J. Lipid Research, January, 1962
Volume 3, Number 1

The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet

Reuben Lasker and Gail H. Theilacker

U.S. Department of the Interior,
Bureau of Commercial Fisheries, Biological Laboratory,
La Jolla, California

[Received for publication March 31, 1961]

SUMMARY

Gas-liquid chromatographic analyses of the C_{12} to C_{22} saturated and unsaturated fatty acids of lipids from Pacific sardine ovaries, mesenteric fat, whole blood, and planktonic eggs are presented and compared. Lipids from planktonic eggs and ovaries, with ova in different stages of maturation, have strikingly similar and constant fatty acid compositions. Changes are shown to occur in the fatty acid pattern of mesenteric fat, blood, and ovarian lipids when sardines are fed an artificial diet rich in oleic and linoleic acids. Lipids from sardines fed chiefly on a natural crustacean diet were analyzed for comparison. In general, whole blood lipids have a fatty acid composition similar to the ovary; mesenteric fat differs from ovarian and blood lipids by having a lower content of linoleic acid.

In the Pacific sardine, *Sardinops caerulea* Girard, the gonads increase greatly in weight and size preparatory to spawning. Each ripening egg contains a small (0.2 mm diameter) oil droplet. After the eggs are spawned into sea water and during embryological development, the larvae consume the oil and the proteinaceous yolk. An active synthesis and turnover of organic constituents seem likely during ovarian deposition of this oil. Although the complex mixture of fatty acids in fish fats seems to reflect the composition of the fat in the food eaten (1), fishes occasionally alter the fatty acid composition of their fat by virtue of their own metabolism (2).

The purpose of this study was to investigate the changes occurring in the fatty acid composition of lipid during its deposition in the maturing sardine ovary. Because diet usually alters the fatty acid composition of fish lipids, an artificial diet was fed to sardines and its effect on mesenteric fat, blood, and ovarian lipids was examined.

EXPERIMENTAL PROCEDURE

Saponification and Methylation. Approximately 0.6 g of sardine ovary was added to a 15-ml centrifuge tube containing 5 ml 2.5 N KOH in 85% methanol. The tube contents were occasionally stirred and kept at 75° in a waterbath. After cooling, the nonsaponifiable materials were extracted with three successive washes of 2 ml petroleum ether. Washes were conveniently removed with long-needled hypodermic syringes. The sample was acidified with 3 ml 5 N H_2SO_4. The fatty acids were removed with three 2-ml washes of petroleum ether. After the combined petroleum ether extracts were evaporated almost to dryness, 2 ml of 1% H_2SO_4 in methanol was added and the mixture kept at 75° for 1 hour to complete methylation. The volume was then reduced to about one-fifth of the original, water was added, and the methyl esters were extracted with three 2-ml rinses of petroleum ether. In all operations, mild centrifugation was used to effect sharp interfaces between immiscible solvents. The entire experimental procedure was performed under nitrogen.

Hydrogenation. Complete hydrogenation of the unsaturated fatty acids of sardine mesenteric fat was accomplished by the method of Farquhar *et al.* (3) in a Warburg manometer as suggested by Mead and Howton (4). This method utilizes the Warburg manometer for visualizing the hydrogenation reaction and its completion. Positive identification of chain length for each saturated and unsaturated fatty acid was

possible by gas-liquid chromatography (GLC) after hydrogenation.

Extractions. We found that extraction of sardine tissues with fat solvents such as chloroform-methanol, 2:1 (v/v), petroleum ether, or diethyl ether, was time-consuming and incomplete, particularly when complete homogenization of tissue was not possible. The extractions effected by these methods, however, although incomplete, showed upon analysis the same relative proportions of fatty acids as did the KOH digestion procedure described above.

Gas-Liquid Chromatography. An Aerograph® gas-liquid chromatograph[1] was used to separate and identify the fatty acids. Two columns were used, with helium as the mobile phase: a butanediol-succinate column, which gave excellent separation of the longer chain unsaturated methyl esters, and a silicone-Dow 11 column, which gave precise chain-length information. Columns consisted of 20% stationary phase coated on 60 to 80 mesh firebrick and were 5 feet in length. Helium flow was 35 ml per minute at 204°. Samples were dissolved in 2 to 10 μl hexane for injection.

