

LIPID CONTENTS OF MARINE FISH: CARCHARHINUS MELANOPTERUS (BLACK SHARK) AND LUTJANUS JOHNNII (HIRA)

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ABSTRACT

Total lipid composition of liver oil of two marine fish *Carcharhinus melanopterus* (Black Shark) and *Lutjanus Johnnie* (Hire) was determined. The fatty acid contents of total liver lipids were identified through gas chromatography-mass spectrometry. It was found that fatty acid methyl esters mainly consist of myristic, pentadecylic, palmitic, stearic acids as saturated, and palmitoleic, oleic, elaidic eicosapentaenoic, Docosahexaenoic, and clupanodonic acids as unsaturated components. Appreciable quantities of polyunsaturated fatty acids (PUFA) and ω -3 fatty acids were detected.

Introduction

Fish are of major importance as human food commodity. Epidemiological, clinical and biochemical studies performed during the last two decades have suggested that consumption of fish and other seafood is beneficial in reducing the risk of coronary disease [Dyer berg, 1986; Glom set, 1985; Harold and Kinchella, 1986]. This beneficial effect is attributed to the lipid in marine animals which is rich in polyunsaturated fatty acids (PUFA) of ω -3 class particularly eicosapentaenoic acid (EPA) [Chanmugam *et al.*, 1986]. The fish oils are not only good hypoglycemic agent [Olivieri *et al.*, 1988] but also have their risk reducing role in blood clotting, renal damage [Ito *et al.*, 1988] and rheumatoid arthritis [Gonnerman *et al.*, 1988]. Protective effect of fish oil against cancer has recently been established [Hunter, 1987].

Pakistan, like other developing countries, has observed a rising trend in heart disease owing to various corollaries of modernization. Besides coronary ailments, renal problems and cancer incidences are also stepping upward significantly. There is dire need to explore natural resources to fight against these diseases. Seafood from our own coast specially fish and fish oil offers a luring promise to remedy the situation though studies pertaining to chemical evaluation will be necessary.

Present study deals with the fatty acids and lipid characterization of liver oils from two marine fish abundantly available the year around.

Materials and Methods

Sample collection

Marine fish *Carcharhinus melanopterus* (Black Reef Shark, locally called as Mangra) and *Lutjanus johnii* (John's Snapper, locally known as Hira) were purchased fresh direct from fishing boats at fish harbor, Karachi and stored at -20°C until used for assay. Fish were dissected and liver was collected, soaked on a filter paper to remove moisture and weighed.

Extraction of lipids

The liver of both fish species was homogenized separately with Janke and Kunkel IKA Wert Ultra-turrax Type TP 18/10 (Germany) homogenizer and extracted with CHCl₃ MeOH (2:1, v/v) [Folch et al, 1957]. The homogenized tissues were shaken vigorously with CHCl₃: MeOH (2:1, v/v) for three times, and the combined extract so obtained was fractionated and washed with distilled water so as to remove the impurities. The solvent layer was separated and evaporated under vacuum, which afforded the oily component.

Qualitative determination

The oily component was chromatographed on silica gel G 60 (Merck, 230-400 mesh) with petroleum ether-diethyl ether-acetic acid (80:20:1; 85:15:1, v/v) [Khan *et al.*, 1970] along with corn oil as an authentic standard, detected with iodine vapors or Rhodamin 6G (under uv). It showed that both types of fish consist of steryl esters, triacylglycerol, sterols, diacylglycerol, monoacylglycerol and phospholipids. The different constituents of lipid classes were scrapped from TLC plate and etherified.

Etherification

The total lipids of the two marine fish oils (25 mg) as well as the lipid classes so separated through TLC were esterified with methanolic sulphuric acid (85:15, v/v). The reaction mixture in vials was heated at 80°C for 2 hours in an oven, it was then cooled, diluted with water, extracted with diethyl ether and analyzed by gas liquid chromatography.

Gas liquid chromatography

The methylated esters of fatty acids were analyzed first by gas liquid chromatography (TLC) on a Shimadzu GC-14A with C-R6A Chromatopac integrator using 2.1 m x 3 mm (I'd.) glass column packed with GP 10% SP 2330 on

100/120 Chromosorb (R) WAW (Suppelco, USA). Isothermal temperature at 190°C was used, the injection port temperature and detector temperature were 200° and 220°C respectively, and nitrogen and hydrogen flow rates were 30 ml /min for each of the two gases.

