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The Component Acids of the Fats of Some Indian Fresh-water Fishes

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The component acids of fats from different parts of bhakur (*Catla buehanani*) were investigated by Pathak & Agarwal (1952). The fats obtained from the body and viscera of two other species of Indian fresh-water fishes have now been studied.

EXPERIMENTAL

The fresh-water fishes used were rohu (*Labeo rohita*) and nain (*Cirrhina mrigala*) caught in the River Ganges near Banaras. The body and viscera (excluding the liver) were separately cut into small pieces and exhaustively extracted with hot acetone. The extracted material was taken up in light petroleum (b.p. 40–60°), the aqueous fraction was separated and the lipids were recovered and dried at 100° under reduced pressure. The viscera of these fish contained nearly 45% of fat. The fats were kept in 10 vol. of acetone at 0° for 4 weeks to separate the phosphatides, if present, from the glycerides.

The fats were hydrolysed, and the mixed fatty acids resolved into groups of simpler mixtures of acids by the Li salt-acetone method (Tsujimoto, 1920; Tsujimoto & Kimura, 1923), followed by further resolution of the insoluble fraction by the Pb salt-ethanol method as modified by Hilditch (1949). Each group of acids was separately converted into methyl esters, with the precautions recommended by Bjarnson & Meara (1944), and fractionated at approx. 0.1 mm. pressure through an electrically heated, packed column (Longenecker, 1937). The compositions of the ester fractions were calculated from the iodine values and the saponification equivalents by the method of

Hilditch (1949). The mean unsaturation, expressed by the fractional number of hydrogen atoms short of saturation (e.g. -2.0, mono-ethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each group. The compositions of the original fats were obtained from those of the separated groups of fatty acids.

RESULTS

The results of the preliminary fractionation of 180 g. mixed body fatty acids and 175 g. mixed visceral fatty acids of rohu are given in Table 1. This table also gives results for 162.9 g. mixed body fatty acids and 134.7 g. mixed visceral fatty acids of nain.

The component acids of each group and of the whole of the body and the visceral fats are recorded in Tables 2 and 3 for rohu and in Tables 4 and 5 for nain.

DISCUSSION

Rohu body and visceral fats

These fats show similarities to typical fats of fresh-water fishes in having unsaturated C₁₈ acids as the predominant group, palmitic acid being the major saturated acid component. There are traces of unsaturated C₂₄ acids (Table 2), and this has also been observed in the body fat of bhakur, another species of Indian fresh-water fish (Table 6).

Table 1. *Fractionation of the fats of rohu and nain fish*

Group	Rohu fats				Nain fats			
	Body		Viscera		Body		Viscera	
	Wt. (%)	Iodine value	Wt. (%)	Iodine value	Wt. (%)	Iodine value	Wt. (%)	Iodine value
A. Pb salt, ethanol-insoluble	28.3	9.9	43.3	3.6	25.9	6.6	27.3	6.3
B. Li salt, acetone-insoluble, but Pb salt, ethanol-soluble	52.2	96.3	33.5	165.2	57.2	117.1	59.1	109.7
C. Li salt, acetone-soluble	19.5	238.0	23.2	213.0	16.9	167.8	13.6	163.9

Table 2. *Component acids of rohu body fat*

Acid	Component acids in groups (as % of total)			Sum (%)	Total as % of whole fat, excluding unsaponifiable fraction	
	A	B	C		% (w/w)	% (moles)
Myristic	1.2	.2	—	3.4	3.4	4.0
Palmitic	19.0	2.1	—	21.1	21.2	22.7
Stearic	5.2	6.3	—	11.5	11.5	11.5
Arachidic	—	0.4	—	0.4	0.5	0.4
Unsaturated fractions						
C ₁₄	Trace	3.6	—	3.6	3.7	4.5
C ₁₆	1.2	5.8	1.2	8.2	8.1	8.5
C ₁₈	1.7	24.3	6.2	32.2	32.2	31.5
C ₂₀	—	7.4	5.0	12.4	12.4	11.1
C ₂₂	—	—	6.7	6.7	6.7	5.5
C ₂₄	—	—	0.3	0.3	0.3	0.3
Unsaponifiable fraction	Trace	0.1	0.1	0.2	—	—
Mean unsaturation of fractions						
C ₁₄	-2.0	-2.0	—	-2.0	—	—
C ₁₆	-2.0	-2.0	-3.3	-2.2	—	—
C ₁₈	-2.0	-3.3	-4.6	-3.3	—	—
C ₂₀	—	-4.7	-6.0	-5.2	—	—
C ₂₂	—	—	-6.9	-6.9	—	—
C ₂₄	—	—	-8.0	-8.0	—	—

