CCXL. NEW ZEALAND FISH OILS
III. THE COMPOSITION OF THE DEPOT FATS OF THE LING (*GENYPTERUS BLACODES*)

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THE available data on marine animal fats indicate that the composition of the fatty acids of a given species varies according to the depot from which the samples were taken [cf. Hilditch & Lovern, 1928; Lovern, 1934; 1937; Shorland & Hilditch, 1938]. Lovern [1934] suggested that one of the mechanisms controlling selective deposition of fat depends on molecular size and is probably concerned with molecular filtration, whereby acids of lower molecular weight are permitted to enter all depots with equal facility while those of higher molecular weight are not so readily admitted to the less permeable depots. Whatever the mechanism of selective deposition, the data so far obtained generally show a correlation between the size and fat content of the depot of a given species and the composition of the fatty acids. In this connexion the ling is especially interesting as the fat is concentrated almost entirely in the liver [Shorland, 1937], the other depots being presumably relatively impermeable and therefore likely to show to a marked degree the effects of selective deposition of the fatty acids.

It has been shown [cf. especially Harper & Hilditch, 1937; Hilditch & Terleski, 1937; Lovern, 1938] that the composition of oil from a given species may vary considerably according to locality and season. The analysis of one sample only may not therefore give a satisfactory picture of the general composition of a particular depot fat within a given species. In this investigation 7 samples of ling liver oil, collected at different periods from Cook Strait, and the viscera and roe lipins previously described [Shorland, 1938] were analysed by the ester fractionation method. The general characteristics of these oils and also of a specimen of oil extracted from a whiptail (*Macruronus novae zelandiae*), the chief source of food of the ling, are given in Table I.

The liver oils (1–3) were analysed according to the procedure of Guha *et al.* [1930], but for the remaining samples advantage was taken of the improved technique of Harper *et al.* [1937] whose methods of calculation were followed throughout. 100–300 g. oil were taken for ester fractionation wherever possible. In the case of the viscera and roe lipins, however, 5–8 g. only of the sample were available, and the esters were fractionated in a Vigreux column (26 × 1 cm.) with a bulb capacity of 20 ml. The column was lagged with asbestos string and heated in a glycerol bath contained in a wide test tube (20 × 5 cm.). As the distillation proceeded the column was suitably lowered into the bath to facilitate fractionation of the higher boiling point esters. The iodine values and saponification equivalents of the fractions obtained with this column were determined by standard methods using a 10 ml. microburette and reduced amounts of reagent. In Table II the efficiency of this column is compared with that of a 250 ml. Willstätter flask packed as described by Hilditch & Houlbrooke [1928].

Table I. *General characteristics of ling* (Genypterus blacodes) *lipins*

Depot	Date of catch	Mean wt. tissue per fish g.	Lipid %	No. of specimens	Blue value	Sap. equiv.	I.V. (Wijs 1 hr.)	Unsaponifiable matter %	P %	Free fatty acid (as oleic acid) %
Liver (1)	31. i. 35	453		17	780	301·9	142·9	3·05		—
(2)	6. vi. 35	580		94	430	303·0	136·5	3·37		—
(3)	9. vii. 34	525		36	420	304·6	142·0	3·10		0·31
(4)	28. vii. 38	—	35–40	(Bulk sample)	410	302·0	146·6	2·95	Trace only	—
(5)	2. viii. 35	648		28	230	300·9	142·2	2·11		—
(6)	22. viii. 35	942		25	320	303·9	139·8	2·23		—
(7)	4. ix. 35	—		(Bulk sample)	420	306·8	142·6	2·87		—
Viscera (excluding liver)	2. vii. 35	1210	0·6	2	820	357·2	178·8	18·2	0·14	34·9
Roe glyceride	28. ix. 35	566	0·5	4	No test	387·2	169·6	18·3		—
Roe phosphatide	28. ix. 35		0·4			302·1	150·2		3·74	
Whiptail (whole fish)	2. vii. 35	1246	0·7	1	75	303·0	143·6	4·79	0·047	3·0

Table II. *Comparative distillation data for methyl esters of "liquids" of ling liver oil 5*

	Willstätter bulb				Semi-micro column			
	Wt. g.	B.P. (0·1 mm.)	S.E.	I.V.	Wt. g.	B.P. (0·1 mm.)	S.E.	I.V.
L 1	4·73	103/129°	275·1	77·2	1·20	75/109°	277·4	88·7
L 2	3·83	129/130°	287·0	103·5	1·13	109/117°	286·4	99·2
L 3	3·83	130/132°	294·2	117·3	1·55	117/130°	299·1	129·6
L 4	4·06	132/137°	297·6	132·3	0·79	130/134°	302·6	159·4
L 5	4·05	137/145°	306·4	183·2	1·09	134/142°	324·2	264·5
L 6	5·46	145/155°	325·8	277·0	0·37	142°/falling	331·6	319·5
L 7	4·20	—	350·5	315·8	0·80	—	351·6	308·5
	30·16		(332·2)*		6·93		(332·2)*	

* Saponification equivalents of residual esters freed from unsaponifiable matter.