Gas chromatography-mass spectrometry

The GC-mass spectrometry of the methylated fatty acids was performed on Hitachi M-80B GC-MS using 115 m x 0.2 mm (i.d.) column packed with OV-101. The column temperature was programmed between 200-250°C with rate of increase of 5°C per minute, injector and interface temperatures were 280°C and 250°C respectively, whereas ionization voltage was 70 eV and total emission current 100 μ A.

Results and Discussion

The liver oil of two marine fish *Carcharhinus melanopterus* and *Lutjanus john* has been analyzed in terms of lipid composition. Total lipid contents of both fish have been shown in Table 1. Fatty acid composition of total lipids and individual lipid classes of *Carcharhinus melanopterus* and *Lutjanus* is presented in Table 2 and 3 respectively. Results from mass spectrometric fragmentation pattern are summarized in Table 4.

Table 1
Total lipids in the liver of marine fish

Name of specie	Wet weight of the tissue (g)	Weight of the lipid extracted (g)	Percentage wet tissue
<i>Carcharhinus melanopterus</i>	79	3654	4625
<i>Lutjanus johnii</i>	43	350	8.14

The lipid content of fish is highly variable. Environmental factors such as diet, season and temperature besides biological differences such as age, sex and size are known to affect fish lipid content and composition and to account for differences between and within species [Stansby, 1976]. The lipid content of *Carcharhinus melanopterus* (nick named Mangra) is 46.25 g/100 g liver tissue (wet weight) which is five times higher than that of *Lutjanus johnii* (nick named [lira]), 8.14 g/100 g. There are reasons to explain this large difference. Fish species that do not migrate over wide areas do not need to store fat as an energy source and have relatively low fat content [Stansby, 1976]. The site of fat storage in the body is different; in some fish, fat is stored in the liver; in others, it is concentrated

immediately under the skin. However, in case of *Lutjanus johnii* both these reasons can not be accommodated before further investigations are carried out.

Table 2

Fatty acid composition of total lipid and individual lipid classes* in the liver oil of *Carcharhinus melanopterus* (Black Shark)

Fatty acids	RRT	Weight %						
		TL	TG	DG	MG	FFA	SE	PL
Saturated								
C10:0	-	-	3.06	-	-	-	0.63	-
C12:0	0.31	0.23	0.51	-	-	8.68	2.48	0.42
C14:0	0.48	4.15	4.72	5.13	7.96	7.82	1.74	4.59
C15:0	0.61	1.04	1.54	1.64	1.85	1.96	1.10	1.09
C16:0	0.78	22.31	26.48	39.46	53.41	43.94	18.72	38.19
C18:0	1.30	7.97	8.99	12.71	14.72	19.02	9.51	16.52
Unsaturated								
Unknown	0.52	1.10	2.09	-	-	-	1.19	-
C16:1	0.86	7.47	3.55	5.26	-	3.47	-	-
C16:1 (ISO)	0.91	4.09	5.83	0.90	5.85	3.80	7.98	4.84
C16:2	1.11	2.53	2.27	-	-	-	38.46	-
C18:1	1.49	17.42	17.78	10.47	10.65	11.31	15.82	12.75
C18:1 (ISO) 1.65	3.82	1.12	0.49	-	-	-	0.44	-
C18:2	1.83	0.90	0.68	0.28	-	-	0.84	0.92
C18:3	2.21	2.00	0.40	0.39	5.56	-	0.53	0.55
C20:1	2.52	2.21	1.23	1.32	-	-	1.00	1.05
C20:2	-	-	1.46	-	-	-	-	-
C20:5 (ω -3)	5.04	3.90	3.11	-	-	-	-	0.79
C22:1	3.93	2.94	1.72	-	-	-	-	1.60
C22:4	6.82	1.58	0.62	-	-	-	-	11.82
C22:5 (ω -3)	7.46	3.95	1.32	-	-	-	-	-
C22:5 (ISO) (ω -3)	8.70	1.47	3.23	-	-	-	-	-
C22:6 (ω -3)	9.50	8.73	9.75	-	-	-	-	4.43
Unknown	10.81	-	-	20.49	-	-	-	-

* Abbreviations used are: TL, total lipids; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FFA, free fatty acids; SE, steryl esters; PL, phospholipids; RRT, relative retention time.

