Increase in acid value is generally associated with the lipase activity originating from microorganism or biological tissue (Boran *et al.*, 2006). The suitable limit for AV was reported to be 7–8 mg KOH/g (Bimbo and Crowther, 1991). In this study, AV of hilsa fish oil in all selected regions were within the acceptable ranged and significantly difference ($P < 0.05$). Peroxide value (PV) of the hilsa fish oil displayed substantial difference ($P < 0.05$) among the selected regions. PV is the milligram equivalents of peroxide oxygen combined in a kilogram of oil and PV of oil is used as a measurement of rancidity which occurs by autoxidation. The lowest PV (5.02 meq O_2/kg) is associated with fishes collected from Barisal region and highest PV (6.01 meq O_2/kg) was associated with the Chandpur region fish. Young (1986) found the PV of fish oil was between 3 to 20 meq O_2/kg. PV findings of hilsa fish oil specimens from the selected regions did not surpass the later range. To be adequate for human consumption the maximum limit of PV of fish oil is 8 meq O_2/kg (Boran *et al.*, 2006). Iodine value gives an estimate of the degree of unsaturation and so of the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. The IV of hilsa fish lipid displayed the highest for Shariatpur region and the lowest for Bhola region which was ranged between 54.90 and 72.06 g I_2/100 g. Endo *et al.* (2005) reported that a wide range of iodine value (IV) between 55 and 188 g I_2/100 g was represented by fish. Iodine value of the waste oil specimens displayed lesser quantity than fish flesh oil such as sardine (156.2 g I_2/100 g) and tuna (162.0 g I_2/100g) flesh (Endo *et al.*, 2005). To check the oil quality free fatty acid (FFA) value is one of the most significant indicators (Khoddami *et al.*, 2012). The lower FFA content exhibited higher quality and lower further to be 7% (Bimbo, 1998). The oil specimens from hilsa fish was lower than this limit in all selected regions. Substantial difference ($P < 0.05$) was found in contrast among selected regions of hilsa fish oil. Hilsa fish with lower FFA content can be least associated with bacterial and enzyme activity from microorganisms or biological tissues (Ashie *et al.*, 1996). Different regions, sex, capture season and type of specimens can be the core reason of difference of AV, PV, IV and FFA of hilsa fish in all selected locations.

Table 1 Chemical characteristics of hilsa fish lipid

Parameters	Barisal	Patuakhali	Bhola	Cox's Bazar	Chandpur	Shariatpur
Acid value (mg KOH/g)	4.04 ± 1.04^{d}	4.14 ± 0.67^{d}	4.08 ± 1.21^{d}	4.37 ± 0.85^{c}	6.45 ± 1.91^{a}	5.41 ± 0.67^{b}
Peroxide value (meq O_2/kg)	5.02 ± 2.11^{e}	5.24 ± 0.98^{d}	5.15 ± 1.65^{d}	5.32 ± 0.79^{c}	6.01 ± 3.29^{a}	5.51 ± 1.53^{b}
Iodine value (g I_2/100g)	62.59 ± 6.65^{d}	69.71 ± 9.06^{b}	54.90 ± 1.59^{f}	56.39 ± 3.98^{e}	68.97 ± 14.91^{c}	72.06 ± 3.43^{a}
Free fatty acid (% palmitic acid)	2.03 ± 0.74^{e}	2.09 ± 0.17^{d}	2.05 ± 0.22^{d}	2.18 ± 0.93^{c}	2.54 ± 0.38^{b}	2.72 ± 0.09^{a}

Note: Values are presented as mean ± SD; values of a same row that do not share a same superscript are significantly different ($P < 0.05$)

Fatty Acid Profile of Hilsa Fish

The fatty acid (FA) composition of the oil extracted from hilsa fish is shown in Table 2. The hilsa fish oil specimens were generally consisted of myristic acid (C14 : 0), palmitic acid (C16 : 0), palmitoleic acid (C16 : 1), stearic acid (C18 : 0), oleic acid (C18 : 1), eicosapentaenoic acid (C20 : 5n-3) and docosahexaenoic acid (C22 : 6n-3). Among the saturated fatty acids (SFAs), Palmitic acid was the highest with the range of 28.93% (Coxs Bazar region) to 31.86% (Shariatpur region) followed by stearic acid ranged between 5.96% (Bhola region) and 7.48% (Patuakhali region) of the total FA. Among the fish specimens the most abundant monounsaturated fatty acids (MUFAs) were oleic acid with the range of 14.36% (Bhola region) to 21.87% (Patuakhali region). Highest quantity of PUFAs was for EPA made up of 4.76% (Patuakhali region) to 8.61% (Shariatpur region) of total FA followed by eicosapentaenoic acid (C20 : 5n-3), 44.61% (Barisal region) to 47.94% (Chandpur region), 32.76% (Bhola region) to 36.74% (Patuakhali region) and 16.34% (Patuakhali region) to 22.04% (Barisal region) were he SFAs, MUFAs and PUFAs content range respectively. The present study coincided with Zuraini *et al.* (2006) in the percentage of SFAs except the percentage of PUFAs and MUFAs. Study on fatty acid profile of some fish species by Zuraini *et al.* (2006) showed higher content of SFAs (45.57%), followed by PUFAs (32.54%) and MUFAs (15.02%). Following this, some studies recommended that about 25% oleic acid in diet provide an effective means of influencing the outcome of breast cancer. In this study, the range of oleic acid found to be between14.36 and 21.87% in all the hilsa fish samples. Thus, the consumption of hilsa fish would be beneficial to the females as it can help in reducing the risk of breast cancer through supplementation of oleic acid. Along with that, a good number of n-3 and n-6 PUFAs was also present in hilsa fish oil and that would be excellent food item for health concerned consumers owing to its high PUFA content.

