Fatty Acid Composition of Muscle and Skin Lipids of Oil Sardine

P. G. VISWANATHAN NAIR and M. RAJENDRANATHAN NAIR
Central Institute of Fisheries Technology, Cochin-682 029

Monthly variation in the fatty acid composition of muscle and skin lipids of oil sardine *(Sardinella longiceps)*, for a period of two years are presented. Data shows wide variations in the proportions of the various acids in these lipids during different seasons. Total saturated acids in muscle lipids varied from 33% to 42% with an average of 38%. Total monounsaturated acids averaged 22.2%, lowest value being 14% and highest 30.1%.Proportions of total polyunsaturated acids ranged between 28% and 52.5% (average 40.1%). Similar variations were observed in the case of all major component acids. The pattern was similar in skin lipids also. Skin lipids had a slightly higher level (by about 3%) of total monounsaturated acids and correspondingly lower proportions of polyunsaturated acids.

Fatty acid composition of the lipids of any species of fish is subject to various influences. One of the most important factor is the food available for the fish. Fish have the ability to alter the ingested fatty acids to a pattern characteristic of the species. But this ability is overridden when the availability of fatty acids from foods is in favour of any one or more of these acids. In such cases the acids present in excessive amounts in food may be deposited in the fish as such. Another factor that may influence the fatty acid composition of fish lipids is the spawning cycle. Certain fatty acids may be preferentially utilised during the spawning cycle, thus affecting the overall composition. Thus, the fatty acid composition of any particular species may vary with season, geographical location of the catch etc.

It has been well recognised that intra-species variations in fatty acid composition is as important as inter-species variations (Ueda, 1977). Individual variations in the same species caught from the same location at the same time are substatial and Stansby (1979, 1981) had warned against use of data obtained by the analysis of a few number of fish as representative of the fatty acid pattern of that species. Even commercial oils, each batch produced from a very large number of fish, show wide variations in their fatty acid composition. A detailed study on menhaden oil (Gruger, 1976) had shown that of the 14 different fatty acids, 10 have maximum values of the ranges more than double that of the minimum and in some cases the maximum value is about nine times the minimum value. These data also show that seasonal variation in fatty acid composition is more important than the effects of geographical location of the catch. Analysis of the data for herring oil (Robisch & Gruger, 1968; Ackman & Eaten, 1966) also gives similar results. Stansby (1981) concludes that only in the case of mehaden and herring oils do sufficient data exist to arrive at any reasonable conclusion about the average ranges of the individual fatty acids.

The fatty acid composition of a commercial oil may not give a correct picture that exists in the lipids of edible portions of fish. Phospholipids are not efficiently extracted by the processes employed for production of commercial oil (Ackman *et al.*, 1976) and hence the fatty acid composition of the oil thus obtanied does not represent that of the total lipids. What is important from human nutritional point of view is the fatty acid composition of the total lipids of the edible portions of fish. Use of solvent extraction procedures are essential for this. But then the serious limitation is that of obtaining a truly representative sample. Stansby (1981) had made a number of suggestions to obtain reasonably representative and useful values of fatty acid composition of any species of fish.

Oil sardine *(Sardinella longiceps)* is the single largest fishery on the Kerala-Karnataka coast (South-west coast of India) and it is the one species employed in this part of the country for production of oil on a commercial scale. But a detailed analysis of the fatty acid composition of this fish, either on the commercial oil or on the total lipids, has not so far been conducted. Therefore the extent of variations in the fatty acid composition and the average values of the individual acids are not known. This study was taken up to find out the extent to which the fatty acid composition of the total lipids varies with season and the other factors to establish the average values for the major acids.

Materials and Methods

Fish caught from an area around Cochin from August 1980 to October 1982 were utilised in this study. Sample collection was done in the middle of each month during the above period. Each time about 250 to 300 fish were taken at random from the fish landed by a number of boats in the fishing harbour. Fish were kept fresh in the boat by icing. Skin was

pealed off from the fish along with the subcutaneous layer of fat, if any, for extratcion of lipids. Care was taken to see that no portion of the muscle was included with this. Muscle was collected separately. Muscle and skin samples were minced separately and aliquots from these were used for extraction of lipids.

Lipid extraction was carried out with chloroform-methanol mixture (2:1, v/v) (Bligh & Dyer, 1959). Extraction of lipids and all further steps were carried out in an atmosphere of nitrogen to minimise autoxidation of the polyunsaturated fatty acids. The washed chloroform layer was concentrated to a known volume in vacuum at about 45°C and an aliquot from this was evaporated to dryness (till constant weight) in vacuum at 50°C to determine the lipid content. Rest of the lipid extract was evaporated to dryness under vacuum and stored at –20°C in nitrogen atmosphere pending analysis.