Saturated fatty acids in both fish are represented by a set of five fatty acids, C12:0, C14:0, C15:0, C16:0, C18:0, though in Mangra, a sixth type is also present which is C10:0. The first three saturated fatty acids in order of abundance in Mangra are C16:0 [62.16% of saturated fatty acid (SFA) 22.31% Total fatty acids (TFA)], C18:0 (22.22% SFA/7.97% TFA) and C14:0 (11.56% SFA/4.15% TFA). The order of saturated fatty acid abundance in Hira is C16:0 (63.60% SFA/25.27% TFA), C18:0 (28% SFA/ 9.8% TFA) and C14:0 (5.57% SFA/1.93% TFA).

In Mangra, C_{18:0}, C_{16:0} and C_{14:0} are fairly evenly distributed in all lipid classes with the exception of steryl esters in which they are expressed feebly. The major fatty acid of all lipid classes in liver oil of this species is C_{16:0}. Hira is also no exception; C_{16:0} and C_{18:0} predominate in all the lipid types with C_{16:0} the highest and both these fatty acids claim above 90% fatty acid compositions. Lauric acid C_{12:0} appears as TG, DG and SE constituent in Hira but in Mangra it restricts its representation to TG and SE only.

Table 3
Fatty acid composition of total lipid and individual lipid classes* in the liver oil of *Lutjanus johnii* (Hira)

Fatty acids	RRT	Weight %					
		TL	TG	DG	MG	SE	PL
Saturated							
C12:0	0.31	-	0.20	1.23	-	1.60	-
C14:0	0.48	2.21	3.17	2.41	2.81	2.19	2.01
C15:0	0.61	0.88	0.91	0.60	1.10	-	0.81
C16:0	0.78	25.27	29.30	25.61	26.45	20.06	33.52
C18:0	1.30	11.25	10.75	9.85	12.34	5.61	19.27
Unsaturated							
C16:1	0.86	6.41	6.73	-	2.43	3.61	3.26
C16:2	1.11	0.81	-	-	-	-	0.80
C18:1	1.49	14.29	24.20	7.16	7.58	13.73	12.37
C18:1 (iso) 0.29	-	-	0.58	-	-	-	-
C18:2	1.83	2.12	2.61	0.59	0.55	2.04	1.23
C18:3	2.21	1.28	0.71	-	0.52	-	0.83
C20:1	2.52	2.03	3.41	1.40	1.19	-	1.29
C20:2	-	-	-	-	0.50	-	-
C20:4	-	-	-	-	1.88	2.94	2.05
C20:5 (ω-3)	5.04	3.94	3.19	1.55	1.59	5.01	3.12
C22:1	3.93	3.81	3.80	0.40	1.89	8.52	4.23
C22:3	-	4.54	0.59	-	-	-	-
C22:4	6.82	0.84	1.34	-	-	-	0.24
C22:5 (ω-3)	7.46	1.67	2.60	-	10.58	-	2.76
C22:5 (iso) (ω-3)	8.70	3.19	-	-	3.15	-	0.46
C22:6 (ω-3)	9.50	15.77	6.10	-	19.82	2.40	10.82
Unknown	10.80	13.94	0.98	-	4.11	32.29	0.48
Unknown	11.73	-	-	44.66	0.34	-	0.45

* Abbreviations used are: TL, total lipids; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FFA, free fatty acids; SE, steryl esters; PL, phospholipids; RRT, relative retention time.

