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A Study on the Fatty Acid Composition of Fish Liver Oil from Two Marine Fish, *Eusphyra blochii* and *Carcharhinus bleekeri*

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Two species of shark found in the coastal waters Karachi (Pakistan) were studied, *Eusphyra blochii* (winghead snark) and *Carcharhinus bleekeri* (sandbar shark) for their liver oil fatty acid composition. Since liver has high lipid content and traditionally the liver oil of these species been used to relieve muscular pain and arthritis in Pakistan this study was conducted. The isolation, identification and characterization of these fatty acids were carried out by gas liquid chromatography (GLC) and a combination of the thin layer chromatography (TLC)-GLC technique. A large variation was observed between winghead shark liver oil and sandbar shark liver oil. Twenty-five individual fatty acids from the oil of marine fish were analysed. Among those studied, palmitic acid was a major saturated fatty acid while stearic acid was the other major constituent. Unsaturated monoenoic fatty acids e.g. oleic and palmitoleic acids, were major constituents and traces of dienoic and trienoic fatty acids were also found. In addition, medicinally important polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acids, were also identified.

Key Words: Marine fish liver oil, *Eusphyra blochii*, *Carcharhinus bleekeri*, Fatty acid composition, TLC-GLC.

Introduction

Fish and other sea foods remain an important source of white meat for the human diet due to its beneficial effect in reducing coronary heart diseases. This effect is especially due to the fat in fish, which is probably responsible for converting fatty dienoic acid into tetraenoic, pentaenoic and hexaenoic acid[1−5].

Like other oils and fats, fish oils may be divided into their lipid fractions such as triacylglycerol, diacylglycerol, monoacylglycerol, phospholipids, sterylesters, sterols and free fatty acids. The quantity of total lipids may differ between various tissues and organs and also between different species. The type of fatty acids present as free acid or as neutral lipid differs to a great extent from species to species and water environments[6−8].

In a study of both freshwater and ocean fish oil preparations, it was observed that fish oil preparations from ocean fish contained 35 unsaturated fatty acids with 1-6 double bonds. In contrast, freshwater fish oil preparations contained 14 unsaturated fatty acids having 1-5 double bonds. Ocean fish oil preparations contained considerable amounts of unsaturated fatty acids of $\geq=$ 20 C, such as $C_{20:5}$ and $C_{22:6}$, whereas all freshwater fish fatty acids showed a chain length of $<$ 20 of carbon atoms[9]. Fatty acids except 20:1 and 22:1, which are of exogenous origin, are a basic composition fish oil from temperate and northern latitudes, with similar totals for saturated (14:0 and 16:0), monounsaturated (16:1 and 18:1) and polyunsaturated (primarily ω-3) fatty acids[10].

The fatty acid pattern of triacylglycerol and phospholipids of various fish oils were also assessed, showing that they contained highly unsaturated fatty acids. The polyunsaturated fatty acids of triacylglycerol and lecithin were preferentially located in the β-position[11]. Various scientists have studied the composition of fish liver oil and found that 91-99% of the fatty acids in the β-position are unsaturated and 36-86% of the fatty acids in the $\propto$-position are saturated[12].

Liver has high lipid content and traditionally the liver oil of the winghead and sandbar shark has been used to relieve muscular pain as well as arthritis in Pakistan. Various reports on the study and identification of fish liver oil having high pharmacological activity potential as a hypolipidemic agent[13−15], an antiarthritic agent[16−18] and preventing an agent renal damage[19−21] inspired us to undertake the present study. This investigation deals with the fatty acid and lipid characterization of fish liver oils from two local marine fish available in the coastal waters off Karachi, Pakistan.

Materials and Methods

Sample collection

Marine fish *Eusphyra blochii* (commonly, winghead shark; locally, Julia-Mangar) and *Carcharhinus bleekeri* (shark; locally, Kanatyan, Mangra) were purchased from a local supplier in Karachi and stored at -20 °C until used for assay. Fish were dissected after 10-15 days and the livers were collected and soaked on filter paper to remove moisture and weighed.

Extraction of lipids

A homogenizer, Janke and Kunkel IKA Wert Ultra Turax Type TP 18/10 (Germany), was used to homogenize the livers of both species of fish separately. The homogenized tissues were shaken vigorously with $CHCl_3$:MeOH (2:1, v/v) and the combined extract was fractionated and washed with distilled water to

remove the impurities. The solvent layer was evaporated in vacuo, which in turn became enriched with the oil components[22].

