STUDIES ON FISH LIPIDS

II. FATTY ACID COMPOSITION OF LIPIDS OF OIL SARDINE *(SARDINELLA LONGICEPS)*

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Gas–liquid chromatography has been employed for the qualitative and quantitative analysis of the component fatty acids in lipids of oil sardine *(Sardinella longiceps)*. Phospholipids and triglycerides of the lipids were previously separated by column chromatography before they were converted into the methyl esters of the fatty acids. The predominant acids present in the depot fat of the fish have been found to be $C_{14:0}$ = 8.13%, $C_{16:0}$ = 27.9%, $C_{18:0}$ = 3.8%, $C_{18:1}$ = 15.4%. $C_{20:5}$ = 10.6% and $C_{22:6}$ =8.8%. Apart from the above acids the distribution of minor acids belonging to C_{18}, C_{20} and C_{22} groups have also been worked out. The separated phospholipid fraction contained more than 70% polyunsaturated acids of which the important constituents were docosahexaenoic ($C_{22:6}$ =28%) and eicosapentaenoic ($C_{20:5}$ = 10.6%). A marked reduction was found in the amounts of polyunsaturated acids in triglycerides, their total amount registering about 20 percent. This fraction recorded about 48 per cent of C_{16} acids of which palmitic and palmitoleic acids amounted to 25.8 per cent and 19.1 per cent respectively. Occurrence of odd numbered fatty acids C_{15} and C_{17} has also been noted in the phospholipid and composite samples of the fish.

INTRODUCTION

Recent investigations brought out the trend of the seasonal variation of the total lipid of the fish (Vasavan et al., 1960) and of the component triglycerides, phospholipids and unsaponifiable matter of the lipids (Gopakumar, 1966). However, no quantitative data are available regarding the fatty acid composition of the lipid of the fish. On the other hand the composition of fatty acid in lipids of fishes like pilchard (de Koning, 1966), herring (Gruger et al., 1964) and Japanese sardine (Hilditch, 1956) have been studied extensively. The present investigation is therefore aimed at working out the distribution of component fatty acids in lipids of oil sardine separately in phospholipid and triglyceride fractions.

Material And Methods

Collection of samples: The oil sardine *(Sardinella longiceps)* landed at Cochin in the month of April were used for extraction of lipids. The fish were chilled in ice, descaled and deboned. The muscle, with the skin kept intact, was blended in mechanical blendor and lots of 100 gm of minced muscle were taken for analysis.

Extraction and purification: The lipids were extracted from weighed lots of the muscle successively four times with 300ml of chloroform-methanol mixture (2:1 v/v) according to the procedure of Bligh and Dyer (1959). The crude lipids obtained were washed thrice according to the procedure of Folch *et al* (1957). 100 gm of muscle yielded 6 gm of lipids.

Chromatography: The lipids so obtained were separated into phospholipids and nonphosphorylated lipids by chromatography on activated silicic acid. Silicic acid 100 gm (100 mesh, A. G. Fluka, for chromatography according to Ramsey and Patterson) heated for 24 hours at 120°C (Rouser *et al.*, 1965) was used. The column bed was prepared in chloroform. The nonphospholipids were eluted from the column using chloroform containing 0.7% methanol.

The phospholipids were subsequently eluted out from the column with mixtures of increasing concentration of methanol in chloroform and finally methanol.

The triglycerides present in the nonphosphorylated lipids were separated by chromatography on florisil according to the method of Litchfield *et al* (1964).

Preparation of methyl esters: The methyl seters were prepared directly from the lipids by methylating them using borontrifluoride -methanol reagent (Morrison and Smith, 1964).