Table 3. *Component acids of rohu visceral fat*

Acids	Component acids in groups (as % of total)			Component acids in whole fat	
	A	B	C	% (w/w)	% (moles)
Myristic	1.6	—	—	1.6	2.0
Palmitic	23.4	2.6	—	26.0	28.0
Stearic	13.3	1.3	—	14.6	14.1
Arachidic	3.1	Trace	—	3.1	2.7
Unsaturated fractions					
C ₁₄	Trace	—	0.7	0.7	0.9
C ₁₆	0.7	4.1	3.8	8.6	9.4
C ₁₈	0.7	22.0	7.6	30.3	29.8
C ₂₀	0.5	3.5	5.6	9.6	8.6
C ₂₂	—	—	5.5	5.5	4.5
Unsaponifiable fraction	Trace	Trace	Trace	—	—
Mean unsaturation of fractions					
C ₁₄	-2.0	-2.0	-2.0	-2.0	—
C ₁₆	-2.0	-2.0	-3.6	-2.7	—
C ₁₈	-2.0	-3.2	-4.9	-3.6	—
C ₂₀	-2.0	4.0	-8.0	-6.3	—
C ₂₂	—	—	-9.0	-9.0	—

Table 4. *Component acids of nain body fat*

Acids	Component acids of groups (%)			Sum (%)	Total as % of whole fat excluding unsaponifiable fraction	
	A	B	C		% (w/w)	% (moles)
Myristic	1.7	0.2	—	1.9	1.9	2.2
Palmitic	19.2	2.1	—	21.3	21.4	22.2
Stearic	3.1	—	—	3.1	3.1	2.9
Unsaturated fractions						
C ₁₄	Trace	3.0	0.6	3.6	3.7	4.3
C ₁₆	1.1	27.7	3.7	32.5	32.6	34.0
C ₁₈	0.7	22.2	6.5	29.4	29.5	27.9
C ₂₀	—	1.9	3.0	4.9	5.0	4.3
C ₂₂	—	—	2.8	2.8	2.8	2.2
Unsaponifiable fraction	0.1	0.1	0.3	0.5	—	—
Mean unsaturation of fractions						
C ₁₄	-2.0	-2.0	-2.0	-2.0	—	—
C ₁₆	-2.0	-2.1	-3.4	-2.2	—	—
C ₁₈	-2.0	-3.5	-4.3	-3.6	—	—
C ₂₀	—	-4.5	-5.2	-4.9	—	—
C ₂₂	—	—	-6.0	-6.0	—	—

Table 5. *Component acids of nain visceral fat*

Acids	Component acids of groups (%)			Total as % of whole fat excluding unsaponifiable fraction	
	A	B	C	% (w/w)	% (moles)
Myristic	1.9	3.9	—	5.7	6.6
Palmitic	17.6	2.4	—	20.1	20.7
Stearic	5.6	0.1	—	5.7	5.3
Arachidic	0.5	—	—	0.5	0.4
Unsaturated fractions					
C ₁₄	Trace	2.7	1.5	4.2	4.9
C ₁₆	0.4	21.8	4.2	26.5	27.5
C ₁₈	1.0	26.0	5.3	32.3	30.3
C ₂₀	0.2	2.1	2.6	5.0	4.3
Unsaponifiable fraction	0.1	0.1	Trace	—	—
Mean unsaturation of fractions					
C ₁₄	-2.0	-2.0	-2.0	-2.0	—
C ₁₆	-2.0	-2.0	-3.5	-2.2	—
C ₁₈	-2.0	-2.6	-4.6	-2.9	—
C ₂₀	-2.0	-3.1	-6.0	-4.6	—

The composition of the visceral fat is somewhat different from that of the body fat in both saturated and unsaturated fractions (Tables 2 and 3). Unsaturated C₂₄ acids are absent. Similar small variations in the proportions of various component acids in fats from different tissues of the same fish have also been observed, e.g. in pike (Lovern, 1932a) sturgeon (Lovern, 1932b) and salmon (Lovern, 1934, 1936a, 1937).