Qualitative determination

Chromatography of oil components on silica gel G-60, 230-400 (Merck, Germany) with petroleum ether:diethyl ether:acetic acid (80:20:1, 85:15:1, v/v) was performed[23], corn oil was used as reference standard and the chromatogram was developed with iodine vapours[24] or Rhodamin 6G (Basic Red 1) and detected under UV. It indicated that both types of fish contained sterylesters, triacylglycerol, sterols, diacylglycerol, monoacylglycerol and phospholipids. These different lipid constituents were separated by preparative thin layer chromatography (TLC) and esterified.

Esterification

A total lipids of both fish oils (25 mg) as well as the lipid class separated through preparative TLC were esterified with methanolic sulphuric acid (85:15, v/v)[25]. The reaction mixture in vials was heated at 80 °C for 2 h in an oven, cooled and then diluted with water, extracted with diethyl ether and analysed by gas liquid chromatography (GLC).

Gas-liquid chromatography

The methylated esters of fatty acids, were analysed by GLC on a Shimadzu GC-14A (Japan) with a CR-6A Chromatopac integrator using a 2.1 m × 3 mm (i.d.) glass column packed with GP 10% SP 2330 on a 100/120 Chromosorb (R) WAW (Suppelco, USA) 190 °C as isothermal temperature. The injection port temperature was 200 °C and detector temperature was 220 °C. Nitrogen and hydrogen flow rates were 30 ml/min for each gas.

Identification of lipid components

In general, the components were identified. The total fatty acid composition of fish oil been known for many years to be complex. Only after the development and widespread application of GLC by co-chromatography with reference standards was it possible to identify these components. The results are quoted as an average of three fish in all cases.

Results and Discussion

In the present study, two sharks, *Eusphyra blochii* and *Carcharhinus bleekeri* were selected for the analysis of total lipids from the liver. Various solvents have been used to extract lipids from fish tissues out of which a chloroform:methanol (2:1, v/v) solution system was found to be superior.

Table 1 provides data on the total lipid contents of the liver of *Eusphyra blochii* (Winghead shark) and *Carcharhinus bleekeri* (Sandbar). The lipid content in the liver of *Eusphyra blochii* was 66.19% and that of *Carcharhinus bleekeri* 39.94% [26].

Table 1. Lipid content in the liver of various fish.

Name of species	West weight of tissue	Weight of the lipid extracted	% of wet tissue
Eusphyra blochii (Winghead shark)	125 g	82.74 g	66.19%
Carcharhinus bleekeri (Sandbar shark)	39.57 g	15.81 g	39.94%

For the complete analysis of lipid classes derived from fish liver oil a combined TLC-GLC technique was adapted. Known amounts of total lipid were applied on silica gel G-60 thin layer plates in the form of a band and the lipid classes were separated after development with appropriate solvent systems. The materials from the bands representing triglycerides, diglycerides, monoglycerides, free fatty acids, steryl esters and phospholipids were scraped off quantitatively and placed in a culture tube for transmethylation.

In order to establish the identity of individual fatty acids present either in free or combined forms two methods were employed. In general, the components were identified by co-chromatography with reference standards. Where standards were not available, the identity of the peaks was determined on the basis of the relative retention time (RRT) plots against carbon number on a log scale shown in the Figure.

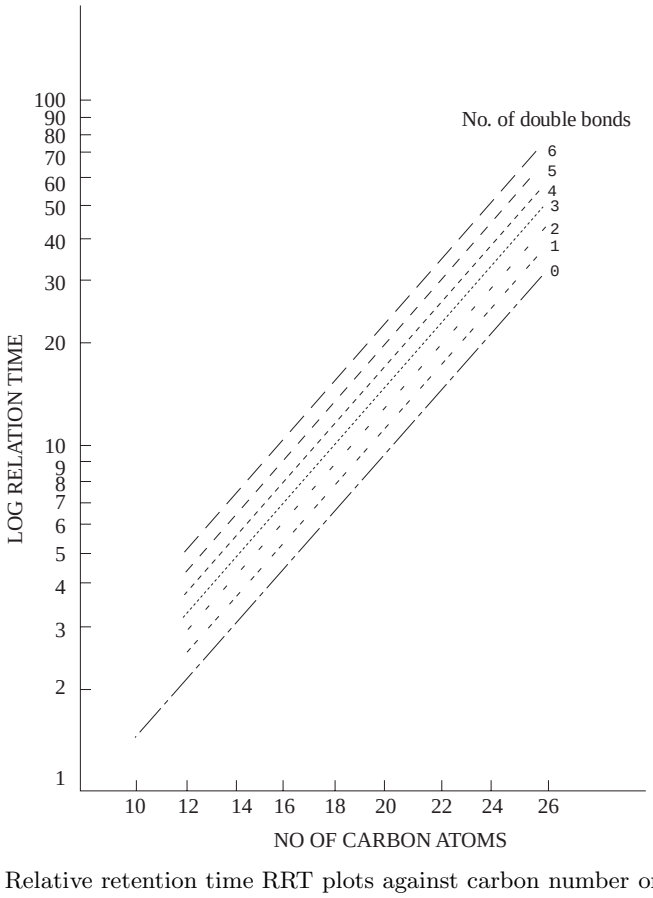

Figure 1. Relative retention time RRT plots against carbon number on log scale.