The "solids" were not fractionated, but for the purposes of comparison the composition was ascertained by attributing the small iodine value of the "solids" (3·6) to oleic acid and expressing the results in terms of saturated and component unsaturated acids.

Table III. *Effect of method of fractionation on the calculated composition of ling liver oil 5*

	Fatty acids (wt. %)				
		Unsaturated			
Method	Saturated	C_{16}	C_{18}	C_{20}	C_{22}
Simple fractionation by semi-micro column	20·9	9·1 (2·0)*	37·3 (2·8)	23·0 (6·0)	9·7 (10·8)
Simple fractionation by Willstätter bulb	21·4	8·4 (2·0)	37·5 (2·8)	22·9 (6·2)	9·8 (10·0)
Detailed fractionation according to procedure of Harper *et al.* [1937]	21·4	8·9 (2·0)	35·4 (2·8)	25·4 (5·5)	8·9 (10·0)

* Figures in brackets indicate mean unsaturation expressed in terms of hydrogen.

Although it is not possible to obtain a precise estimate of the accuracy of simple fractionation for a complex oil, the data given in Table III suggest that a single distillation by means of the Willstätter bulb or by the semi-micro column gives approximately the same result as the more detailed fractionation.

To test the accuracy of the macro-fractionation process sample 2, after analysis by the usual procedure, was hydrogenated (I.V. 5·8) and again analysed by detailed fractionation using a Willstätter bulb. The analysis of another sample, no. 4, was checked by distillation of the "liquids" in a heated and packed column of the kind described by Longenecker [1937], after fractionation by the usual method employing a Willstätter bulb. As an illustration of the method used, the latter fractionation data are given in Table IV.

Table IV. *Comparison of fractionation data obtained by use of the heated and packed column and by the Willstätter bulb*

Heated and packed column

	Wt. g.	B.P. (0·1 mm.)	S.E.	I.V.
L 1	1·81	110/111°	266·2	51·1
L 2	2·00	111/140°	277·7	72·2
L 3	3·08	140/146°	288·6	87·1
L 4	2·57	146/147°	295·6	93·1
L 5	4·97	147/149°	297·8	117·1
*L 6	3·69	149/156°	304·9	142·8
*L 7	4·94	156/166°	328·0	221·9
*L 8	3·04	166/176°	345·2	314·6
*L 9	2·68	—	495·3	206·0

* Sap. equiv. of esters freed from unsaponifiable matter: L 6 304·9, L 7 322·5, L 8 339·6, L 9 340·9.

Willstätter bulb

	Wt. g.	B.P. (0·1 mm.)		Wt. g.	B.P. (0·1 mm.)	S.E.	I.V.
L 1	15·43	132/145°	L 11	2·84	125/130°	269·5	59·5
			L 12	3·22	130/132°	274·0	76·3
			L 13	2·97	132/133°	282·4	87·8
			L 14	6·38	—	297·1	114·6
L 2	9·25	145/152°	L 21	2·15	125/128°	284·5	91·9
			L 22	2·59	128/135°	288·9	97·4
			L 23	2·38	135/140°	297·2	114·5
			L 24	1·86	—	308·6	151·7
L 3	13·61	152/155°	L 31	2·82	130/135°	296·8	113·9
			L 32	3·47	135°	298·0	117·7
			L 33	4·48	135/146°	303·2	127·6
			*L 34	2·58	—	315·9	203·8
†L 4	9·03	155/174°	L 41	2·03	138/165°	307·4	147·0
			L 42	2·63	165/174°	315·3	179·4
			L 43	2·05	174°/falling	318·8	206·1
			*L 44	2·16	—	334·9	218·2
†L 5	16·57	—	L 51	2·33	175/182°	327·5	242·4
			L 52	2·18	182/175°	332·0	258·7
			L 53	2·51	175°/falling	336·6	279·8
			*L 54	6·46	—	389·5	261·4

* Sap. equiv. of esters freed from unsaponifiable matter: L 34, 311·0; L 44, 323·8; L 54, 344·6.
† Unsaponifiable matter extracted prior to refractionation.