Table 4
Mass and fragmentation pattern of fatty acids from fish liver oil

Systemic name	Common name	Mol. formula	Mol. wt.	Mass and fragmentation pattern
Saturated fatty acids				
Methyl tetra-decanoate	Methyl myristate	C ₁₅ H ₃₀ O ₂	242	GC-MS, m/z 242 (M ⁺ , C ₁₅ H ₃₀ O ₂ , 13%), 211 (M ⁺ -31, 14%), 199 (M ⁺ -43, 11%), 185 (2%), 157 (2%), 143 (M ⁺ -99) (12%), 129 (5%), 111 (2%), 101 (7%), 87 (65%), 74 (100%), 55 (22%).
Methyl n-penta-decanoate	Methyl pentadecylate	C ₁₆ H ₃₂ O ₂	256	GC-MS, m/z 256 (M ⁺ , C ₁₆ H ₃₂ O ₂ , 12%), 225 (M ⁺ -31, 7%), 213 (M ⁺ -43, 10%), 185 (2%), 171 (1%), 157 (5%), 143 (14%), 129 (4%), 115 (2%), 101 (5%), 87 (77%), 74 (100%).
Methyl n-hexa-decanoate	Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	GC-MS, m/z 270 (M ⁺ , C ₁₇ H ₃₄ O ₂ , 56%), 239 (M ⁺ -31, 29%), 227 (M ⁺ -43, 39%), 199 (11%), 185 (17%), 171 (14%), 157 (7%), 143 (C ₁₇ H ₃₄ O ₂ , 62%), 129 (26%), 101 (20%), 87 (100%), 74 (C ₁₇ H ₃₄ O ₂ , 92%), 55 (73%).
Methyl n-hepta-decanoate	Methyl margarate	C ₁₈ H ₃₆ O ₂	284	GC-MS, m/z 284 (M ⁺ , C ₁₈ H ₃₆ O ₂ , 32%), 253 (M ⁺ -31, 10%), 241 (M ⁺ -43, 17%), 227 (2%), 213 (1%), 199 (7%), 185 (7%), 171 (1%), 157 (1.5%), 143 (24%), 129 (10%), 115 (2.5%), 101 (9%), 87 (100%), 74 (99%), 55 (42%).
Methyl n-octa-decanoate	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	GC-MS, m/z 298 (M ⁺ , C ₁₉ H ₃₈ O ₂ , 27%), 267 (M ⁺ -31, 5%), 255 (M ⁺ -43, 9%), 241 (1.5%), 227 (0.2%), 213 (2%), 199 (6%), 185 (2%), 171 (0.5%), 57 (1.5%), 143 (17%), 129 (7%), 115 (2%), 101 (7.5%), 87 (82%), 74 (100%).
Unsaturated fatty acids				
Methyl hexa-decenoate	Methyl palmitoleate	C ₁₇ H ₃₂ O ₂	268	GC-M, m/z 268 (M ⁺ , C ₁₇ H ₃₂ O ₂ , 7%), 236 (M ⁺ -32, 25%), 194 (M ⁺ -74, 17%), 180 (2.5%), 166 (4%), 152 (17%), 138 (10%), 124 (13%), 110 (22%), 96 (33%), 82 (47%), 74 (64%), 55 (100%).

(Table 4 Continued)