Gas–Liquid Chromatography: The methyl esters prepared were analysed using a gas chromatograph (The oven chromatograph type S_3, of Gas Chromatography Limited, Maidenhead, Berkshire, England). The instrument was equipped with a flame ionisation detector. The column used was 6.35mm × 183cm copper column packed with chromosorb G (60-80 mesh, acid washed with dimethyl chlorosilane, Koch Light Laboratories Ltd., Colnbrook, Bucks, England) coated with DEGS (10% w/w of the inert phase). The operating conditions were as follows:

Oven temperature	198° C
Flash heat temperature	245° C
Detector Voltage	400 V
Carrier gas	Nitrogen
Nitrogen gas pressure	1.75Kg/cm^2
Hydrogen pressure	1.41Kg/cm^2
Air pressure	0.71Kg/cm^2

Reference Standards:

a) Unsaturated fatty acid methyl esters C_{18}–C_{24} (8 Nos.) prepared by Hormel Institute (Courtesy, National Iustitute ofHealth. U. S. A.)

b) Saturated acids: methyl laurate, methyl myristate, methyl tridecanoate, methyl palmitate, methyl heptadecanoate, and methyl stearate (A. G. Fluka, Switzerland, purity checked by GLC under above conditions).

c) methyl linoleate and methyl linolenate were prepared from the corresponding pure acids (A. G. Fluka, Switzerland).

Straight line graphs were obtained when logarithms of the retention times of these acids were plotted against the corresponding carbon numbers. Two sample mixtures were analysed.

TABLE I FATTY ACID COMPOSITION OF TOTAL LIPIDS, PHOSPHOLIPIDS AND TRIGLYCERIDES OF OIL SARDINE.

(As weight percent of Methyl esters)

	C_{14}		C_{15}	C_{16}			C_{17}	C_{18}					C_{20}					C_{22}					C_{24}
Double bonds	0	1	0	0	1	2	0	0	1	2	3	4	1	2	3	4	5	1	1?	4	5	6	1
Total lipid	8.13	–	0.29	27.0	6.8	–	1.0	3.8	15.4	4.3	0.8	1.7	2.3	–	0.8	0.7	10.6	2.9	0.4	1.2	0.8	8.8	0.8
Phospho lipids	3.2	0.6	0.25	17.5	6.5	–	0.4	5.9	10.8	2.3	1.7	1.5	1.5	–	–	–	10.6	5.6	–	1.2	1.2	28.0	1.7
Trigly cerides	10.4	–	–	25.8	19.1	3.5	–	5.0	13.6	2.5	0.5	1.3	2.8	–	–	–	7.9	3.4	–	–	–	4.6	–

? Unidentified.

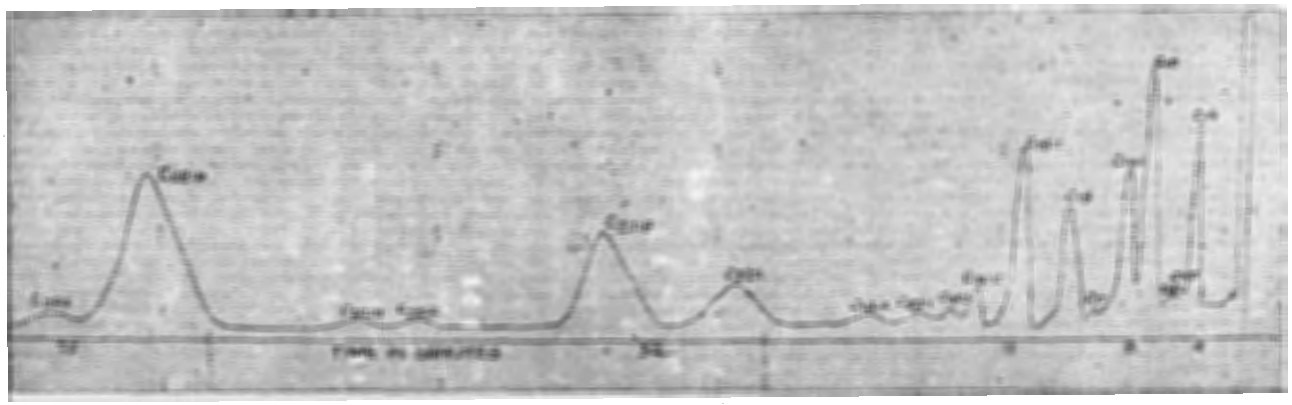

Fig I. "GLC recorder curves obtained from phospholipids of oil sardine".

a) methyl laurate, methyl myristate, methyl palmitate and methyl stearate. (Recovery 98 percent)

b) $C_{18:1}$, $C_{20:5}$, $C_{22:6}$ and $C_{24:1}$. (Recovery 96 per cent)

Fatty acids of unknown samples were identified by comparison of the retention times of these reference standards and confirmed by the graphic procedure of Miwa (1963).

