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Lipid of Hilsa Fish (*Hilsa ilisa*)

SAGARMAY SAHA, ISHANI BANDYOPADHYAYA and JYOTIRMOY DUTTA*

Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta-700 009

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The major fatty acids of the flesh-lipid of hilsa fish (*Hilsa ilisa*) are 16 : 0 (32.0±5.0%), 18 : 0 (8.2±0.5%), 16 : 1 n7 (17.7±1.0%), 18 : 1 n9 (29.6±1.9%), 20 : 5 n3 (4.9±0.5%), 20 : 4 n6 (1.7±0.3%) and 22 : 6 n3 (1.5±0.6%). In this respect this lipid resembles the dietary fat of the Greenland Eskimos. In fish at minimum egg formation, the flesh contains 15.0±0.7% lipid, composed of neutral (88.4±1.7%), phospho-(5.1±1 6%) and glyco-(6.5±1.2%) lipids. Lipid contents and lipid-class compositions of the flesh-lipid of hilsa fish at different degrees of egg maturation apparently indicate that the energy required for upstream migration by this fish, for spawning, is supplied by the oxidation of flesh-lipid without any preference for a particular lipid class.

HILSA fish (*Hilsa ilisa*; English : Hilsa shad), a member of the clupeidae family, inhabits coastal, esturian and river waters. This fish is available in the entire coastal region of India extended upto Parsian gulf in the west and Vietnam in the east. Like salmon, hilsa goes up rivers to breed and is harvested in these rivers[1]. Hilsa is a very popular food fish in the eastern part of India, Pakistan, Bangladesh and Burma[2].

It is now well-understood that the polyunsaturated fatty acids, particularly those belonging to the n3 family, when ingested through diet, exert a benefical effect against heart diseases[3,4]. As these fatty acids are known to be more abundant in the lipids of marine animals than in the terrestrial ones, a special interest has been aroused to understand fatty acid compositions of different food fishes belonging to the different parts of the world.

The hilsa fish has not been properly studied from this standpoint. Pathak and Ojha[5] analysed the fatty acid composition of this fish-fat before the introduction of chromatographic methods in lipid analysis. This fish lipid requires reassessment through the modern techniques of lipid analysis. It has long been observed that the fat content of the flesh of female hilsa fish varies widely at different seasons causing sharp rise and fall in the quality of the taste and the market price of this fish. It is also a matter of experience that this depletion of flesh-lipid of this fish is related to the maturation of eggs.

In this communication we have presented the lipid-content and lipid-class compositions of the flesh of hilsa fish when the egg maturation is minimum. The fatty acid compositions of the total flesh-lipid have been reported and compared with that of a fresh water food fish rohu (*Labeo rohita*). We have also presented the total lipid content and lipid class composition of hilsa-flesh at different stages of egg-maturation in order to show the correlation between the two parameters.

Experimental

Fresh fish samples were collected from local market. The flesh was removed from the bones, scales, fins and head, chopped into small pieces, mixed throughly and stored under N_2 at $-20°$ for further analysis.

Lipid extraction : The lipid was extracted from a known quantity of flesh by chloroform-methanol mixtures according to the method of Bligh and Dyer[6]. The lipid was dried over anhydrous Na_2SO_4 in cold under N_2 and dissolved in chloroform to a known volume. The solvents from an aliquot of this solution was completely removed, the lipid content measured by direct weighing and expressed as percentage of total flesh.

Fractionation of lipid by silicic acid column chromatography : Fractionation into lipid subclasses was carried out in glass columns (40×2 cm) packed with silicic acid (100−200 mesh ; 15 g) according to the reported method[7]. The lipid (~150 mg) was separated into neutral, glyco and phospholipid fractions by stepwise elution using 10 column volumes (ca. 175 ml) of chloroform, 40 column volumes (ca. 700 ml) of acetone and 10 column volumes (ca. 175 ml) of methanol respectively. The content of each fraction was determined by direct weighing after removal of solvent as in the case of total lipids. The amount of phospholipid in the total lipid was also measured from the total phosphorous content[8].