The more remarkable feature of these two fats is, however, their high saturated acid contents (36.6 and 45.3 % respectively). The body and visceral fats of bhakur contain 38.4 and 37.4 % of saturated acids respectively (Pathak & Agarwal, 1952). Except for the doubtful case of the Chinese fan fish (Tsujimoto, 1916), the highest percentage of

saturated acids recorded in other fish fats is about 28.0 % in groper liver (Shorland & Hilditch, 1939) and in the heart and spleen of tunny from the Atlantic Ocean (Lovern, 1936b). The proportion of stearic acid (11.5 and 14.6 % in body and visceral fats) is still more remarkable, as the highest percentage previously reported (excluding the Chinese fan fish) is about 8 % in tunny (Lovern, 1936b). The total C₁₈ acid content in these fats is similar (43.7 and 44.9 % of the total), and is of the usual order for the fat of fresh-water fishes.

This suggests that an otherwise normal fish fat has undergone bio-hydrogenation with the appearance of larger amounts of stearic acid and a consequent increase in the total saturated acid.

Table 6. *Fatty acid compositions of fresh-water and marine fish fats from different parts of the same fish*

All values are as percentage of total. The figures in parentheses give the mean unsaturation of the component.

Acids	Herring body*	Herring viscera†	Bhakur body‡	Bhakur viscera‡	Bhakur liver‡	Nain body	Nain viscera	Rohu body	Rohu viscera
Myristic	8.3	5.8	2.9	2.8	0.6	1.9	5.7	3.4	1.6
Palmitic	12.1	15.7	29.0	25.3	19.6	21.4	20.1	21.2	26.0
Stearic	0.3	2.8	6.5	7.9	6.4	3.1	5.7	11.5	14.6
Arachidic	—	0.3	—	1.4	3.1	—	0.5	0.5	3.1
Unsaturated fractions									
C ₁₄	0.5 (-2.0)	1.4 (-2.0)	0.2 (-2.0)	1.4 (-2.0)	—	3.7 (-2.0)	4.2 (-2.0)	3.7 (-2.0)	0.7 (-2.0)
C ₁₆	6.4 (-3.4)	10.5 (-2.5)	25.3 (-2.7)	10.0 (-2.7)	7.0 (-2.0)	32.6 (-2.2)	26.5 (-2.2)	8.1 (-2.2)	8.6 (-2.7)
C ₁₈	31.0 (-4.5)	31.8 (-2.6)	17.9 (-2.5)	37.2 (-2.5)	25.4 (-2.7)	29.5 (-3.6)	32.3 (-2.9)	32.2 (-3.3)	30.3 (-3.6)
C ₂₀	28.3 (-5.5)	22.4 (-7.1)	7.5 (-5.7)	11.9 (-5.7)	26.5 (-4.8)	5.0 (-4.9)	5.0 (-4.0)	12.4 (-5.2)	9.6 (-6.3)
C ₂₂	23.1 (-4.6)	9.3 (-10.5)	10.1 (-9.0)	2.1 (-9.0)	9.4 (-6.5)	2.8 (-6.0)	—	6.7 (-6.9)	5.5 (-9.0)
C ₂₄	—	—	0.6 (-11.0)	—	2.6 (-8.0)	—	—	0.3 (-8.0)	—

* Lovern (1938a).

† Hilditch & Pathak (1948).

‡ Pathak & Agarwal (1952).

Nain body and visceral fats

Nain belongs to a different genus from rohu, and this is clearly reflected in the nature of its body and visceral fats. Differences are apparent in the lower stearic acid content, in the lower proportions of saturated acids, and more especially in the unsaturated C₁₆ acids (Tables 2-5). Unsaturated C₁₆ acids comprise only 8.1 and 8.6 % of the rohu body and visceral fats but form major components in the fats of nain, being 32.6 and 26.5 % respectively. Palmitic acid, which constitutes 21.4 and 20.1 % respectively of the body and visceral fats of nain, together with unsaturated C₁₆ (32.6 and 26.5 %) and C₁₈ (29.5 and 32.3 %) acids, account for nearly 80-85 % of the total component acids of these nain fats. The proportions of C₂₀₋₂₂ acids (7.8 and 5.0 %) in these fats are much smaller than those in rohu fats.