Table 2 Fatty acid profile of hilsa fish in selected regions

Fatty acid (%)	Barisal	Patuakhali	Bhola	Cox's Bazar	Chandpur	Shariatpur
C14:0	8.22 ± 3.34^{d}	7.34 ± 1.67^{f}	9.14 ± 1.41^{b}	8.67 ± 0.97^{c}	9.56 ± 2.01^{a}	7.89 ± 2.17^{e}
C15:0	0.42 ± 0.13^{b}	0.39 ± 0.09^{d}	0.30 ± 0.10^{f}	0.41 ± 0.12^{c}	0.59 ± 0.05^{a}	0.35 ± 0.13^{e}
C16:0	29.48 ± 2.89^{e}	31.45 ± 5.66^{b}	29.84 ± 3.89^{d}	28.93 ± 1.77^{f}	31.15 ± 7.22^{c}	31.86 ± 4.83^{a}
C18:0	6.32 ± 1.04^{d}	7.48 ± 2.08^{a}	5.96 ± 1.02^{f}	6.26 ± 0.77^{e}	6.64 ± 0.71^{b}	6.57 ± 2.17^{c}
C20:0	0.17 ± 0.07^{c}	0.27 ± 0.11^{b}	ND*	0.50 ± 0.13^{a}	ND*	ND*
∑SFA	44.61	46.93	45.24	44.77	47.94	46.67
C16:1	12.37 ± 1.47^{d}	10.15 ± 1.97^{f}	14.10 ± 1.92^{a}	13.34 ± 0.89^{b}	12.59 ± 3.68^{c}	11.46 ± 1.63^{e}
C18:1 n-9 cis	3.52 ± 0.44^{d}	3.44 ± 0.71^{f}	4.03 ± 0.17^{a}	3.47 ± 0.07^{e}	3.57 ± 0.03^{c}	3.71 ± 0.01^{b}
C18:1	16.55 ± 4.81^{d}	21.87 ± 3.43^{a}	14.36 ± 1.33^{f}	15.62 ± 5.71^{e}	16.94 ± 0.93^{c}	18.81 ± 3.19^{b}
C20:1	0.90 ± 0.23^{c}	1.28 ± 0.11^{a}	0.27 ± 0.09^{f}	0.85 ± 0.13d	0.82 ± 0.22^{e}	1.13 ± 0.03^{b}
∑MUFA	33.34	36.74	32.76	33.28	33.92	35.11
C16:2	1.46 ± 0.47^{b}	1.26 ± 0.19^{f}	1.88 ± 0.25^{a}	1.30 ± 0.42^{d}	1.43 ± 0.33^{c}	1.27 ± 0.54^{e}
C16:3 n-3	2.11 ± 0.21^{c}	2.14 ± 0.95^{b}	2.46 ± 0.43^{a}	1.80 ± 0.29^{e}	1.94 ± 0.17^{d}	1.47 ± 0.34^{f}
C18:2 n-6	1.42 ± 0.78^{c}	1.17 ± 0.49^{e}	1.38 ± 0.24^{d}	1.38 ± 0.22^{d}	1.90 ± 0.28^{a}	1.47 ± 0.47^{b}
C18:3 n-3	0.89 ± 0.37^{a}	0.60 ± 0.15^{f}	0.78 ± 0.14^{c}	0.73 ± 0.07^{d}	0.88 ± 0.39^{b}	0.65 ± 0.12^{e}