Determination of fatty acid composition, glc analysis of fatty acid methyl esters (FAME) : FAMEs were prepared from lipids by methanolysis in presence of concentrated H_2SO_4[9]. These were purified by preparative tlc on silicic acid plates

using hexane-diethylether-acetic acid (80 : 20 : 1) as the developing solvent and analysed by glc on stainless steel columns (2 m × 3 mm) packed with 15% DEGS on chromosorb W(HP) (100 – 120 mesh) kept at 180°. The carrier gas (nitrogen) flow-rate was kept at 40 ml min^{-1}. A Hewlett Packard 5840A dual column, dual FID, computerised gas chromatograph was used. The injection ports and FIDs were kept at 200°. The peaks were identified by comparing the retention times with those of standard compounds, co-injecting authentic FAME mixtures with the sample and using the log-retention time vs carbon chainlength plots. Sometimes FAMEs were hydrogenated catalytically (PtO_2) and the reduced FAMEs were chromatographed to aid peak identification.

Results and Discussion

Flesh lipid content : Hilsa flesh contains 15.0± 0.7% (w/w) of fat when egg maturation is minimum (average of 6 measurements). This value is about 3 times that of pomphret (*Pompus argenteus*; 5.3±0.9) and Indian mackerel (*Rastrelliger kanagurta*; 5.6±1.1)—the two other popular food fish of marine origin. In fact hilsa can be placed in the category of high oil containing fish of the world. The major component of hilsa flesh lipid is neutral lipid (average of 6 measurements). This class comprises 88.4±1.7% (w/w) of the total lipid while phospholipid and glycolipid levels are 5.1±1.6% and 6.5±1.2% respectively. Such low levels of polar lipids and predominance of neutral lipids are also observed in the flesh of other fish like mullets[10], tuna[11] and menhaden[12]. These fish are also known to store appreciable amounts of lipids in their flesh.

The total flesh-lipid content and the percentages of each lipid class in hilsa fish at different degrees of egg maturation, are presented in Table 1. The increase in weight has been taken as the measure of egg maturation. The results clearly show that the lipid content in flesh drastically decreases with the maturation of eggs, but the class composition remains almost unaltered. These results imply that the kinetic energy spent in moving upstream by hilsa fish is obtained primarily from the oxidation of the stored lipid in the flesh and none of the lipid class is preferentially oxidised over the other in this process. This loss of fat in the flesh of hilsa fish with egg maturation results in gradual loss of the fine taste of the flesh.

TABLE 1—TOTAL LIPID CONTENT AND LIPID CLASS COMPOSITION OF FLESH-LIPIDS OF HILSA FISH AT DIFFERENT DEGREE OF EGG-MATURATION*

Amount of egg (g/kg flesh)	Total lipid (% flesh)	Class composition (% total lipid)		
		Neutral	Glyco-	Phospho-
0	15.9	88.4	6.5	5.1
0 – 5	15.5	88.0	6.8	5.2
5 – 50	5.1	87.6	6.8	5.6
50 – 100	1.6	91.4	4.4	4.2

*Average of 6 fish in each group.

Fatty acid composition of flesh lipid of hilsa fish is presented in Table 2 alongwith the fatty acid compositions of the flesh-lipid of a fresh water fish rohu (*Labeo rohita*) and the dietary fat of Greenland Eskimos[14] for comparison. The results show some distinct differences and similarities between these three lipids. The difference between the marine fish hilsa and sweet water fish rohu regarding the distribution of the total saturates and unsaturates is not very wide. Hilsa flesh lipid contains 40.9% of saturated and 59.1% unsaturated fatty acids, while for rohu these figures are 32.1 and 67.9% respectively. But regarding the percentages of total monoenoic and polyenoic fatty acids, the two fishes are widely different. Of the unsaturated fatty acids the major components are the monoenoics in hilsa and these account for 47.5% of the total lipid, twice of that in rohu (24.5%). The rohu lipid on the other hand is richer in polyenoic fatty acids. The total polyenoic percentage in rohu (43.4%) is four times that of hilsa (11.6%). Amongst the polyenoic fatty acids, a remarkable difference can be marked in the percentage of 20 : 4 n6 in the two