The phosphorus content of the lipins (cf. Table I) shows the absence of appreciable amount of phosphatide except in the case of the roe lipins. These were submitted to an acetone separation and the resultant glyceride and phosphatide fractions were analysed separately.

Table V. *Composition of ling* (Genypterus blacodes) *fats*

(*a*) Fatty acids (wt. %)

Depot	Date collected	Saturated			Unsaturated			
		C_{14}	C_{16}	C_{18}	C_{16}	C_{18}	C_{20}	C_{22}
Liver (1)	31. i. 35	1·9	16·9	2·6	6·5	34·9	25·1	12·1
					(2·0)	(2·5)	(5·0)	(7·6)
(2)	6. vi. 35	2·2	18·0	1·8	5·5	38·4	25·8	8·3
					(2·0)	(2·6)	(5·4)	(6·9)
(3)	9. vii. 34	1·3	15·8	1·2	7·8	37·6	24·4	11·9
					(2·0)	(2·4)	(4·9)	(8·8)
(4)	28. vii. 38	0·7	15·8	2·6	6·6	35·5	23·7	15·1
					(2·0)	(2·6)	(5·4)	(8·5)
(4)	28. vii. 38 (Heated and packed column)	1·1	16·4	2·6	6·4*	37·4	21·9	14·2
					(2·0)	(2·2)	(5·3)	(9·0)
(5)	2. viii. 35	2·2	16·2	2·6	9·4	35·3	25·3	9·0
					(2·0)	(2·7)	(5·7)	(10·0)
(6)	22. viii. 35	2·0	15·8	3·8	7·6	34·3	23·3	13·2
					(2·0)	(2·6)	(5·4)	(9·7)
(7)	4. ix. 35 (Heated and packed column)	1·9	16·1	2·7	7·2†	34·5	24·2	13·4
					(2·0)	(2·1)	(5·3)	(8·5)
Viscera (excluding liver)	2. vii. 35	0·9	18·9	2·9	6·7	16·9	36·6	17·1
					(2·0)	(2·9)	(5·6)	(9·4)
Roe (phosphatide)	28. ix. 35	1·3	25·0	0·9	2·1	20·2	34·4	16·1
					(2·0)	(2·7)	(7·1)	(10·0)
Roe (glyceride)	28. ix. 35	—	20·4	2·0	7·0	30·8	28·7	11·1
					(2·0)	(3·1)	(7·3)	(7·3)

* Includes 0·1 % myristoleic acid. † Includes 1·1 % myristoleic acid.

(*b*) Total groups of acids (mol. %)

Depot	Date collected	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Liver (1)	31. i. 35	2·3	26·1	37·9	23·3	10·4
(2)	6. vi. 35	2·7	26·1	40·5	23·9	6·8
(2)	6. vi. 35 (Hydrogenated)	2·5	27·4	39·8	22·2	8·1
(3)	9. vii. 34	1·6	26·6	39·4	22·9	9·5
(4)	28. vii. 38	0·9	25·1	38·8	22·1	13·1
(4)	28. vii. 38 (Heated and packed column)	1·3	25·7	40·3	20·4	12·3
(5)	2. viii. 35	2·8	28·2	38·0	23·3	7·7
(6)	22. viii. 35	2·5	26·1	38·3	21·7	11·4
(7)	4. ix. 35 (Heated and packed column)	3·5	25·1	37·6	22·5	11·3
Viscera (excluding liver)	2. vii. 35	1·2	29·0	20·3	34·5	15·0
Roe phosphatide	28. ix. 35	1·7	30·4	21·5	32·4	14·0
Roe glyceride	28. ix. 35	—	30·5	33·2	26·8	9·5

(*c*) Deviations from mean values of total groups of acids (mol. %) of the liver oils

Liver oil	Date collected	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Mean value ...		2·2	26·3	38·9	22·5	10·1
(1)	31. i. 35	+0·1	−0·2	−1·0	+0·8	+0·3
(2)	6. vi. 35	+0·5	−0·2	+1·6	+1·4	−3·3
(2)	6. vi. 35 (Hydrogenated)	+0·3	+1·1	+0·9	−0·3	−2·0
(3)	9. vii. 34	−0·6	+0·3	+0·5	+0·4	−0·6
(4)	28. vii. 38	−0·3	−1·2	−0·1	−0·4	+3·0
(4)	28. vii. 38 (Heated and packed column)	−0·9	−0·6	+1·4	−2·1	+2·2
(5)	2. viii. 35	+0·6	+1·9	−0·9	+0·8	−2·4
(6)	22. viii. 35	+0·3	−0·2	−0·6	−0·8	+1·3
(7)	4. ix. 35 (Heated and packed column)	+1·3	−1·2	−1·3	—	+1·2