The logarithmic retention times of sardine fatty acid methyl esters were compared with those of known mixtures run on the same column under the same conditions prior to analysis of a sample. Chain length was confirmed with GLC by a plot of the logarithmic retention time of a completely hydrogenated sample. In all cases, retention times matched those of purified fatty acid methyl ester standards: laurate (12:0), myristate (14:0), palmitate (16:0), stearate (18:0), arachidonate (20:4), eicosapentaenoate (20:5), and docosahexaenoate (22:6).[2] A chromatogram of sardine planktonic egg fatty acids is shown in Figure 1. Quantitative estimates of individual fatty acids were obtained by the triangulation method described by Farquhar *et al.* (3).

Ovarian Development. The stage of ovarian development was determined for each ovary by microscopic examination and measurement of the ova. The yolk diameter of the largest eggs was recorded. Thus, a series of ovaries having eggs with yolk diameters ranging from 0.5 mm in diameter to 1.04 mm in diameter was obtained and analyzed for fatty acids. The ova of one ovary appeared to be in the process of degeneration or resorption. The eggs were smaller than 0.4 mm in diameter; some contained yolk, but the yolk was without the smooth granular appearance common to developing ova.

[1] Wilkins Corp., Walnut Creek, California.

[2] Obtained from the Hormel Institute, University of Minnesota, Austin, Minnesota.

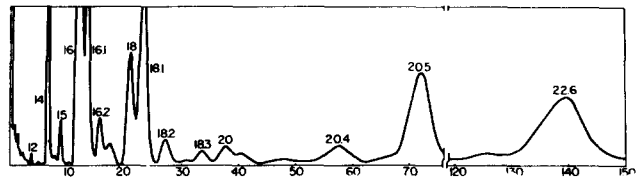

FIG. 1. Gas-liquid chromatogram of saturated and unsaturated methyl esters obtained from sardine planktonic eggs. Ten-minute intervals are indicated on the abscissa. Five-foot column of 20% butanediol-succinate, coated on 60 to 80 mesh firebrick; helium flow, 35 ml per minute; temperature, 204°.

RESULTS

Fatty Acids of the Sardine Ovary and Fertilized Eggs. Sardine ovaries in any stage of maturation, and even in a possible state of resorption, present strikingly similar fatty acid patterns (Table 1). The one ovary identified as "resorbing" showed no significant difference in any fatty acid constituent from any other ovary tested.

It was important to ascertain whether the oil in the individual eggs was similar to, or different from, that found by examining whole ovaries. Newly spawned and fertilized eggs were separated manually from plankton hauls and analyzed for fatty acids. The results are also shown in Table 1. The oil in planktonic sardine eggs is very similar in all components to ovarian lipid in any stage of ova development.

Effect of Diet. Lovern (5) presented convincing evidence that the fat of ingested copepods is deposited largely unchanged by herring. Hand and Berner (6) found that the diet of the adult Pacific sardine is comprised largely (over 80%) of small copepods. A comparison of the fat of *Calanus helgolandicus*, taken in a net haul off San Diego, with the average fatty acid composition of lipids found in sardine ovaries (Tables 1 and 2), shows that there is a great similarity in percentage composition between most of the fatty acid constituents, although some differences exist in the myristic and stearic components. *Calanus* fat serves as an illustration of the kind of fat ingested by sardines in nature. The close correspondence of the fatty acid constituents of the sardine ovarian lipid to those of *Calanus* is good evidence that deposition of dietary fat probably proceeds in the sardine, as in the herring, without major alteration; nor does the fat become altered significantly by the special production of oil for the eggs. Blood taken from a San Diego Bay sardine and from one caught off Santa Barbara, California (Table 3), showed much the same fatty acid composition as did the gonads. This suggests that mobilization of fats from the intestines to the ovaries is relatively direct.