Systemic name	Common name	Mol. formula	Mol. wt.	Mass and fragmentation pattern
Methyl n-octa-decenoate	Methyl oleate	C ₁₉ H ₃₆ O ₂	296	GC-MS, m/z 296 (M ⁺ , C ₁₉ H ₃₆ O ₂ , 6%), 264 (M ⁺ -32, 25%), 222 (M ⁺ -74, 12.5%), 180 (10%), 166 (7%), 152 (3.5%), 138 (*%), 124 (8.2%), 110 (17.5%), 97 (40%), 83 (42%), 69 (76%), 55 (100%).
Methyl octa-decenoate	Methyl elaidate (methyl oleate isomer)	C ₁₉ H ₃₆ O ₂	296	GC-MS, m/z 296 (M ⁺ , C ₁₉ H ₃₆ O ₂ , 7.5%), 265 (M ⁺ -31, 44%), 222 (M ⁺ -74, 15%), 180 (12.5%), 167 (4%), 153 (8%), 139 (9%), 123 (16%), 111 (19%), 97 (42%), 83 (49%), 69 (67%), 55 (100%).
Methyl (all-cis)-eico-sapentaenoate $\Delta^{5,8,11,14,17}$	Methyl eicosa-pentaenoate	C ₂₁ H ₃₂ O ₂	316	GC-MS, m/z 316 (M ⁺ , C ₂₁ H ₃₂ O ₂ , 0.2%), 273 (M ⁺ -43, 0.3%), 242 (M ⁺ -74, 0.2%), 217 (5%), 203 (4.5%), 189 (0.2%), 175 (8%), 161 (7%), 147 (13.5%), 133 (25%), 199 (43%), 105 (38%), 91 (75%), 79 (100%), 67 (62%), 55 (33%).
Methyl (all-cis)-do-cosa-hexaenoate $\Delta^{4,7,10,13,16,19}$	Methyl docosa-hexaenoate	C ₂₃ H ₃₄ O ₂	342	GC-MS, m/z 342 (M ⁺ , C ₂₃ H ₃₄ O ₂), 311 (M ⁺ -31, 0.2%), 236 (M ⁺ -106, 0.5%), 222 (1%), 208 (5.2%), 194 (1%), 180 (2.5%), 175 (5%), 161 (12%), 147 (12%), 133 (22%), 119 (43%), 105 (43%), 91 (86%), 79 (100%), 67 (65%), 55 (33%).
Methyl docosapen-taenoate $\Delta^{4,6,12,15,19}$	Clupano-denoate	C ₂₃ H ₃₆ O ₂	344	GC-MS, m/z 344 (M ⁺ , C ₂₃ H ₃₆ O ₂ , 0.5%), 301 (M ⁺ -43, 0.5%), 233 (0.4%), 205 (0.2%), 191 (0.1%), 177 (8%), 163 (5%), 147 (12%), 133 (17%), 119 (42%), 93 -CH ₃ (67%), 79 (100%), 67 (78%), 55 (50%).

Total unsaturated fatty acids in the two fish types are comparable (In Mangra 69.66 g %; In Hira 6531g %). Not only it is the major fraction of fatty acids, it is also significant both in terms of multiplicity and medicinal value of PUFA. Oleic acid, C18:1, predominates]27.17% unsaturated fatty acids (UFA) /18.93% of total fatty acids (TEA)] followed by docosahexenoic acid (DHA), C22:6, (13.61% UFA/ 9.4% TFA) and palmitoleic acid, C16:1 (11.65% UFA/81.17% TFA) in Mangra, out of 15 identified unsaturated fatty acids. In case of Hira DHA, C_{22:6}, is highest in quantity (21.2% UFA/13.8% TFA) followed by oleic acid, C18:1 (19.14% UFA/1250% TFA) and palmitoleic acid, 016:1 (8.5% UFA/5.6% TFA). The rest of 12 unsaturated fatty acid types make 47.55% of total

unsaturated fatty acids (TUFA) in this species compared with 41.47% TUFA in Mangra.

The quantity of PUFA in the two types of fish are not significantly different (Table 5), however, there is considerable difference on the basis of TUFA, being higher in Hira. The EPA and DHA values in two species are considerably close and the same is true for total w-3 fatty acids despite the fact that Hira has low (1/6th) fat content compared with that of Mangra. It has been reported that EPA and DHA make up 30-50% of some marine fish cellular lipids and 10-30% of the oils produced from fish depot fats [Ackman, 1986]. Both DHA and EPA originate from C_{18:3} in marine and other invertebrates who feed on plants. DHA, C_{22:6}, is also directly taken as part of dietary lipid.

The present study can be considered as an attempt to evaluate local marine resources in terms of total lipids, lipid types, specially PUFA and w-3 fatty acids. Liver was selected for study due to its high lipid content. Compared with liver, the other edible parts of fish have very little lipid content. Only in Pacific *Herring* Atlantic *Mackerel*, Atlantic *Menhaden* or *Pollock* total lipid ranges between 10% to 20%; in other fish types it is < 10%, whereas our results show 46% lipid content of Mangra liver though *Lutjanus* has low percentage (8%). In both fish types PUFA ranges between 27% to 30% (Table 5) which is almost equal to $\pm 2\%$ of the reported values for cod liver, *Menhaden* and *Salmon*. Even *Herring* has lower value (16.1g%) whereas Max EPA has higher value (41.1g%) than that of the two fish studied [Hepburn *et al*, 1985; Buckner *et al*, 1984].