It was also possible to predict the carbon number and the number of double bonds unknown on the basis of such plots[27]. In order to assess the concentration of various fatty acids in various lipid classes,

a combination of TLC and GLC techniques was carried out. The fractions such as triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG), free fatty acids (FFA), phospholipids (PL) and sterylesterase (SE) were analysed separately.

Table 2 shows the percentage composition of fatty acids in the lipid classes of winghead shark's liver in which the saturated fatty acid ranges from 56% to 70.12%. Palmitic acid is predominant and its composition ranges from 36.63% to 46.97% while stearic acid ranges from 9.34% to 17.49%.

Among unsaturated fatty acids, monoenoic are the major fatty acids. Oleic acid ranges from 11.10% to 26.45% . The dienoic and trienoic are minor constituents. Polyunsaturated fatty acids (PUFA) range from 4.25% to 15.21% in which EPA is present from 0.41% to 1.65% and DHA ranges from 0.24% to 3.07%. The same components have been examined in silver carp and bighead carp at similar ratios[28]. However, monoacylglycerol, free fatty acids and sterylester fractions of lipid classes do not contain EPA.

Table 3 shows the fatty acid composition in the class lipid of liver the sandbar shark.

Table 2. Fatty acid composition of lipid class* in the liver of *Eusphyra blochii* (Winghead shark).

Fatty acid**	RRT	Weight (%)					
		PL	MG	DG	FFA	TG	SE
Saturated							
$C_{12:0}$	0.32	0.26	-	0.42	-	0.25	1.83
$C_{14:0}$	0.48	4.35	4.27	3.95	7.81	4.87	7.50
$C_{15:0}$	0.61	1.05	1.17	1.18	-	0.60	0.70
$C_{16:0}$	0.78	46.97	42.68	38.44	37.80	40.36	36.63
$C_{18:0}$	1.29	17.49	16.35	15.42	13.19	11.00	9.34
Unsaturated							
$C_{16:1}$	0.86	-	-	-	4.98	-	-
$C_{16:1}$(ISO)	0.91	5.54	-	4.84	-	8.15	-
$C_{16:2}$	1.14	0.72	0.75.	0.41	-	-	-
$C_{18:1}$	1.49	15.76	23.94	18.54	11.10	26.45	18.67
$C_{18:1}$(ISO)	1.63	-	-	-	1.93	-	-
$C_{18:2}$	1.80	-	0.74	0.60	-	0.76	0.36
$C_{18:3}$	2.18	0.59	-	0.33	-	-	0.60
$C_{18:4}$	2.64	0.42	0.91	1.48	-	0.51	0.69
$C_{20:2.}$	2.48	1.95	2.78	3.17	6.20	1.51	3.81
$C_{20:3}$	3.06	0.26	1.17	2.48	3.01	0.53	-
$C_{20:4}$	3.87	1.63	0.97	-	3.26	2.19	1.86
$C_{20:5}$	4.92	0.41	-	0.90	-	1.65	-
$C_{22:3}$	5.67	-	0.52	0.40	1.30	-	-
$C_{22:4}$	3.63	-	0.74	2.34	-	-	-
$C_{22:5}$	7.21	1.63	1.11	-	3.55	0.33	11.70
$C_{22:5}$ (ISO)	8.37	-	-	-	-	-	0.29
$C_{22:6}$	9.20	0.37	0.52	0.80	3.07	0.24	0.67
Unkown		0.60	1.38	4.30	2.80	0.60	5.35

*The short hand designation of class lipids are represented as: PL = Phospholipids, MG = Monoglycerides, DG = Diglycerides, FFA = Free fatty acids, TG = Triglycerides,

**The short hand designation[32]. RRT = Relative retention time.

Table 3. Fatty acid composition of lipid class* in the liver of *Carcharhinus bleekeri* (Sandbar shark).