TABLE 1. PERCENTAGE FATTY ACID COMPOSITION OF SARDINE OVARIES AND PLANKTONIC EGGS*

Whole Ovaries	14:0	†	15:0	†	16:0	16:1	16:2	†	18:0	18:1	18:2	18:3	18:4‡	20:0	20:4	20:5	21:0‡	22:5	22:6
1. 0.5 mm§	4.6	0.0	0.6	0.0	33.0	7.2	1.4	1.0	5.0	9.6	1.5	0.7	0.3	1.2	1.2	15.4	0.0	0.0	17.4
2. 0.7 mm	5.3	0.3	0.9	0.1	39.6	9.1	1.8	0.9	4.7	9.4	1.1	0.6	0.4	0.9	1.3	9.6	0.0	1.6	12.3
3. 0.7 mm	4.8	0.2	0.8	0.2	39.0	8.9	2.0	1.0	4.8	11.2	0.8	0.4	0.3	0.5	1.5	10.2	0.0	2.4	11.0
4. 0.8 mm	4.1	0.2	0.9	0.2	40.6	8.8	2.1	1.3	4.8	10.2	0.9	0.2	0.5	0.8	1.3	9.8	0.0	0.5	12.7
5. 1.04 mm	3.6	0.3	0.7	0.1	37.6	7.1	1.5	0.6	4.9	10.4	0.8	0.5	0.4	1.2	1.0	13.4	0.0	1.2	14.8
6. 1.04 mm	4.8	0.2	0.7	0.1	38.5	7.0	1.7	0.6	5.1	9.9	1.3	0.5	0.4	1.6	1.5	13.0	0.0	1.6	11.6
7. Planktonic sardine eggs	3.8	0.3	0.8	0.1	36.4	6.7	1.7	0.9	5.0	9.5	1.3	0.7	0.4	1.2	2.2	12.7	0.2	1.2	15.0
8. "Resorbing" ovary	3.9	0.3	0.9	0.4	32.8	8.4	2.2	1.0	5.5	9.6	0.9	0.4	0.2	0.7	1.9	13.6	0.0	0.0	17.2
Average (ovaries & eggs)	4.4	0.2	0.8	0.2	37.2	7.9	1.8	0.9	5.0	10.0	1.1	0.5	0.4	1.0	1.5	12.2	0.1	1.1	14.0

* Acids present in concentrations of 0.1% or less have been omitted.
† Denotes unidentified fatty acid.
‡ Tentative identification.
§ Diameter of largest yolk.

A test of the effect of diet on the composition of ovarian fat, mesenteric fat, and blood fat of the sardine was made. Depot fatty acids are known to vary with diet in some fish (7, 8, 9). Adult sardines were fed trout food[3] exclusively for three months. Sea water, however, was pumped directly into the sardine holding tank, and it is possible that planktonic food was present. The amount of trout food fed daily was enough to cram the stomachs of the fish, and it is concluded that most, if not all, of the food of these fishes was trout food.

Trout food is very rich in oleic (18:1) and linoleic (18:2) acids but poor in stearic (18:0) and the unsaturated eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids. The complete analysis is given in Table 2.

In trout food, 18:2 is in much greater relative quantity than in any sardine tissue tested. The mesenteric fat laid down by sardines fed on trout food contained at least 3 times the quantity of 18:2 usually found in sardines feeding in the sea. Marked reductions in 20:5 and 22:6 occurred in the ovaries, mesenteric fat, and blood, and are correlated with the low quantity of these substances found in the food. These results are summarized in Table 4.

One consistent difference between mesenteric fat and ovarian fat is that the former contains a high quantity of 18:1 regardless of diet, while the latter seems to respond to the diet (Table 4).

The high resolving power of GLC permitted the identification of most of the saturated and unsaturated fatty acids of the sardine. The occurrence of odd-numbered carbon fatty acids has been demonstrated in menhaden (10, 11) but not previously in sardine tissues. Pentadecanoic acid (15:0) appeared in small quantities in every sardine tissue we tested. Heneicosanoic (21:0) was tentatively identified in planktonic eggs, mesenteric fat, and blood.