Lipid samples were saponified and the unsaponifiable matter was removed (AOAC, 1975). Free fatty acids were liberated by acidifying with dilute hydrochloric acid and the acids were separated with petroleum ether. The solvent was removed from this and fatty acid methyl esters were prepared by using 14% boron trifluoride in methanol (AOAC, 1975). The methyl esters of fatty acids were analysed on a gas chromatograph (Toshniwal) with dual column and flame ionisation detectors. The column used was stainless steel, 200 cm × 0.6 cm packed with 10% Silar 5 cp on Gaschrom Q 80–100 (both from Applied Science Laboratories, U.S.A.) Peak identification and quantitation was as described in a previous communication (Viswanathan Nair & Gopakumar, 1978). Reference standards of fatty acid methyl esters used were those obtained from Applied Science Laboratories, U.S.A., Sigma, U.S.A. and Kochlight, U.K.

Results and Discussion

Data presented in Table 1 show the variations in the lipid content of muscle and skin of oil sardine. Lipid contents of both muscle and skin vary with season. Maximum lipid content was during October to December and the minimum was during April to June. These results are in agreement with an earlier report (Gopakumar, 1974) where data for four years had been presented. According to this report the minimum lipid content of the fish was 2% and the maximum, about 16% of the fresh tissue. However, in the present case muscle and skin were studied separately and it was seen that the difference between the minimum and maximum was much greater in skin lipids when compared to muscle lipids. Lipid content of mucle varied from about 2 to 12% (on wet weight basis) while that of skin varied from about 2 to 47%. When the lipid levels were at the lowest, muscle and skin did not differ very much in their lipid content. As the build up of lipids in the tissues progresses, the difference become wider and at the maximum lipid content, the differences are also maximum. Increase in the lipid content of whole fish is mainly due to the build up of the subcutaneous layer of depot fat.

Table 1. *Lipid content of muscle and skin of oil sardine samples from August* 1980 *to June* 1982

Date of sampling	Lipid content % of fresh tissue	
	Muscle	Skin
August 1980	6.0	18.5
September	9.0	46.7
October	11.7	40.4
November	8.5	36.5
December	10.4	29.3
January 1981	—	12.6
March	6.6	11·0
April	3.0	10.7
May	2.8	5.0
June	1.8	2.4
July	7.4	16.6
September	10.2	41.3
October	10.8	46.5
January 1982	7.5	18.3
February	—	13.7
March	6.5	16.8
April	3.8	10.5
May	2.1	2.8
June	3.5	6.4

Available reports on the fatty acid composition of oil sardine (Gopakumar & Rajendranathan Nair, 1966; Venkateswara Rao & Gedam, 1975), show wide variations in the fatty acid composition. Fatty acid composition of the fish samples analysed during the present study are reported in Tables 2 and 3. It can be seen that the proportions of the individual acids vary considerably from sample to sample. The ranges are quite wide in certain cases. Although the lipid content of muscle and skin follow a well defined pattern of changes during the year, any such regular change in the proportion of the various fatty acids was not obvious from the available data. Only in the case of C 22:6 acid the changes could be correlated to some extent with the changes in lipid content. In muscle, the proportion of this acid was found to be low (about 7%) during the months of October-November, when the lipid content was the maximum. During May-June the lipid content was the minimum and the proportion of C 22:6 was the maximum (about 20%). Except for these, there was no indication of a clear relation between the content of any fatty acid and the total lipid content or the season. Therefore, what we have attempted in the present study is to establish the average ranges for the individual acids.

Data in Tables 3 and 4 show that variations in the proportion of individual acids is quite large. These differences are, in many cases much larger than

Table 2. *Fatty acid pattern of muscle lipids of oil sardine showing the mean values, minimum and maximum of the range and 95% confidence interval values (in weight percentage)**

Fatty acids	Range Min.	Max.	Mean ± SD	95% confidence interval values	
10:0 and lower	0.2	1.7	0.6 ± 0.4	0.41	0.75
12:0	0.1	0.4	0.2 ± 0.1	0.17	0.25
13:0	0.1	0.3	0.16 ± 0.07	0.12	0.20
14:0	6.1	11.6	8.8 ± 1.5	8.17	9.49
15:0	0.6	1.5	0.9 ± 0.2	0.79	1.03
16:0	11.9	22.9	18.3 ± 3.4	16.84	19.82
17:0	1.0	4.6	2.8 ± 0.9	1.79	3.31
18:0	5.0	11.2	6.9 ± 1.2	6.40	7.48
14:1	0.2	1.5	0.5 ± 0.3	0.39	0.69
16:1	6.4	15.3	10.6 ± 2.5	9.45	11.69
18:1	6.4	13.8	9.7 ± 2.1	8.73	10.57
20:1	0.3	3.8	1.3 ± 0.9	0.89	1.75
16:2	1.8	5.6	3.7 ± 1.0	3.14	4.16
18:2	1.6	5.3	2.9 ± 0.9	2.53	3.29
18:3	0.3	2.6	1.1 ± 0.6	0.77	1.33
18:4	1.3	5.9	3.0 ± 1.0	2.55	3.43
20:4	1.4	4.5	2.8 ± 1.0	2.37	3.25
20:5	9.2	17.2	12.4 ± 2.1	11.46	13.36
22:3	0.5	1.6	0.7 ± 0.4	0.50	0.88
22:4	0.8	2.9	1.3 ± 0.6	1.01	1.53
22:6	6.6	20.9	11.6 ± 3.7	9.94	13.20
Total saturated acids	33.1	41.5	37.7 ± 2.50	36.63	38.85
Total monoenes	14.3	30.1	22.2 ± 4.44	20.19	24.13
Total polyunsaturates	28.7	52.5	40.1 ± 5.36	37.76	42.52

