

STUDIES ON THE FATTY ACID COMPOSITION OF HILSA OIL

by

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Abstract

With the establishment of a hilsa canning plant at Dacca, it has become necessary to keep hilsa in cold storage for some time. It has been observed that during storage even for a short time, the fish develops a predominantly rancid taint which persists in the canned product.

In order to investigate changes in fat components during frozen storage, a preliminary study has been completed on the fatty acid composition of fresh hilsa oil by argentation chromatography and gas liquid chromatography. The results obtained indicated that the fatty acid chain length of hilsa oil was between C₁₂ to C₂₂ and the degree of unsaturation between 0 to 6 double bonds. The saturated and monounsaturated acids amounted to about 88 percent of the total acids.

The unsaponifiable content and iodine value of the same oil was estimated to be 1.25 and 90 respectively. The total fat content was 18 percent.

INTRODUCTION

The fish fats of marine or freshwater fish contain a large number of fatty acids and their triglycerides are extremely heterogeneous in character (Hilditch and Williams, 1964). The presence of large amounts of polyunsaturated acids of C_{16:4}, C_{18:4}, C_{20:5} and C_{20:6} which have associated with equal amounts of saturated and monounsaturated acids of C_{14:0}, C_{16:0}, C_{16:1}, C_{18:0} and C_{18:0} makes the mixture very complex and studies of their fatty acid composition by conventional methods are very difficult. Moreover, the presence of polyenic acids in fish fats accelerates the rate of autoxidation and, as a result, the fat becomes rancid within a short period (Hilditch and Williams, 1964).

The oil of freshwater fish contains fewer polyenic acids than that of marine fish but the amount of saturated and monounsaturated acids is very high (85-90 percent). In marine fish oils, the polyunsaturated acids are very high (40-50 percent) and are associated with an equal amount of saturated acids (Hilditch and Williams, 1964).

EXPERIMENT

- (a) Raw materials: six hilsa were collected from the local market, minced, dried and the oil extracted by chloroform-methanol mixture (Scorry, 1965).
- (b) The physical and chemical characteristics of the oil were determined by conventional methods.
- (c) The methyl ether of fatty acids of the oil were prepared (Lambertsen, 1972).
- (d) The methyl ether were fractionated by argentation chromatography (De Vries, 1963; Morris, 1966) and total methyl ether were analysed before and after hydrogenation by gas liquid chromatography (James and Martin, 1956; James, 1959), followed by analysis of each fraction obtained with the help of argentation chromatography.
- (e) The methyl ether were hydrogenated and analysed by GLC (Hoque *et al.*, 1973).

RESULTS

Analysis of the total methyl ether of fresh hilsa oil before and after hydrogenation by GLC in two types of column stationary phases (polar and non-polar), followed by the analysis of each fraction obtained after the fractionation with the help of argentation chromatography (column and TLC), permitted the determination of the total fatty acids in hilsa oil as shown in Tables 1 and 2.

Table 1

Fatty acids before hydrogenation by GLC

Fatty acids	Weight (%)	Fatty acids	Weight (%)
C _{12:0}	0.60	C _{18:3}	0.40
C _{14:0}	7.80	C _{18:4}	0.50
C _{15:0}	0.25	C _{20:0}	1.50
C _{16:0}	31.80	C _{20:1}	0.80
C _{16:1}	12.20	C _{20:2}	0.07
C _{16:2}	0.90	C _{20:3}	0.40
C _{16:3}	1.30	C _{20:4}	0.46
C _{18:0}	8.10	C _{20:5}	5.70
C _{18:1}	25.54	C _{22:5}	0.60
C _{18:2}	0.50	C _{22:6}	0.38

Table 2

Fatty acids after hydrogenation

Fatty acids	Polar phase	Non-polar phase
	Weight (%)	Weight (%)
C _{12:0}	0.70	0.60
C _{14:0}	7.80	7.70
C _{15:0}	0.20	0.20
C _{16:0}	46.30	46.40
C _{18:0}	35.00	34.90
C _{20:0}	8.80	8.90
C _{22:0}	1.20	1.30

A comparative study on the fatty acid composition of the fresh and canned hilsa oil after one year showed no significant changes on the fatty acid composition (Table 3).

Table 3

Fatty acids of fresh and canned hilsa oil

Fatty acids	Fresh oil	Canned oil	Fatty acids	Fresh oil	Canned oil
12:0	0.60	0.50	18:3	0.40	0.80
14:0	7.80	7.40	18:4	0.50	0.70
15:0	0.25	0.40	20:0	1.50	1.30
16:0	31.80	31.00	20:1	0.80	0.70
16:1	12.20	11.00	20:2	0.07	0.10
16:2	0.90	1.40	20:3	0.40	0.40
16:3	1.30	2.50	20:4	0.40	0.40
18:0	8.10	7.10	20:5	5.10	6.50
18:1	25.54	23.30	22:5	0.80	0.80
18:2	0.50	1.30	22:6	0.38	0.50

DISCUSSION

The physical and chemical characteristics of the hilsa oil were within normal limits. Five fractions were obtained by argentation chromatography (column and preparative TLC) (De Vries, 1963; Morris, 1966). The analysis of each fraction by GLC was corroborated with the analysis of total methyl ether before and after hydrogenation by gas liquid chromatography (James and Martin, 1956; James, 1959).