Table 2 Continue

Fatty acid (%)	Barisal	Patuakhali	Bhola	Cox's Bazar	Chandpur	Shariatpur
C18:4 n-3	1.35 ± 0.34[a]	0.92 ± 0.67[f]	1.32 ± 0.41[b]	1.02 ± 0.25[d]	1.07 ± 0.07[c]	0.94 ± 0.17[e]
C20:2 n-6	0.70 ± 0.17[c]	0.96 ± 0.06[a]	0.14 ± 0.04[e]	0.56 ± 0.09[d]	ND*	0.79 ± 0.21[b]
C20:3 n-3	1.01 ± 0.14[d]	0.93 ± 0.19[f]	0.96 ± 0.25[e]	1.51 ± 0.21[b]	1.10 ± 0.03[c]	1.59 ± 0.21[a]
C20:4 n-6	1.08 ± 0.21[b]	0.91 ± 0.22[e]	0.93 ± 0.16[c]	1.17 ± 0.18[a]	0.81 ± 0.04[f]	0.92 ± 0.34[d]
C20:5 n-3	7.83 ± 2.14[c]	4.76 ± 1.38[f]	8.15 ± 2.72[b]	8.61 ± 2.50[a]	6.81 ± 0.94[d]	6.16 ± 1.37[e]
C22:5 n-3	1.55 ± 0.23[a]	1.24 ± 0.43[e]	1.46 ± 0.43[b]	1.31 ± 0.21[d]	0.72 ± 0.12[f]	1.41 ± 0.43[c]
C22:6 n-3	2.64 ± 1.04[a]	1.45 ± 0.21[e]	2.55 ± 0.37[c]	2.55 ± 1.07[c]	1.50 ± 0.33[d]	1.59 ± 0.09[b]
∑PUFA	22.04	16.34	22.01	21.94	18.16	18.26
PUFA/SFA	0.49	0.35	0.49	0.49	0.39	0.39
∑n-6	3.20	3.04	2.45	3.11	2.71	3.18
∑n-3	17.38	12.04	17.68	17.53	14.02	13.81
n-6/n-3	0.19	0.25	0.14	0.18	0.19	0.23
DHA/EPA	0.34	0.30	0.31	0.30	0.22	0.26

Note: ND = Not Detected

Values are presented as mean ± SD; values of a same row that do not share a same superscript are significantly different ($P < 0.05$)

High content of palmitic acid, oleic acid and DHA were found in the fatty acid of hilsa fish. These results coincided with the study of fatty fish by Garcia-Arias *et al.* (2003) and some lean fish obtained by Osman *et al.* (2001). The seasonal changes and environmental effect on tropical fish species, and along with the changes in post-spawning period might be the explanations of the characteristic difference of the saturated, monounsaturated and polyunsaturated fatty acid contents of hilsa fish lipids in different regions (Colin *et al.*, 1993; Gamez-Meza *et al.*, 1999). For comparing relative nutritional value of fish lipidn-6/n-3 ratio is a decent index (Piggott and Tucker, 1990; Jamilah *et al.*, 2008). The n-6/n-3 ratio of 0.24 to 0.66 for some selected marine fish was pointed out by Osman *et al.* (2001). In human body optimal balance for this ratio is 1 : 1 (Simopoulos, 1989), while n-6/n-3 ratio of not more than 5.0 in total human diet is recommended by World Health Organisation (WHO) (Vujkovic *et al.*, 1999). Several studies showed that with the reduction of the intake of seafood or fish lipid rich in EPA and DHA, risks of heart attack and many common disorders can expressively increase. An ideal ratio of n-6/n-3 of 4.0 at maximum is recommended by UK Department of Health (HMSO, 1994), which is greater than the maximum value is harmful to health and may promote cardiovascular diseases (Moreira *et al.*, 2001). Ratio of n-6/n-3 in this study for hilsa fish lipid samples ranged between 0.14 (Bhola region) and 0.25 (Patuakhali region) (Table 2). These findings are in covenant with those found from fatty fish (Jamilah *et al.*, 2008). Improvement of the nutritional value and protection against diseases could be done through addition of n-3 PUFA (Moreira *et al.*, 2001). Higher value of PUFA/SFA ratio recommended is 0.45 (HMSO, 1994) than those obtained from hilsa fish studied. Highest value (0.49) was obtained in Barisal, Bhola and Cox's Bazar regions and the lowest PUFA/SFA ratio (0.35) was obtained from Patuakhali region hilsa fish. Eicosapentaenoic acid (EPA, C20 : 5n-3) and docosahexaenoic acid (DHA, C22 : 6n-3) are identified as major polyunsaturated fatty acids. In the human diet EPA is the most

essential fatty acid of the n-3 series because it is the precursor to the 3-series eicosanoids (Chen *et al.*, 1995). It was reported that DHA and EPA were interchangeable by retrogradation and DHA to be decreased the concentration of low concentration lipoprotein cholesterol in plasma (Childs *et al.*, 1990). Additionally, DHA is an important structural component of gray matter of the brain, the retina of the eye, and specific cell membranes and is found in high levels in the testes and sperm. However, the habitat, natural diet, size, age, reproductive status of fish, environmental conditions, and particularly water temperature, influence lipid content and fatty acid composition of fish muscle to a certain extent which was the crucial source of differences in fatty acids of hilsa fish (Alasalvar and Konno, 1999; Pal *et al.*, 2011; Hossain *et al.*, 2014; Łuczyńska *et al.*, 2014). Following this, the unique taste of hilsa might be attributed to the environment where it lives or to the feed it takes.

CONCLUSION

The study demonstrates the nutritional profile in terms of fatty acid composition of hilsa fish in six selected regions of Bangladesh and proved that fatty acids especially EPA and DHA were present in adequate amount in hilsa fish. Moreover, the acid value, peroxide value, iodine value and percentage of FFA value suggest that the hilsa fish oil might be suitable for edible purpose. Although, hilsa fish oil are rich in n-3 PUFAs in Barisal, Bhola and Cox's Bazar region than Patuakhali, Chandpur and Shariatpur region, it can be concluded that all the hilsa fish samples might be considered good diet for fish consumers due to the presence of appreciable amount of nutritional components.

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