A number of fatty acid constituents appeared in small quantities and are as yet unidentified. Three peaks appeared in trace amounts on the chromatograms after 12:0 and are probably C_{12} unsaturated fatty acids judging from their positions and disappearance after hydrogenation. A peak after 14:0 was also probably unsaturated. One C_{18} unsaturated compound, making up less than 1% of the total fatty acids, was tentatively identified as 18:4. The substances appearing after 15:0 and 16:2 could not be hydrogenated.

Stoffel and Ahrens (11) reported 16:3 and 16:4 in menhaden body oil as 1.3% and 2.0%, respectively, of the total fatty acids. We could not separate these acids from the others in sardine lipids, because they are probably masked by the stearate and oleate peaks. Where stearate is low (e.g., in *Calanus* lipid, Table 2) a significant error may be introduced in the reported quantity of stearate. Our conclusions, however, remain unaffected by this possible source of error.

DISCUSSION

Brocklesby and Harding (12) published an analysis of oil obtained as a commercial product from Pacific sardines. Our results differ in certain important respects from theirs. They found a considerable amount (15.2%) of C_{24} unsaturated acid in British Columbia sardines. We have never observed this

[3] Rangens' complete fingerling food.® Rangens' Inc., Buhl, Idaho.

TABLE 2. PERCENTAGE FATTY ACID COMPOSITION OF THE COPEPOD *Calanus helgolandicus* AND TROUT FOOD*

	12:0	14:0	†	15:0	†	16:0	16:1	16:2	†	18:0	18:1	18:2	18:3	18:4‡	20:0	20:4	20:5	21:0‡	22:5	22:6
Calanus helgolandicus	0.2	10.7	0.9	0.8	0.2	34.6	8.2	0.5	0.2	1.6	7.3	1.1	0.5	1.3	1.7	0.3	14.3	0.2	0.0	15.4
Trout food	0.2	4.3	0.2	0.3	0.1	27.1	3.4	1.0	0.8	2.9	25.2	26.1	1.8	0.0	2.2	0.0	2.0	0.0	0.0	0.8

* Acids present in concentrations of 0.1% or less have been omitted.
† Denotes unidentified fatty acid.
‡ Tentative identification.

TABLE 3. PERCENTAGE FATTY ACID COMPOSITION OF SARDINE MESENTERIC FAT AND WHOLE BLOOD*

Fish No.	Tissue Analyzed	12:0	14:0	†	15:0	†	16:0	16:1	16:2	†	18:0	18:1	18:2	18:3	18:4‡	20:0	20:4	20:5	21:0‡	22:5	22:6
1.	Fat ♀	0.1	6.1	0.4	0.6	0.1	28.0	4.9	2.3	1.3	5.4	20.3	2.3	2.0	2.1	3.8	0.1	10.9	1.2	1.4	6.9
2.	Fat ♂	1.0	3.4	0.5	0.6	0.1	27.3	10.9	1.4	0.6	3.5	32.1	0.5	0.3	0.3	1.0	0.2	8.5	0.0	0.6	7.4
3.	Fat ♀	0.1	6.6	0.1	0.4	0.0	32.2	5.0	2.3	1.2	8.1	18.4	1.0	0.7	2.8	1.7	0.2	10.3	0.7	2.2	6.2
4.	Whole Blood ♀§	0.1	3.8	0.1	0.4	0.1	36.7	5.0	1.6	0.6	5.0	8.8	0.4	0.4	0.3	1.8	0.9	13.2	0.6	1.1	19.5
5.	Whole Blood‖	0.4	4.9	0.1	0.5	0.1	30.7	5.6	2.3	0.1	4.6	6.7	0.6	0.1	0.5	1.6	1.5	20.2	0.0	0.0	19.6

* Acids present in concentrations of 0.1% or less have been omitted.
† Denotes unidentified fatty acid.
‡ Tentative identification.
§ San Diego fish.
‖ Sex unknown, Santa Barbara fish.

fatty acid in our sardines. Their report of 14.4% of palmitic acid should be contrasted with our analyses showing an average quantity of 37% for sardine ovaries. However, Hart and Wailes (13) have shown that sardines taken in British Columbia feed chiefly on diatoms during the summer. This may have influenced the fatty acid composition of the fish.