The percentages of the various component acids are different in the body and in the visceral fat; the chief component acid in the fat of nain body is C₁₆ while in that of viscera it is C₁₈. Thus these fats are similar in this respect to the body and visceral fats of bhakur (Table 6). There are variations in the composition of fats from different parts of nain, but these are not so outstanding as in the case of fats from different tissues of bhakur and herring (Table 6). Fats of the latter fish are typically marine in nature and are included in the table as they show

clearly the differences between fats from marine and fresh-water sources. There are much increased proportions of the C₂₀ and C₂₂ groups of unsaturated acids with corresponding reductions in the amount of unsaturated C₁₆ and C₁₈ acids. The high percentage of stearic acid and total content of saturated acid are not so marked in the case of nain fats as in rohu fats, and bio-hydrogenation has evidently not proceeded to a great extent in this case.

The comparatively saturated nature of these fats may be related to the fact that the fishes belong to warm tropical waters, since Lovern has observed that in the cases of eels (Lovern, 1938b) and tunny (Lovern, 1936b) there is a definite tendency towards the deposition of a less unsaturated fat at a higher temperature.

Rapson, Schwartz & Van Rensburg (1945) noted that head and body tissues of groper contained about 4 times as much fat as the mesentrium, and Pathak & Agarwal (1952) found that in bhakur the main fat depot was in the viscera, which contained about 45 % fat. Our results show that the viscera of rohu and nain also serve as the main fat depot, and contain on the average 45 % of fat.

SUMMARY

1. The composition of body and visceral fats from rohu (*Labeo rohita*) and nain (*Cirrhina mrigala*) have been studied. Preliminary separations of the fatty acids into groups differing in unsaturation

were carried out using the lithium and lead salts in acetone and ethanol respectively. Their compositions were studied by the ester-fractionation procedure.

2. The fishes rohu and nain belong to different genera, and significant differences have been found in the proportions and degree of unsaturation of their C_{16} , C_{18} , C_{20} and C_{22} groups of unsaturated acids, and also in the amounts of total saturated acids and of stearic acid.

3. The high saturated acid content of the fats is remarkable, especially in fats from rohu. The increase in the percentage of stearic acid seems to have been at the cost of unsaturated C_{18} acids, and thus may indicate bio-hydrogenation. Small differences in the composition of the fats from body and viscera of the same fish have been observed in both species.

4. The fat content of the viscera of these fish is about 45 %.

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The Organic Constituents of Mammalian Compact Bone

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The inorganic matter which characterizes bone as a tissue has been studied by a large number of workers (Neuman & Neuman, 1953). On the other hand, the material composing the so-called 'organic matrix' has received very little attention.

Collagen, the principal organic constituent of bone, has for a long time been assumed to be similar to the collagen of the softer connective tissues (Huggins, 1937), although hardly anything was known about it, except that under mild hydrolytic conditions, it gave rise to gelatin. Quite recently Neuman (1949), using microbiological methods of analysis, has shown that the amino acid composition of collagen from bone does not differ significantly from that of collagen in other tissues. The same conclusion has been reached by Rogers, Weidmann & Parkinson (1952). Randall, Fraser, Jackson, Martin & North (1952) have examined collagens from a number of tissues, including bone, with the electron microscope, and have found typical non-branching striated fibrils in all specimens.

Hawk & Gies (1901) isolated a mucopolysaccharide complex which they termed 'osseomucoid'

from ox bone, and Seifert & Gies (1904) showed that it was invariably present, as a minor constituent, in the bones of a number of mammalian species. Hisamura (1938) separated osseomucoid into two components, one of which contained a hexosamine and a hexuronic acid and the other a hexosamine and galactose. Two hexosamines, glucosamine and galactosamine, were found in osseomucoid by Masamune, Yosizawa & Maki (1951), who separated them by paper chromatography. Rogers (1949) reported the presence of 0.1–0.25 % of hexosamines and 2–4 % of reducing substances in human bone and later (Rogers, 1951) isolated a substance containing equimolar weights of a hexosamine, a hexuronic acid and the hydrogen sulphate group.

The presence in bone of two proteins, one soluble in cold water, and similar in composition to collagen, and a second which resists solution in hot water, has been shown by Rogers *et al.* (1952).

The citrate content of bone has been extensively examined by Dickens (1941). This ion is generally considered to be intimately associated with the inorganic crystal lattice (Neuman & Neuman, 1953).