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LIPID COMPOSITION OF MARINE FISH: *PARASTOMATEUS NUGER* (KALA PAPLET) AND *TRICHIURUS LEPTURUS* (TALWAR)

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ABSTRACT

Total lipid composition of liver of two fish *Parastomateus nuger* (Kala Paplet) and *Trichiurus lepturus* (Talwar) were determined. The fatty acid components of total liver lipids were identified by Gas-Liquid-Chromatography (GLC) and a combination of TLC-GLC technique. It is found that fatty acid methyl esters mainly consist of muristic, palmitic, stearic acid as saturated and palmitoleic and oleic acid as major unsaturated fatty acid. In addition medicinally important poly unsaturated fatty acid, eicosapentaenoic and docosahexaenoic acids were also identified.

INTRODUCTION

In the past two decades views about dietary omega-3 (ω-3) fatty acid have moved from speculation about their functions to solid evidences that they are not only essential nutrient but also may favorable modulate many diseases. The discovery that fish oil may have unique benefits in the fight against coronary heart disease has stimulated a tremendous amount of research. Although the subject is complex the study of fish oil is very worthwhile because a totally new perspective on the relationship of diet to coronary heart disease. Reported research have shown that fish oil ω-3 fatty acid reduce the activity of monocyte and platelet cell production, narrowing of arteries and change of the

blood clot and ultimate heart attack. Extensive data indicates that fish oil reduce the risk of heart attack even in people who already have atherosclerosis.

There are more than 25 different types of fatty acids occurred in the oil of marine fish. In most of the fish saturated fatty acids have carbon length ranging from C_{12} (Lauric acid) to C_{24} (Lignoceric acid). The unsaturated fatty acids range generally from C_{14} (Tetradecaenoic) to C_{22} (docosahexaenoic) acid(Stansby & Stansby, 1967) Fatty acids except $C_{20:1}$ and $C_{22:1}$ are of exogenous origin are a basic composition for the fish oil for temperature and northern latitudes, with similar totals for saturated ($C_{14:0}$ and $C_{16:0}$), mono unsaturated ($C_{16:1}$ and $C_{18:1}$) and poly unsaturated (primarily- GƆ-3) fatty acids (Ackman, Ratnayake, & Olsson, 1988).

Omega-3 fatty acids are one of the two groups of fatty acids, the omega-3 and omega-6 which are vital to human life and they are called the essential fatty acids. These fats must be supplied by diet. People living in industrialized countries eat up to 30 times more omega-6 than omega-3 fatty acids, resulting in a relative deficiency of omega-3 fats omega-6 metabolic products (inflammatory prostaglandins, thromboxanes and leukotrienes) are formed in excessive amount causing allergic and inflammatory disorders and make the body more prone to heart attack and cancer (Simopoulos, 1999). Eating diet rich in omega-3 fatty acids or taking fish oil supplements can restore the balance between the two fatty acids and can possibly reverse these disease processes. Omega-3 fatty acids are found mostly in fish but are contained in other foods as well. Fish oil is the best food source of these fatty acids.

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 incidence are also stepping upward significantly. There is dire need to explore natural resources to fight against these diseases. Sea food from our own coast especially fish and fish oil offers a luring promise to remedy the situation through studies pertaining to chemical evaluation will be necessary.

Pakistan has a large coast and its marine sources are boundless. A study on oils from Pakistani fish may not be only anti- atherosclerotic dietary substituent but also give way to discover cheaper lubricating oils and food coloring and texturing additives.

Present work 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 *Parastomateus nuger* (Kala Paplet) and *Trichiurus lepturus* (Talwar) were purchased fresh direct from fishing boats at fish harbor, Karachi and stored at -20°C until used for assay. Fish was dissected and liver was collected, soaked on a filter paper to remove moisture and weighted.

Extraction of lipids

The liver of both fish species was homogenized separately with Janke and Kunkel IKL Wert Ultra-turrax Typ TP 18/10 (Germany) homogenizer. The homogenized tissues were shaken vigorously with $CHCl_3$: MeOH (2:1,v/v) (Folch, Lees, & Sloane-Stanley, 1957) 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 vaccum, 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, Khalid, & Ali, 1970) along with corn oil as an authentic standard, detected with iodine vapours or Rhodamin 6G (under uv). It showed that both types of fish consist of steryl esters, triacylglycerol, and phospholipids. The different constituents of lipid classes were scrapper from plate and esterified.