Our sardines were selected at random from those collected by bait boats in the San Diego area. In any single school a large variation can exist in the degree of gonad maturation and in the amount of mesenteric fat present. The ovaries analyzed were selected according to maximum egg size to provide a complete range of maturation. No systematic difference in any fatty acid was discovered as ovary maturation proceeds; all the ovaries tested had remarkably consistent fatty acid patterns. This consistency may be the result of a similarity in diet in all these fishes and serves to emphasize the small metabolic control exerted over fatty acid deposition in the ovary.

The difference found in 18:1 in the mesenteric fat when compared to ovarian fat could be the result of a metabolic emphasis on storage of fatty acids in C_{18} form. If so, this may mean that mesenteric fat does not contribute to the fat of the ovary directly but is used chiefly for energy by the fish. Lovern (14, 15) found that changes appear in salmon-egg oil as the eggs develop and that, as ripening proceeds, there is a marked rise in the proportion of C_{18} acids, while, at the same time, unsaturation decreases. This change is in marked contrast to the present findings.

TABLE 4. PERCENTAGE COMPOSITION OF FOUR FATTY ACIDS IN INDIVIDUAL SARDINES AS RELATED TO DIET

Tissue	Conditions	18:1	18:2	20:5	22:6
Mesenteric fat ♀	Crustacean-fed	20.3	2.3	10.9	6.9
Mesenteric fat ♂	Crustacean-fed	32.1	0.5	8.5	7.4
Mesenteric fat ♀	Crustacean-fed	18.4	1.0	10.3	6.2
Mesenteric fat ♀	Trout food-fed	32.8	11.1	1.5	0.8
Mesenteric fat ♀	Trout food-fed	22.6	8.1	7.3	1.4
Ovary*	Crustacean-fed	10.0	1.1	12.2	14.0
Ovary	Trout food-fed	20.3	3.7	7.2	11.4
Ovary	Trout food-fed	21.1	7.1	8.6	6.4
Blood ♀	Crustacean-fed	8.8	0.4	13.2	19.5
Blood ♀	Trout food-fed	13.7	4.2	4.1	1.8
Calanus		7.3	1.1	14.3	15.4
Trout food		25.2	26.1	2.0	0.8

* Average of all ovaries listed in Table 1.

The authors are indebted to Mr. James Kittredge for his interest and advice during the progress of this work.

REFERENCES

1. Bailey, B. E. *Fisheries Research Board Can. Bull.* **89**: 3, 1952.
2. Lovern, J. A. *Biochem. Soc. Symposia* (*Cambridge, England*) No. **6**: 49, 1951.
3. Farquhar, J. W., W. Insull, Jr., P. Rosen, W. Stoffel, and E. H. Ahrens, Jr. *Nutrition Revs.* **17**: No. 18, Part II (Suppl.) 1959.
4. Mead, J. F., and D. R. Howton. *Anal. Chem.* **22**: 1204, 1950.
5. Lovern, J. A. *Biochem. J.* **29**: 847, 1935.
6. Hand, C. H., and L. Berner, Jr. *U.S. Fish Wildlife Serv. Fishery Bull.* **60**: 175, 1959.
7. Kelly, P. B., R. Reiser, and D. W. Hood. *J. Am. Oil Chemists' Soc.* **35**: 189, 1958.
8. Kelly, P. B., R. Reiser, and D. W. Hood. *J. Am. Oil Chemists' Soc.* **35**: 503, 1958.
9. Mead, J. F., M. Kayama, and R. Reiser. *J. Am. Oil Chemists' Soc.* **37**: 438, 1960.
10. Gellerman, J. L., and H. Schlenk. *Experientia* **15**: 387, 1959.
11. Stoffel, W., and E. H. Ahrens, Jr. *J. Lipid Research* **1**: 139, 1960.
12. Brocklesby, H. N., and K. F. Harding. *J. Fisheries Research Board Can.* **4**: 59, 1938.
13. Hart, J. L., and G. H. Wailes. *Biol. Board. Can.* **7**: 247, 1931.
14. Lovern, J. A. *Biochem. J.* **28**: 1955, 1934.
15. Lovern, J. A. *Biochem. J.* **30**: 20, 1936.