The changes in the fatty acid composition in canned fish showed some minor alteration in the fat component. This difference may be due to batch and seasonal variation.

SATURATED ACIDS

The saturated acids made up about 50 percent of the total acids. The acids of chain length of C_{14:0}, C_{16:0} and C_{18:0} were the major acids and those of C_{12:0}, C_{15:0} and C_{20:0} were minor acids. In this regard, freshwater hilsa oil from Bangladesh corresponded with Group IV according to the classification of Tsujimoto (1932) where the unsaponifiable content was lower than 2 percent and there was an exceptionally high proportion of saturated acids (up to 50 percent). These characteristics are not generally found in common marine fish fats (Hilditch and Williams, 1964).

Monoenic acids

The monoenic acids made up about 38 percent of the total acids. The palmitoleic acid and oleic acid were the two significant monoenic acids. The distribution of monoenic acids in hilsa oil also differed from that of marine fish fats where the amount of these two acids was not so prominent (Hilditch and Williams, 1964).

Dienic and trienic acids

In the present study the dienic and trienic acids were of minor importance. In this respect the hilsa fish oil is similar to other marine fish fats (Hilditch and Williams, 1964; Tsujimoto, 1932; Gedam *et al.*, 1971; Graille *et al.*, 1975; Huq *et al.*, 1977).

Polyenic acids

The polyenic acids were significantly lower in hilsa oil in comparison with other fish fats. Among the C²⁰ and C²² series, C_{20:5} was the major acid and others were of minor importance (Table 1), whereas the acids of these two series are highly polyunsaturated and prominent ranging from 4 to 6 double bonds and frequently associated with the acids of C_{16:4} and C_{18:4} (Hilditch and Williams, 1964; Tsujimoto, 1932; Gedam *et al.*, 1971; Graille *et al.*, 1975; Huq *et al.*, 1977).

Odd and iso-acids

Only one odd carbon number acid (C_{15:0}) was found in hilsa oil; no iso-acid was observed. These types of minor acids have been reported in fish oils (Hilditch and Williams, 1964; Graille *et al.*, 1975).

CONCLUSION

The fatty acid composition and the general characteristics of hilsa oil were studied to find out the nature of unsaturation and the chain length. The investigation revealed that the saturated and monounsaturated acids amounted to about 88 percent of the total acids and 7.8 polyenic acids. The dienic and trienic acids were insignificant. The unsaturation varied from 0 to 6 double bonds and the chain length from C₁₂ to C₂₂.

The present study is being extended to investigate (a) the seasonal variation in the fat component and (b) changes in the oil during frozen storage (glazed and unglazed) of these fish to find out (i) the nature of oxidation or autoxidation (ii), the best possible conditions to improve the quality of fish during frozen storage. The work will be extended further to investigate the composition of the unsaponifiable matter.

REFERENCES

- De Vries, B., Separation of triglycerides by column chromatography on silica impregnated with silver nitrate. 1963 *J. Am. Oil Chem. Soc.*, 49:184
- Gedam, P.H., M.R. Subbaram and J.S. Agarwal, Consecutive chromatographic techniques in the component fatty acid analysis of sardine oil. *Fette Sefen Anstrichmitt.*, 73:748 1971
- Graille, J., M.S. Huq and M. Nandet, Etude d'une huile de Sardines du Maroc. *Rev. Fr. Corps Gras*, 23:151 1975
- Hilditch, T.P. and P.N. Williams, The chemical constitution of natural fats. London, Chapman and Hall, pp. 10, 1964 32-48 4th ed.
- Hoque, M., A. Ghose and J. Dutta, Use of argentation column chromatography in the identification of fish oil fatty acids by GLC: application to cod liver oil. *J. Am. Oil Chem. Soc.*, 50:29 1973

- Huq, M.S., M.S. Khan and S.F. Rubbi, Determination of chain length and the degrees of unsaturation of fatty acid composition of Hilsa fish oil by argentation chromatography and gas liquid chromatography. *Bangladesh J.Sci.Ind.Res.*, vol. for 1977 (in press)
- James, A.T., Determination of the degree of unsaturation of long chain fatty acids by gas-liquid chromatography, 1959 *J.Chromatogr.*, 2:552
- James, A.T. and A.J.P. Martin, Analysis of fatty acids by gas-liquid chromatography. *J.Appl.Chem.*, 6:105 1956
- Lambertsen, G., Lipids in fish fillet and liver; a comparison of fatty acid composition. *Fiskeridir. Skr.(Teknol. Unders.)*, 5(6):15 p. 1972
- Morris, L.J., Separations of lipids by silver ion chromatography. *J.Lipid Res.*, 7:717 1966
- Scorry, N.M., Lipids analysis. New York, Interscience Publishers Inc., vol. 11:60-100 1965
- Tsujimoto, M., Liver oils of elasmobranch fish. *J.Soc.Chem.Ind.Jap.*, 51:317T 1932