Esterification

The total lipids of the two marine fish oils (25mg) as well as 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 a 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 (GLC) on a Shimadzu GC-14A with C-R6A Chromatopacintegrator using 2.1m x 3mm (i.d) glass column packed with GP 10% SP 2330 on 100/120 Chromosorb ® WAW (Suppelo, USA). Isothermal temperature at 190°C was used, the injection port temperature and detector

temperature were 200°C and 220°C respectively, and nitrogen and hydrogen flow rates were 30ml/min for each of the two gases.

RESULTS AND DISCUSSION

In the present study two marine fish *Parastomateus nuger* and *Trichiurus lepturus* has been analysed interms of lipid composition. Total lipid contents of both the fish have been shown in the table-1.

Table 1: Total lipids in the liver of marine fish.

Name of species	Wet weight of the tissue (g)	Weight of lipid extracted (g)	Percentage wet tissue (%)
Parastomateus nuger	42	2.50	5.95
Trichiurus lepturus	30	1.51	5.03

Fatty acid composition of total lipids and individual lipid classes of *Parastomateus nuger* and *Trichiurus lepturus* is presented in table-2 and table-3 respectively.

Table 2: Fatty acid patterns of lipid classes* in the liver oil of *Parastomateus nuger* (Kala Paplet).

Fatty acids**	Weight%			
	TG	FFA	SE	PL
Saturated				
$C_{14:0}$	2.91	1.62	-	1.30
$C_{15:0}$	2.30	1.00	0.67	0.70
$C_{16:0}$	53.66	29.61	21.12	19.87
$C_{18:0}$	13.90	7.09	5.40	10.52
Saturated				
$C_{16:1}$	-	-	-	3.70
$C_{16:2}$	-	0.39	0.31	0.54
$C_{18:1}$	16.28	9.98	4.90	7.83
$C_{18:1}$	-	-	-	0.82
$C_{18:2}$	0.65	0.20	-	-
$C_{20:1}$	1.84	0.89	1.98	1.71
$C_{20:2}$	2.11	-	1.96	-
$C_{20:5}$	1.38	-	-	1.66
$C_{22:1}$	1.23	15.58	0.30	7.97
$C_{22:1}$	-	-	0.85	-
$C_{22:2}$	0.63	-	-	3.90
$C_{22:3}$	0.73	0.30	5.16	0.76
$C_{22:4}$	0.59	-	2.74	5.30
$C_{22:5}$	-	-	28.33	8.14
$C_{22:5\ (iso)}$	-	0.62	-	4.09
$C_{22:6}$	0.22	1.26	-	14.79
Unknown	0.70	-	25.51	2.65
Unknown	0.87	-	0.77	3.75
Unknown	-	31.46	-	-

Table 3: Fatty acid patterns of lipid classes* in the liver oil of *Trichiurus lepturus* (Talwar).

Fatty acids**	Weight%			
	TG	FFA	SE	PL
Saturated				
$C_{14:0}$	4.54	3.50	4.29	2.59
$C_{15:0}$	0.52	0.69	1.46	0.48
$C_{16:0}$	15.96	30.93	33.03	36.34
$C_{18:0}$	10.96	9.92	13.99	23.29
Saturated				
$C_{16:1}$	5.66	4.11	9.59	3.90
$C_{16:2}$	-	0.40	-	0.76
$C_{18:1}$	33.97	19.64	19.42	18.60
$C_{18:2}$	1.26	1.02	2.31	11.01
$C_{18:3}$	0.39	-	0.86	0.36
$C_{20:1}$	0.96	1.38	0.89	0.20
$C_{20:5}$	1.31	0.65	0.58	2.70
$C_{22:1}$	1.29	0.94	1.97	4.72
$C_{22:4}$	0.74	-	0.70	0.24
$C_{22:5}$	0.92	-	2.30	0.96
$C_{22:6}$	1.46	0.30	2.35	3.85
unknown	20.06	26.52	6.26	-

*The short hand designation of class lipids are represented as: TG= triglycerides; FFA= free fatty acid; SE= steryl esters; PL=phospholipids; ** The short hand designation adopted by Farquhar(Farquhar, Insull Jr, Rosen, Stoffel, & Ahrens Jr, 1959).

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).

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.

