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The Triglyceride Fatty Acids from Heart Lipids of *Puntius Sarana* and Their Variation with Different Sizes

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The triglyceride fatty acid components from the heart lipid of *Puntius sarana* of different sizes have been characterized by thin-layer and gas liquid chromatography. C_{10} to C_{24} acids including both odd-numbered and branched-chain acids were detected. The major constituents were ante-iso C_{10}, C_{10}, $C_{12:2}$, C_{14}, C_{16}, $C_{16:1}$, C_{17}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:4}$ while twenty other acids were detected in lower proportion. The composition of these acids and their variation with size of fish have been investigated and discussed.

The present work was undertaken as a part of the programme under progress in this laboratory to understand the triglyceride fatty acids and their variations with size of fish in various organs. *P. sarana* is an economically important fresh water food fish in India. This species has not been investigated for the fatty acid composition. Jafri & Qasim (1965) reported only the total liver fat content from *P. sarana* along with other carps.

Materials and Methods

All the solvents and the chemicals were analar grade (BDH/E. Merck). The solvents were freshly redistilled under nitrogen before use. The fatty acid methyl ester standards were procured from Sigma Chemicals Company (USA). The fish were collected from the same locale of river Jamuna at Delhi during a three week period in October. Only male fish were selected and transported in ice. Fish were sorted out into three different groups on the basis of their average length (10.2, 16.4, and 23.7 cm) and their hearts were dissected and stored at-20°C until used.

Extraction of lipids

The weighed heart tissue samples were extracted in a homogenizer using chloroform/methanol mixture (Bligh & Dyer, 1959). The lipid extract was dried over anhydrous sodium sulphate, filtered and stored at-20°C in a nitrogen flushed glass stoppered volumetric flask which was kept filled to the neck with solvent until analysed. For storage longer than a week, an antioxidant BHT (2, 6 di-t-butyl 4 methyl-phenol) was added.

Separation of neutral lipids from phospholipids

The solvent from the total lipids was removed at 30°C under a mild vacuum in a Buchi rotary evaporator. The residue was taken up in 40-60°C petroleum ether and again concentrated to a small volume under nitrogen and the phospholipids were separated out by acetone precipitation (Kates, 1972) and neutral lipids were esterified after saponification by boron trifluoride method (Metcalfe *et al.*, 1966).

Thin layer chromatography

Methyl esters were separated and identified by reversed phase TLC using 0.5 mm thick kieselguhr G plates impregnated with 10% v/v liquid paraffin in petroleum ether (60-80°C) at 5°C and developed in nitromethane acetonitrile acetic acid (15:10:10)(Hammonds & Shone, 1964) and by argentation TLC using 10% silver nitrate impregnated 0.5 mm thick silica gel G developed in (i) diethyl ether-hexane (15:85) (Mangold, 1969) and (ii) benzene (Linko & Karinkanta, 1970).

Gas liquid chromatography

Methyl esters were analysed on Perkin Elmer 900 model, dual column chromatograph, equipped with both automatic digital and disc integrator, using flame ionisation detector and employing either

polyester (polar) or silicone (non polar) column with the following working conditions. Polyester column: Stainless steel column (3.6 m x 2 mm i.d.) packed with chromosorb W (mesh 80-100) impregnated with 20% (w/w) diethylene glycol succinate (DEGS); column temperature 200°C; nitrogen flow rate 20 ml/minute; sample size 0.3 to 2 µl (0.2 to 0.4% hexane solution). Silicone column: Stainless steel column (2.4 m x 2 mm i.d.) packed with gas chromo Q (90-100 mesh) impregnated with 5% (w/w) silicone rubber gum (SE 30) programmed from 180° to 320°C at the rate of 6°C per minute with initial and final hold time of 3 and 6 min respectively; nitrogen flow rate 20 ml/min; sample size 0.4 to 1.5µl.

Peak identification

Gas chromatograms of authentic samples were regularly run under exactly identical conditions in all cases. Sample peaks were identified by $\log_{10}$ relative retention time versus carbon number (James & Martin, 1956) and $\log_{10}$ relative retention temperature versus carbon number (Martin *et al.*, 1961) plotting procedures.

Unsaturated acids were confirmed by hydrogenating the methyl esters in methanol in the presence of platinum oxide catalyst and rechromatographing on the DEGS column under identical conditions. Absence of cyclopropane fatty acids and presence of branched-chain acids was established by rechromatographing the hydrogenated samples after bromination in diethyl ether (Kates, 1972).

Determination of percentage composition

The area counts were printed out by automatic digital integrator. Known standards were run regularly to check the linear response of the instrument. The percentage composition of each component was determined by area normalization as follows:

$$\text{Percentage of a component} = \frac{\text{area of that peak} \times 100}{\text{total area of all the component peaks}}$$

Results and Discussion

The number of fish used in each sample, the weight of the organs and the yield of the oil are given in Table 1. There was only limited variation in the lipid content of the heart (12.6%). There was slight fall in the 16.4 cm size (9.4%).

