PART II

SCIENTIFIC AND TECHNICAL

FATTY ACID COMPOSITION OF THREE SPECIES OF FRESHWATER FISHES

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Fatty acid composition of freshwater fish *tilapia, (Tilapia mosambica)*, barbus *(Barbus carnaticus)* and *Varal (Ophicephalus)* is determined by gas-liquid chromatography.

Varal contained the highest amount of $C_{20:5}$ acid compared to other two species. Of the odd-numbered fatty acids C_{17} was the predominant fatty acid present. Palmitic acid was found to be in a lower level in these fish species compared to marine fish.

Barbus recorded unusually high percentage of 23.3% of $C_{18:2}$ Acid.

INTRODUCTION

Tilapia (Tilapia mosambica), barbus *(Barbus carnaticus)* and *varal (Ophicephalus)* are three major freshwater fishes of India. Extensive investigations were carried out by Pathak and Reddy (1962) on the fatty acid composition of Indian freshwater fishes. Gruger *et al.* (1964) and Ackman (1967) have determined the fatty acid composition of a number of North American freshwater fishes.

A comparative study was carried out to understand the differences in the fatty acid build up among these three species. The results are given in this paper.

MATERIALS AND METHODS

Both *tilapia* and barbus were caught from the famous freshwater reservoir, Malampuzha, of Kerala. *Varal* was purchased from the market in Cochin.

EXTRACTION OF LIPIDS

Lipids were quantitatively extracted from the fish samples with chloroform-methanol mixture (2:1v/v) by the method of Bligh and Dyer (1959). The chloroform extracts were concentrated in a current of carbon dioxide and lipids dried and weighed in vacuum.

PREPARATION OF METHYL ESTERS

The lipids were saponified by the

Official Methods of the American Oil Chemists' Society. The fatty acids recovered were esterified with methanol-HCl reagent.

Gas-liquid Chromatography

Gas-liquid chromatographic analysis were carried out on a gas-chromatograph F and M model 1609, equipped with a flame ionisation detector and a Honeywell strip chart recorder (3mV). A stainless steel column 0.9 m. × 4.8 mm. (6 ft. × 3/16 in.) packed with chromosorb W (45 to 60 mesh) coated with 15% Diethylene Glycol Succinate (DEGS) was used. Nitrogen was used as the carrier gas.

The operating conditions were as follows: Column temperature, 200°C, injection port temperature, 300°C, detector port temperature, 300°C, nitrogen 120 ml./min., hydrogen 35ml./min., air 350 ml./min.

Methyl esters of fish oils, diluted in chloroform, were injected with a Hamilton 10μl microsyringe. The analysis was carried out at 8 × 1000 attenuation.

Fatty acids of unknown samples were determined by comparison with the retention times of reference standards as described by Gopakumar and M. R. Nair (1972). The probable errors are for major components (5%), low to medium range components (10%) and upto 50% for the minor components.

Results and Discussion

Examination of the fatty acid composition of three species of common freshwater fish, *varal* (Ophicephalus), *tilapia (Tilapia mosambica)* and barbus *(Barbus carnaticus)* showed that a variety of fatty acids are seen in them. Odd-numbered saturated and monounsaturated acids are present in them, C_{17} acids being the most predominating. *Varal* contained the highest amount of polyunsaturated fatty acid $C_{20:5}$ but C_{22} acids are found to be present in smaller levels, uniformly, in all the three species. This pattern seems to be quite characteristic of the fish in this region.

Hilditch and Williams (1964) observed that triglyceride oils and lipids from freshwater fish, compared to those of marine origin, are richer in C_{16} and C_{18} fatty acids and lower in C_{22} and C_{20} acids. Tropical and subtropical freshwater species were found to contain low levels of C_{22} fatty acids (Pathak and Reddy 1962, Brener *et al.* 1961, 1963).

Ackman (1967) reported that in four North American freshwater fish (sheepshead, tullibee, maria and alewife) both C_{16} and C_{18} acids were higher than in marine species. Ackman in this study observed that total di- and tri-enoic acids were twice as high in freshwater oils as in marine oils and tetraenoic acids were three to four times high in freshwater oils. He also suggested that extension of these to marine type fatty acids $C_{20:5\omega3}$ and $C_{22:6\omega3}$ etc. is not normally obligatory in freshwater fish.

Barbus recorded unusually high percentage of $C_{18:2}$ acid. This was also confirmed by chainlength analysis of the oil by GLC after hydrogenation.

The levels of $C_{18:2}$ and $C_{18:3}$ acids in *varal* and *tilapia* are in the limits usually observed in freshwater species (Ackman 1967, Ito and Fukuzumi 1963b). Fatty acid build up in *varal* showed a marine type pattern containing

TABLE

FATTY ACID COMPOSITION OF FRESHWATER FISHES

Fatty acid designation	Name of Fishes		
	Varal	Barbus	Tilapia
	—Weight per cent—		
Saturated acids			
$C_{12:0}$	1.1	0.3	–
$C_{13:0}$	—	0.1	—
$C_{14:0}$	2.7	2.1	4.7
$C_{15:0}$	2.6	0.4	0.9
$C_{16:0}$	25.2	18.5	29.7
$C_{17:0}$	2.5	1.4	3.0
$C_{18:0}$	15.2	10.9	5.4
$C_{19:0}$	—	—	0.7
Total	49.3	33.7	44.4
Monounsaturated acids			
$C_{12:1}$	—	0.1	—
$C_{14:1}$	1.0	0.7	0.9
$C_{15:1}$	0.4	0.1	0.5
$C_{16:1}$	3.7	4.9	13.8
$C_{17:1}$	3.9	0.4	0.9
$C_{18:1}$	20.2	25.1	19.8
$C_{20:1}$	0.4	0.7	0.8
$C_{22:1}$	0.3	1.0	0.3
Total	29.9	33.0	37.5
Polyunsaturated acids			
$C_{18:2}$	5.6	23.3	4.1
$C_{18:3}$	1.0	3.6	4.0
$C_{18:4}$	0.4	1.1	0.5
$C_{20:2}$	—	—	0.5
$C_{20:3}$	0.4	—	0.7
$C_{20:4}$	0.3	—	2.1
$C_{20:5}$	11.0	4.2	1.5
$C_{22:4}$	—	—	0.4; 0.5
$C_{22:5}$	0.4	0.4	1.8
$C_{22:6}$	1.6	0.6	2.0
Total	20.7	33.2	18.1
Lipids, g./100g. wet muscle	1.8	2.2	3.7

a higher percentage of $C_{20:5}$ (11.0%) than to other two species, barbus and *tilapia*. This is because of the high phospholipid content in the lipids of *varal* (total lipid content being 1.8% and phospholipids, 0.8% by weight of the wet tissues). Palmitic acid was found to be in lower level in these three species of freshwater fish relative to marine oils and C_{18} acids in higher proportions than in marine oils. The key role of palmitic acid ($C_{16:0}$) in saturated fatty acid metabolism in marine depot fats has been well explained by number of workers (Ackman 1967; Ackman and Sipos 1965). The percentage of palmitic acid (60 percent of total saturated) is almost within the range of value of these marine and freshwater oils, proposed by them.

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