The triangulation method was used to determine the corresponding peak areas obtained from the GLC recorder curves. The fatty acid composition in weight per cent was calculated by multiplying each area percentage by molecular weight of the corresponding methyl ester and subsequently dividing each product by the sum of the weighed product.

These conversions had average variations ranging from – 18 per cent for $C_{14:0}$ to + 14 percent for $C_{22:6}$.

Results

Fatty acid compostion of total lipids, phospholipids and triglycerides of oil sardine is recorded in Table I. It has been observed that the saturated acid $C_{16:0}$ and polyunsaturated acids $C_{20:5}$ and $C_{22:6}$ and monounsaturated acid $C_{18:1}$ were the predominating acids present in the depot fat of oil sardine examined. The analysis of the lipid of the fish showed that unsaturated acids formed about 60 per cent of the total fatty acids. The phospholipids analysed contained more than 70 per cent unsaturated acids of which 53 percent being polyunsaturated acids. Docosahexaenoic acid was the major acid present in the phospholipid fraction (28 per cent), the second predominating acid being $C_{20:5}$ which recorded a level of 10.6 per cent.

A marked reduction has been found in the amounts of polyunsaturated acid in triglycerides, their total amount being only about 20 per cent. This fraction recorded about 48 per cent C_{16} acids and among constituent unsaturated acids, the monoenoic acid, palmitoleic acid predominating to the extent of 19.1 per cent.

Discussion

Fatty acid composition of the total lipid of sardine has been found to be of a similiar pattern as for other species of fish examined. The distribution of fatty acids was however found to be remarkably different when determined separately in the phospholipid and triglyceride fractions. The phospholipids were shown to contain higher proportion of polyunsaturated acids than the composite lipid and nonphosphorylated lipid fraction. The lipids of cod (Ackman and Burgher, 1964), tuna (Shuster *et al.*, 1964), hake and pilchard (de Koning, 1966) also showed a similar pattern in their fatty acid build up.

Among the lower acids, C_{16} acid has been found to be the most dominant, recording about 34 per cent. It has been found that the concentration of C_{16} acids in lipids of many fish examined remained fairly constant over the range, 30 to 40 per cent of total acids. Among the Indian fishes the percentage of C_{16} acids observed are as mackerel (*Rastrelliger kanagurta*) 34.2 percent, kilimeen (*Nenipterus japonicus*) 31.3 per cent, jew fish (*Prestipera geraca*) 37.89 percent and pomfret (*Pampus argenteus*) 37.3% (unpublished data). A notable difference has been observed in the levels of palmitoleic acid in the triglyceride and phospholipid fraction of the lipids of oil sardine whereas in the former fraction, its concentration amounted to about 20 per cent, in the latter its level was as low as 6.5 per cent.

A comparison of the data of fatty acid composition of many species also showed that the acid $C_{14:0}$ was present in relatively higher level in the lipids of oil sardine. In general, the composition of saturated fatty acid showed an indentical pattern in many species, the concentration of which following order $C_{16:0}$ greater than $C_{18:0}$ greater than $C_{17:0}$ According to Ackman and Sipos (1965), palmitic acid is the major saturated fatty acid in the metabolic fatty acid pool followed by lower proportions of stearic and still lower proportions of C_{17} acids.

As has been pointed out by Lovern (1964) data are still inadequate for generalization of theories relating to fatty acid distribution in marine lipids. According to Ackman and Sipos (1965), marine organisms build depot fatty acids whose compositions are substantially based on the forms of lipids such as alkoxy diglycerides, fatty alcohols etc. and even in such systems based primarily on fatty acids, the depot fat composition might be governed by variations due to size, nature of feed ingested, sexual maturity and to spawning cycle. A remarkable feature of sardine lipids is the absence of branched chain fatty acids. It is likely that this may not be the case for lipid samples for fish at all seasons over which the lipid content shows a variation in the range 2 to 16 per cent (on wet weight basis).

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