Table 1. *Total lipids from the heart of P. sarana of different size groups*

Average length cm	No. of fish	Total weight of heart g	% Lipid in heart w/w
10.2	103	5.3	11.3
16.4	112	36.9	9.4
23.7	49	33.3	12.6

The fatty acid methyl esters were analysed. As a few peaks in the chromatogram could not be identified unambiguously, the samples were hydrogenated and rechromatographed on DEGS column under identical conditions, confirming the presence of $C_{12:1}$, $C_{12:2}$, $C_{16:1}$, $C_{16:2}$, $C_{17:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ as their peaks shifted to the position normally occupied by the corresponding saturated fatty acids methyl esters. It was suspected from $\log_{10}$ plot that the peak for $C_{20:4}$ could be either $C_{22:1}$ or $C_{20:3}$ or $C_{20:4}$. Hydrogenation confirmed that it was not $C_{22:1}$. The possibility of $C_{20:3}$ was ruled out and this peak was identified as $C_{20:4}$ by following Ackman (1969). The identity of this acid was further confirmed by TLC.

A few peaks in the chromatograms did not shift their position after hydrogenation and therefore suspected to be either branched-chain acids or cyclopropane compounds. In the present investigation bromination of hydrogenated methyl esters did not lead to disappearance of any of the peaks on rechromatographing the sample and these peaks were identified as the following branched-chain fatty acids: Ante-iso $C_{10:0}$ ante-iso $C_{11:0}$, iso $C_{12:0}$, ante-iso $C_{15:0}$, ante-iso $C_{16:0}$, ante-iso $C_{17:0}$ and ante-iso $C_{22:0}$.

All the samples were also analysed on SE 30 column to check the data obtained in DEGS column and also to verify if any acid of chain length higher than C_{22} was

missed in DEGS analysis. The peaks were identified from the plot of $\log_{10}$ relative retention temperature *versus* carbon number. The SE_{30} data of heart lipids from all the three sizes revealed the presence of $C_{19:1}$, iso $C_{10:0}$, ante-iso $C_{20:0}$, $C_{20:0}$, $C_{20:1}$, iso $C_{21:0}$, ante-iso $C_{21:0}$, iso $C_{22:0}$, ante-iso $C_{22:0}$, iso $C_{23:0}$, ante-iso $C_{23:0}$, and iso $C_{24:0}$. These acids were present in traces and were missed in DEGS analysis.

The GLC data for fatty acids from heart of different sizes of fish are compiled in Table 2. While comparing these fatty acids it was observed that ante-iso $C_{11:0}$, iso $C_{20:0}$, ante-iso $C_{20:0}$, iso $C_{21:0}$, and iso $C_{22:0}$ were present only in the heart of 16.4 and 23.7 cm size fish but, absent in the 10.2 cm size fish heart. The $C_{10:0}$ $C_{19:1}$ and iso $C_{23:0}$ were present in the 10.2 and 16.4 cm sizes. The $C_{11:0}$, $C_{14:1}$, $C_{20:0}$, iso $C_{20:1}$ and $C_{24:0}$ were detected only in 10.2 cm size fish heart. The presence of $C_{22:0}$ and ante-iso $C_{23:0}$ were detected only in 23.7 and 16.4 cm size fish heart respectively.

The variations in the total percentage composition of various fatty acids in heart

Table 2. *Estimation of triglyceride fatty acids of heart from various sizes of P. sarana*

		Size groups cm		
	Fatty acids	10.2	16.4	23.7
Ante–iso	10:0	6.16	3.88	4.82
	10:0	8.14	4.81	—
Ante–iso	11:0	—	3.76	4.31
	11:0	2.48	—	—
iso	12:0	Trace	Trace	Trace
	12:0	0.15	0.19	0.03
	12:1	Trace	0.09	Trace
	12:2	1.31	1.94	1.42
	13:0	Trace	Trace	Trace
	14:0	1.20	2.51	1.66
	14:1	Trace	—	—
Ante–iso	15:0	Trace	0.07	Trace
	15:0	0.65	0.56	0.06
Ante–iso	16:0	0.41	0.28	0.07
	16:0	16.26	24.78	26.45
	16:1	2.73	2.32	0.45
	16:2	Trace	0.12	0.04
Ante–iso	17:0	0.65	0.75	0.29
	17:0	1.74	1.73	0.24
	17:1	0.15	0.21	0.02
	18:0	12.12	0.08	10.46
	18:1	34.63	31.07	39.23
	18:2	2.57	5.07	2.09
	18:3	5.23	3.59	0.94
	19:1	Trace	Trace	—
iso	20:0	—	Trace	Trace
Ante-iso	20:0	—	Trace	Trace
	20:0	Trace	—	—
	20:1	Trace	—	—
	20:4	3.41	3.76	1.17
iso	21:0	—	Trace	Trace
Ante–iso	21:0	Trace	Trace	Trace
iso	22:0	—	Trace	Trace
Ante–iso	22:0	Trace	0.42	0.07
	22:0	—	—	0.09
iso	23:0	Trace	Trace	—
Ante–iso	23:0	—	Trace	—
iso	24:0	Trace	—	—