TABLE 2—FATTY ACID COMPOSITION (W/W%) OF FLESH-LIPIDS IN HILSA FISH, ROHU FISH AND THE DIETARY FAT OF GREENLAND ESKIMOS*

Fatty acid	Hilsa	Rohu[a]	Eskimo diet[b]
12 : 0	tr	1.0±0.2	4.8
16 : 0	32.0±5.0	21.8±3.1	13.6
16 : 1n7	17.7±1.0	8.8±0.8	9.8
16 : 2+17 : 1	—	—	0.4
18 : 0	8.2±0.5	9.3±0.5	4.0
18 : 1n9[c]	29.6±1.9	15.3±0.8	24.6
18 : 2n6	0.3±0.1	7.0±1.1	5.0
18 : 3n3	1.1±0.7	6.7±0.9	0.6
18 : 4n3	0.4±0.2	0.2±0.0	—
20 : 0	0.7±0.3	—	0.1
20 : 1	0.2±0.1	0.4±0.1	14.7
20 : 4n6	1.7±0.3	10.2±1.2	0.4
20 : 5n3	4.9±0.5	4.8±0.6	4.6
22 : 1	tr	tr	8.0
22 : 4n6	0.4±0.2	0.9±0.3	8.5
22 : 5n6	0.3±0.1	0.8±0.2	
22 : 5n3	1.0±0.3	1.8±0.7	
22 : 6n3	1.5±0.6	11.0±1.3	
Total saturated	40.9	32.1	22.5
Total unsaturated	59.1	67.9	76.5
Total monoenes	47.5	24.5	57.3
Total polyenes	11.6	43.4	19.2
Total n6	2.7	18.9	5.4
Total n3	8.9	24.5	13.7

*n = 6 (number of measurements).
[a]Rohu lipid was analysed alongwith hilsa. [b]Ref. 14.
[c]Short-hand designation of fatty acids : the number at the extreme left denotes the number of carbon atom, the number followed by the colon is the number of double bonds and the number after n denotes the position of the 1st double bond from the methyl end.
tr = trace (less than 0.1%).

fish, this percentage in hilsa (1.2±0.2%) is about one-tenth of that in rohu (10.2±1.2%). Another distinction is in proportions of 22 : 6 n3 acid, in hilsa this is 1.5±0 06% which is again one-tenth of that in rohu (11.0±1.3%). Regarding fatty acid composition the dietary fat of the Eskimos has a closer resemblance to the hilsa lipid than the rohu lipid, particularly in total polyenoic, total monoenoic and arachidonic acid contents. Like hilsa lipid this fat is also rich in monoenoic (57.3%) and poor in polyenoic (19.2%) fatty acids and has a low arachidonic acid content (0.4%). The percentages of total n6 acids (5.4%) and total n3 acids (13.7%) in this fat are closer to those in hilsa lipid (2.7 and 8.9% respectively) than those in rohu lipid (18.9 and 24.5% respectively). This fat on the other hand resembles rohu lipid regarding the percentage of total saturates and total unsaturates. Like rohu lipid this fat is low in the total saturates (22.5%) and high in unsaturates (76.5%).

It is now well-established[18] that the incidence of heart attack is very low amongst Greenland Eskimos. This phenomenon has been attributed largely to the nature and distribution of the fatty acids, particularly those belonging to the n6 and n3 families, in their dietary fat[14].

In the light of the above findings and resemblance between hilsa fish lipid and the dietary fat of the Eskimos, regarding fatty acid compositions, it will be of interest to study the nutritive as well as the therapeutic values of hilsa flesh lipid against heart diseases.

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