It is a curious fact that as few as ten or even eight major fatty acids fairly accurately describe the composition of most fish oils [Lambersten, 1978]. Among saturated fatty acids palmitic acid, C_{16:0}, as in most animals, is the principal fatty acid (1045% of the total) in fish. In these two fish species, this acid is $23.79 \pm 1.48\%$. The fact that stearic acid, C_{18:0}, content varies from fish to fish is also exhibited in these two species, being 7.97% of total in Mangra compared to 11.25% in Hira. Saturated fatty acids higher than Can were not found which is quite expected as these fatty acids can all be biosynthesized by the organism.

In liaison to other fish oils, odd chain fatty acid, C_{15:0} is present in both fish types, though in small amounts. The odd chain fatty acids in fish are either contributed to the lipids of aquatic food chain by bacteria endogenous to the intestine of all living organisms [Hamid *et al*, 1978] or these arise out of metabolic events known to follow the priming of propionate molecule instead of acetate molecule. The amino acids, leucine and isoleucine, may also suggestively contribute to odd chain fatty acids mostly C_{15:0} and C_{17:0} [Ackman, 1972].

Table 5
Comparison of *Carcharhinus melanopterus* (Black Shark; Mangra) with *Lutjanus johnii* (John's snapper; Hira) in Liver oil composition

Patty Acids	<i>Carcharhinus melanopterus</i> (Mange) (g%)	<i>Lutjanus johnii</i> (Hire) (g%)
Total saturated acids	3034	1167
Total unsaturated acids	69.66	6533
Mono unsaturated aids	41.23 TEA	23.22 TEA
Fatty Acids (MUSFA)	60.30 TUSA ^a	43.72 TUSA
Poly unsaturated	27.03 TFA	29.89 TEA
Fatty Acids (PUPA)	39.70 TUSA	5628 TUSA
Eicosapentaenoic acid (EP A)	13.7 TFA	17.2 TFA
and Docosahexaenoic acid (DHA)	19.7 TUSA	26.4 TUSA
Total ω-3 acids	19.6 TFA	205 TEA
	64.64 TUSA	30.6 TUSA

*TAF = Total fatty acids

**TUSA = Total unsaturated fatty acids.

The monounsaturated fatty acids are represented by C_{16:1}, C_{18:0}, C_{20:1} and C_{22:1} in both species. However, their concentration (Table 5) in Mangra (41.23% TFA) is almost double to that found in Him (23.22% TFA). C_{16:1} and Cum (Homeric forms inclusive) make 80-85% of total monounsaturated acids in both animals. These two acids are most represented in TG and PL fractions (Table 2 and 3), in fact C₁₈ is represented in almost all lipid classes abundantly. At the same time, C_{20:1}, of which both C_{16:1} and C_{18:1} act as precursor (C_{22:1} originates from fatty alcohols) is only 2% of the total, providing the clue that C and C_{18:1} play their role in these fish both as energy source and structural components.

General considerations

The present study opens various future avenues. Presence of considerably high proportion of PUFA and ω-3 fatty acids in local marine fish is strengthening the idea that marine fish of aquatorial waters are also abundant in these medicinally important fatty acids. These acids, when given in high doses (20-25 g/day) have been proved to reduce blood TG, platelet aggregation (clotting tendency) and blood pressure and thus effectively prevent cardiovascular diseases.

In our region seafood or fish do not make staple diet. In the majority of cases in urban population, dietary habits (plant derived foods) and mundane activities (less physical work) concerted with smoking and anxieties are the potential harbingers of hypercholesterolemia and hypertriglycerolemia. In fact a

pilot study of Karachi population [Ali, M. and Ali, S.S., unpublished data] has shown hypertriacylglycerolemia (> 350 mg/dl of plasma) in apparently normal persons.

In view of the large coastal area of Pakistan and abundant marine supplies; the two fish studied are available round the year in ample quantities and general population can be motivated for use of fish. Where fresh fish delivery is a problem, fish oil or fish liver oil has been recommended to be delivered in gelatin capsules [Hunter, 1987]. Besides local usage, such product has a great potential for export as well.

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