Table 3. *Comparison of the triglyceride fatty acids in heart of P. sarana of different sizes determined by GLC*

Average length cm	Saturated: Straight chain Even numbered %	Saturated: Straight chain Odd numbered %	Saturated: Branched chain iso %	Saturated: Branched chain Ante-iso %	Unsaturated: Mono-enoic %	Unsaturated: Dienoic %	Unsaturated: Trienoic %	Unsaturated: Tetra-enoic %
10.2	37.87	4.87	Trace	7.22	37.51	3.88	5.23	3.41
16.4	40.37	2.39	Trace	9.16	33.69	7.13	3.59	3.76
23.7	38.69	0.30	Trace	9.56	39.70	3.55	0.94	1.17

of *P. sarana* of different sizes were compiled in Table 3. The saturated fatty acids in heart neutral lipids varied from 48.55 to 51.82%. The total percentage of saturated even-numbered acids was the highest in 16.4 cm size fish (40.37%) against 37.87 and 38.69% in 10.2 and 23.7 cm size fish respectively. The percentage of $C_{10.0}$ was quite high in heart lipids of 10.2 cm (8.41%) and 16.4 cm (4.81%) size fish, while this acid could not be detected in the heart of 23.7 cm fish.

The percentage of saturated straight chain odd-numbered acids was the highest (4.87%) in 10.2 cm size fish heart. This percentage decreased in heart lipid with size (2.29% in 16.4 cm size and 0.3% in 23.7 cm size fish). The presence of $C_{11:0}$ was significant (2.48%) in heart from 10.2 cm fish while it was absent in the heart from larger varieties of fish.

Among the branched-chain acids the iso acids were present in traces, while the percentage of anteiso acids was significant and was found to increase with size (7.22% in 10.2 cm, 9.16% in 16.4 cm and 9.56% in 23.7 cm size fish).

The presence of odd-numbered fatty acids was first reported by Farquhar *et al.* (1959) in menhaden *(Brevoorita tyrannus)* oil and later confirmed by others (Ito & Fukuzumi, 1962, 1963; Klenk, *et al.*, 1963; Roubal, 1963). Morice & Shorland (1956) first demonstrated the presence of branched chain fatty acids in shark liver oil. Ackman *et al.* (1963) provisionally identified saturated odd numbered straight chain fatty acids in seal oil and later confirmed them along with branched-chain acids in marine mammals and fish (Ackman & Sipos, 1965).

Sen & Sehlenk (1964) who found that mullet *(Mugil cephalus)* oil contained more than 25% straight-chain odd-numbered saturated, monounsaturated and polyunsaturated fatty acids suggested that these acids arise as a result of slow catabolism of the ingested odd-numbered fatty acids from phytoplanktons. On the other hand, Ackman (1965) held the view that odd-numbered fatty acids arise out of dietary thetin, present in a number of algae (Challenger, 1959). On the death of the host algae, thetin on enzymatic degradation may give rise to acrylic acid or propionic acid, a precursor for odd-numbered fatty acids, as was demonstrated by Tove (1959) in mice feeding experiments.

P. sarana feeds mainly on phytoplankton (green algae) and zooplankton consisting of crustacea, rotifers and insects (Sinha, 1972). The branched-chain and odd-numbered acids detected in this fish may be dietary, as Schlenk *et al.* (1960) reported the presence of these fatty acids in a fresh water alga, *Chlorella pyrenoidosa* while Klenk & Eberhagen (1962) found them in both phyto and zooplanktons. Their occurrence may also be expected in other fresh water organisms. Such fatty acids can also be biosynthesized by the fish from branched chain skeleton arising from the oxidative decarboxylation of branched-chain amino acids like valine, leucine and isoleucine and odd-numbered straight-chain from propionic or acrylic acid, the degradation products of thetin. The percentage of the monoenoic acids in the heart neutral lipid was the highest (39.7%) in the 23.7 cm size fish and

lowest (33.69%) in the 16.4 cm size fish. The major constituent was $C_{18:1}$ followed by $C_{16:1}$. The percentage of the dienoic acids in heart neutral lipid was the highest (7.13%) for 16.4 cm size fish while it was the lowest (3.55%) in the 23.7 cm fish. The major dienoic acid was $C_{18:2}$ followed by $C_{12:2}$ and $C_{16:2}$ in that order. The concentration of the trienoic acid ($C_{18:3}$) was the highest (5.23%) in the 10.2 cm fish heart and gradually decreased with size (from 3.59 to 0.94%). The only tetraenoic acid detected in fish heart lipid was $C_{20:4}$ in all the three sizes, the highest percentage being in 16.4 cm size fish heart.

Thus it was noticed that the level of triglyceride fatty acids in heart varied with fish size within narrow limits. A trend in quantitative relations has been established, but comparison is not possible as similar studies on this organ in other species have not been made.

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