Table-2 shows the fatty acid composition in the class lipid of liver of *Parastomateus nuger*. Among these lipid classes saturated fatty acids ranges from 27.19% to 72.77% palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) are the major saturated fatty acids ranges from 19.87% to 53.66% and 5.40% to 13.90% respectively. Among unsaturated fatty acid, oleic acid ($C_{18:1}$) is the major fatty acid ranges from 4.90% to 16.28%. In this fish docosahexaenoic acid (DHA)

was found in large concentration (14.79%) in the phospholipid fraction only. The percentage composition of eicosapentaenoic acid (EPA) ranges from 1.38% to 1.66%.

Table-3 gives the quantitative percent estimate of fatty acids of lipid classes in the liver of *Trichiurus lepturus* in which saturated fatty acid ranges from 31.98% to 62.70%. Palmitic acid($C_{16:0}$) is the predominant fatty acid ranges from 15.96% to 36.34% followed by stearic acid ($C_{18:0}$) 9.92% to 23.29%. Among unsaturated fatty acids, monoenoic are the major fatty acid. Oleic acid ranges from18.60% to 33.97%. In this fish eicosapentaenoic acid ($C_{20:5}$) was high in phospholipid (2.70%) as compared to dicacylglycerol and stearyl esters fractions. Docosahexaenoic acid ($C_{22:6}$) being highest in phospholipid (3.85%) fraction.

CONCLUSION AND GENERAL CONSIDERATIONS

The fish oil isolated from two different fishes provided interesting data regarding their fatty acids composition of the total as well as class lipids. It was concluded from the data that fatty acid mixture were of complex nature as compared to plant oil or mammalian fat. As many as 25 individual fatty acids may occur in the oil of two fish. The individual fatty acids may be divided into three main groups.

The first group comprised saturated fatty acid starting from Lauric ($C_{12:0}$), myristic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), Palmitic ($C_{16:0}$) and stearic acid ($C_{18:0}$). Palmitic acid was the major fatty acid found in both the sample.

In the second group all monoenoic acids were categorized. These acids were identified as palmitoleic ($C_{16:1}$ ω-7), oleic ($C_{18:1}$ ω-9). gadoleic ($C_{20:1}$ ω-9) and cetoleic ($C_{22:1}$ ω-11) acids. Some of the acids were also present in their isomeric form. Oleic acid was the major monoenoic acid in both the sample.

Among di, tri and polyenoic acids linoleic ($C_{18:2}$ ω-6), linolenic ($C_{18:3}$ ω-3), docosatetraenoic ($C_{22:4}$ ω-6), eicosapentaenoic ($C_{20:5}$ ω-3) and docosahexaenoic ($C_{22:6}$ ω-6) acids were also identified in the fatty acid mixture of pertinent interest was the finding of the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in appreciable amount in both the sample. In *Parastomateus nuger* DHA was found in large concentration.

Research work on fish oil attracted many laboratories recently because of its medicinal usage. Omega-3 fatty acids of various chain length has been known to serum cholesterol and TG levels in human subjects (Van Gent, Luten, Bronsgeest-Schoute, & Ruiter, 1979) and thus

has a correlation with CHD. In addition fish oil has been tried to cancer patients with promising anti cancer effect (Dai, Liu, Wang, Rao, & Reddy, 2002). Literatures on the implications of fish oil in diabetes (Mohan & Das, 2001), immunity (Hellerstein et al., 1996) asthma (Nagakura, Matsuda, Shichijyo, Sugimoto, & Hata, 2000) renal diseases (Donadio, 2001) arthritis (Kremer, 2000) and other diseases have been well documented.

Over the past quarter of century many studies have shown that large amount of polyunsaturated fat in the diet exhibited significance hypolipidemic effect. This factor is also confirmed by low incidence of coronary complications in Eskimos where major component of diet is of marine origin which is composed of ɷ-3 PuFA (Harris & Connor, 1980).

The present study opens various future avenues. Polyunsaturated fatty acids like ɷ-3 fatty acids found mostly in marine fish, when given in high dose (20-25g /day) have been proved to reduce blood TG, platelet aggregation and blood pressure and thus effectively prevent cardiovascular diseases(Ahmad, Ali, Usmanghani, & Ali, 1991). Therefore addition of fish and fish oil enriched in ɷ-3 fatty acids in a daily diet are beneficial for human heart health.

The two fish studied above are available round the year in excess quantities and general population can be motivated for the use of fish. When fresh fish delivery is a problem fish oil of fish liver oil has been recommended to be delivered in gelatin capsules (Hunter, 1987).

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