*Small quantities (0.5%) of the following acids were found only in a few of the samples, but not included in the Table, 13:1, 15:1, 22:1, 20:2, 20:3, 22:2, and 22:5

Table 3. *Fatty acid pattern of skin lipids of oil sardine showing the mean values, minimum and maximum of the range and 95% confidence interval values (in weight percentages)**

Fatty acids	Range Min.	Max.	Mean ± SD	95% confidence interval values	
10:0 and lower	0.2	2.0	0.51 ± 0.29	0.37	0.65
12:0	0.1	0.5	0.3 ± 0.1	0.20	0.30
14:0	7.3	12.8	9.9 ± 1.5	9.06	10.70
15:0	0.6	1.2	0.9 ± 0.2	0.77	0.95
16:0	13.5	23.2	18.4 ± 2.8	17.10	19.60
17:0	1.1	5.2	2.9 ± 1.0	2.43	3.42
18:0	5.3	8.8	6.9 ± 0.9	6.46	7.24
14:1	0.3	1.4	0.6 ± 0.4	0.43	0.81
16:1	7.8	17.6	11.4 ± 2.3	10.37	12.45
18:1	7.0	13.3	11.0 ± 2.2	9.98	11.94
20:1	0.5	3.1	1.4 ± 1.0	0.94	1.88
16:2	2.1	5.2	3.6 ± 0.8	3.20	3.99
18:2	2.2	4.9	2.9 ± 0.8	2.54	3.28
18:3	0.1	2.1	0.9 ± 0.7	0.58	1.20
18:4	1.4	5.7	3.3 ± 1.0	2.84	3.70
20:2	0.2	2.4	0.8 ± 0.6	0.50	1.08
20:4	1.0	5.8	2.7 ± 1.0	2.20	3.10
20:5	7.6	20.9	12.7 ± 2.9	11.37	14.01
22:4	0.4	2.0	1.1 ± 0.5	0.87	1.31
22:6	4.9	17.3	8.1 ± 3.1	6.72	9.56
Total saturated acids	31.6	42.5	38.8 ± 2.6	37.62	39.96
Total monoenes	15.1	34.5	24.3 ± 4.6	22.24	26.44
Total polyunsaturated acids	31.3	47.5	36.8 ± 4.4	34.79	38.83

*Small quantities (0.5%) of the following acids were present in some of the samples, but not included in the Table, 13:0, 13:1, 15:1, 20:3, 22:2, 22:3, and 22:5

those pointed by Stansby (1981) for commercial menhaden and herring oils and this might be due to the limitations in sampling inherent in this type of a study. But another important point to be noted is that the 95% confidence interval values for the major acids fall within a narrow range. This means that the extreme variations in fatty acid compositions are found only rarely and most of the time the values are close to the average value. This was found to be true for the skin lipids also.

It is interesting to note that the wide seasonal variations in the lipid contents of muscle and skin do not affect their fatty acid compositions to any appreciable extent. This might be due to several factors. It is known that the phospholipid content of fish tissues is fairly constant, throughout the year (Ackman & Key, 1969) and the variations in lipid content is almost entirely due to changes in depot fat. The fatty acid pattern of muscle lipids does not show any regular change at any stage, as the lipid content increases (except the C 22:6 acid discussed above). This may be due to the fact that the phospholipids and depot fat in this fish have somewhat similar compositions. The fish used in this study was caught from a limited area and hence the effect of geographical location on the fatty acid composition may be minimum. Therefore, the variations observed may be due to the nature of food ingested by the fish.

A comparison of the fatty acid compositions of muscle and skin lipids of oil sardine show that only marginal differences exist between the two. Skin lipids have a low level of C 22:6 acid when compared the muscle lipids and total polyunsaturated acids was

slightly lower in skin lipids (36.81 ± 0.44% in skin lipids and 40.14 ± 5.36% in muscle lipids). The close similarity between the skin and muscle lipids in their fatty acid compositions strengthens our earlier assumption that the depot fat and structural lipids of oil sardine do not show any marked differences in their fatty acid make up.

From the results of this investigation we may conclude that the fatty acid composition of the lipids of oil sardine show considerable intraspecies variations. In a few cases these variations are fairly wide, but statistical analysis shows that the proportion of the individual acids, in a great majority of cases, fall within a reasonably narrow range. Monthly data for two years have not revealed any seasonal trend in the variations of the fatty acid compositions, and it is not clear whether this effect was marked by the scattering of values due to the limitations in sampling techniques. Efforts are on to establish this point. Finally the skin and adipose lipids, which constitute the main depot fat for this fish, was found to have a fatty acid pattern which was only marginally different from the body lipids and the variations in the fatty acid composition of this lipid also were similar to that in the muscle lipids.

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