Fatty acid**	RRT	Weight (%)					
		PL	MG	DG	FFA	TG	SE
Saturated							
$C_{12:0}$	0.32	-	0.35	0.14	0.02	0.30	3.78
$C_{14:0}$	0.48	2.31	2.22	1.45	0.07	1.93	8.08
$C_{15:0}$	0.61	-	0.74	0.40	1.18	0.53	1.20
$C_{16:0}$	0.78	56.46	49.01	48.95	33.50	45.47	38.88
$C_{18:0}$	1.29	9.47	8.60	9.08	-	7.99	11.55
Unsaturated							
$C_{16:1}$	0.86	3.20	-	-	-	-	-
$C_{16:1}$(ISO)	0.91	13.95	7.13	9.50	-	14.04	5.61
$C_{16:2}$	1.14	-	-	0.50	-	0.13	-
$C_{18:1}$	1.49	2.80	18.35	21.96	0.30	27.05	16.50
$C_{18:1}$(ISO)	1.63	0.35	-	-	4.05	-	-
$C_{18:2}$	1.80	-	0.63	-	53.08	-	0.79
$C_{18:3}$	2.18	-	0.20	0.32	-	0.27	-
$C_{18:4}$	2.64	1.54	1.30	0.83	0.06	0.57	1.62
$C_{20:2.}$	2.48	2.12	2.10	1.28	-	0.69	2.40
$C_{20:3}$	3.06	-	2.74	1.59	1.22	-	2.06
$C_{20:4}$	3.87	0.49	0.67	-	3.90	0.22	-
$C_{20:5}$	4.92	0.65	0.36	-	-	0.16	0.85
$C_{22:1}$	4.18	1.00	0.40	0.18	-	0.12	-
$C_{22:3}$	5.67	-	0.42	-	-	-	-
$C_{22:5}$	7.21	2.22	1.22	1.43	0.59	-	3.89
$C_{22:5}$ (ISO)	8.37	0.41	-	-	0.36	0.07	-
$C_{22:6}$	9.20	0.49	0.91	2.39	-	0.06	1.02
Unkown	10.04	2.54	2.65	-	1.67	0.40	1.77

*The short hand designation of class lipids are represented as: PL = Phospholipids, MG = Monoglycerides, DG = Diglycerides, FFA = Free fatty acids, TG = Triglycerides,

**The short hand designation.32 RRT = Relative retention time.

Among these lipid classes, the saturated fatty acid ranges from 34.77% to 68.24%. Palmitic and stearic acids are the major saturated fatty acids and they range from 33.50% to 56.46% and 7.99% to 11.55%, respectively.

Among unsaturated fatty acids monoenoic fatty acids range from 4.35% to 41.21%; oleic acid is predominant ranging from 0.30% to 27.05% . Dienoic and trienoic acid are the minor constituents. Polyunsaturated fatty acids range from 1.08% to 7.38%. The composition of EPA and DHA ranges from 0.16% to 0.85% and 0.06% to 2.39% respectively. Diacylglycerol did not contain EPA while free fatty acid contained neither EPA nor DHA.

Conclusion and Future Prospects

The lipid composition of fish liver oil was determined by means of gas chromatography. Detailed qualitative and quantitative analysis was performed on two species of fish.

The present study can be considered an attempt to evaluate local marine resources for total lipids and lipid types, especially PUFA and ω-3 fatty acids. Due to its high lipid content, the liver was selected for study.

The fish oil isolated from two different fish provided interesting data regarding the fatty acid composition of the total lipid classes. It is evident from the data that the fatty acid mixtures were of a complex nature compared to those of plant oil and mammalian fats. As many as 25 individual fatty acids may be present in the oil of most fish. The identified fatty acids may be divided into two main groups. The first group is comprised of saturated fatty acids starting from lauric ($C_{12:0}$), myristic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), palmitic ($C_{16:0}$) and stearic acid ($C_{18:0}$). The major fatty acid was palmitic acid in all the samples.

The second group was comprised of all the monoenoic acids having an even carbon chain i.e., $C_{16:1}\omega$-7 to $C_{22:1}\omega$-11, and oleic acid was the major oleic acid in most of the lipid samples. Among di-, tri- and polyenoic acid, linoleic ($C_{18:2}\omega$-6), and linolenic ($C_{18:3}\omega$-3), eicosatetraenoic ($C_{20:4}\omega$-6), eicosapentaenoic ($C_{20:5}\omega$-3) and docosahexaenoic ($C_{22:6}\omega$-3) were also identified in the fatty acid mixture.

Of pertinent interest was the discovery of eicosapentaenoic acid (EPA, $C_{20:5}\omega$-3) and docosahexaenoic acid (DHA, $C_{22:6}\omega$-3) in appreciable amounts in marine fish. Recently, these two acids have attracted the attention of several workers because of their medicinal application[29].

The present study is very valuable for dieticians. Medicinally important fatty acids like PUFA and ω-3 are abundant in the marine fish of equatorial waters. These acids when given in high doses (20-25 g/day) have been proven to reduce blood triglycerides, platelet aggregation and blood pressure and thus effectively prevent cardiovascular diseases[30]. Diets enriched with seafood or fish would be helpful in avoiding preventing heart problems.

The two fish studied are available round the year in abundance the coast of Pakistan. Fish liver oil can be recommended in the form of gelatin capsules[31] as a dietary supplement.

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