The results in Table V give general confirmation of the accuracy of the ester fractionation technique which has already been tested for fish oils by Harper *et al.* [1937]. The outstanding feature, however, of the present investigation is the apparent difference in the values obtained for the mean unsaturation of C_{18} esters by the heated and packed column on the one hand ($-2{\cdot}1$ to $-2{\cdot}2$ H) and by the less efficient Willstätter bulb on the other ($-2{\cdot}4$ to $-2{\cdot}7$ H). It has already been shown in connexion with animal liver fats [Hilditch & Shorland, 1937] that the Willstätter bulb does not permit complete separation of C_{20} unsaturated esters from C_{18} ester concentrates which give on bromination of the corresponding acids varying proportions of high melting point bromides characteristic of highly unsaturated C_{20} acids. An investigation of the C_{18} unsaturated acids (corresponding esters, S.E. 295·4, I.V. 90·2) prepared by refractionation of a C_{18} ester concentrate in the heated and packed column gave the results reported in Tables VI*a* and VI*b*.

Table VI*a*. *Separation of C_{18} unsaturated acids of ling liver oil by lithium salts*

A. Acetone-soluble			B. Acetone-insoluble alcohol-soluble			C. Insoluble both in acetone and in alcohol		
%	S.E.	I.V.	%	S.E.	I.V.	%	S.E.	I.V.
6·0	282·4	142·2	8·1	280·5	99·0	85·9	280·6	79·4

Table IV*b*. *Insoluble bromo-additive products of each fraction*

	Insoluble in ether			Soluble in ether, insoluble in petroleum		
Fraction	M.P.	% Br	% total acids	M.P.	% Br	% total acids
A	231°	67·7	0·4	110/118°	52·3	0·5
B	224°	66·1	0·1	155°	—	*ca.* 0·1
C	235°	—	*ca.* 0·1	170/175°	59·7	0·2

The saponification equivalent of the acetone-soluble fraction A and the presence of high-melting point ether-insoluble bromides shown in Table VI suggest that even the relatively efficient heated and packed column does not separate completely in one distillation a pure C_{18} ester fraction. In order to eliminate the last traces of C_{20} esters the C_{18} concentrate was twice redistilled, giving finally a series of similar fractions.

Table VII. *Distillation of C_{18} esters prepared by repeated distillation from a heated and packed column*

Fraction	g.	B.P./0·1 mm.	S.E.	I.V.
C_{18} 1	2·82	134/135°	295·2	88·3
C_{18} 2	12·96	135°	295·9	89·3
C_{18} 3	2·79	—	295·9	88·2

Bromination of corresponding acids of fraction C_{18} 2 gave the results shown in Table VIII.

Table VIII. *Bromination of acids from "highly purified" C_{18} esters*

Ether-insoluble			Soluble in ether, insoluble in petroleum		
M.P.	% Br	% total C_{18} acids	M.P.	% Br	% total C_{18} acids
217/218°	65·6	0·4	173/174°	57·1	0·5

As a result of repeated fractionation the proportions of acids giving rise to ether-insoluble and petroleum-insoluble bromides were found to be reduced respectively from 0·6 to 0·4% and from 0·8 to 0·5% respectively. The % Br (65·6) accords with the presence of a mixture of octadecatetraenoic (69·8% Br) and octadecatrienoic (63·3% Br) acids which Toyama & Tsuchiya [1929] have shown to exist in sardine oil, the ether-insoluble bromide from the octadecatetraenoic (stearidonic) acid melting at approximately 220° as compared with 217–218° observed for the ether-insoluble C_{18} bromides reported in this investigation.

Discussion

If allowance is made for experimental errors in the ester fractionation method [cf. Harper *et al.* 1937] the liver oils, except perhaps in the case of the C_{22} acids, were not found to vary appreciably in the proportions of the total groups of acids (cf. Table V*c*). This observation may have considerable significance in regard to the average composition of ling liver oil, since the samples differed as regards average liver weight and covered a wide range of blue values (cf. Table I). Variations in the content of hexadecenoic and palmitic acids are suggestive of dehydrogenation and hydrogenation processes which have been inferred already by Lovern [1937] in connexion with several North Sea species.

Comparison with previous results [Shorland & Hilditch, 1938] shows that "English" hake (*Merluccius gayi*) liver oil from Cook Strait is not significantly different in composition from ling liver oil, whereas groper (*Polyprion oxygeneios*) liver oil from the same locality is characterized by greatly reduced proportions of highly unsaturated C_{20} and C_{22} acids and correspondingly increased amounts of palmitic and palmitoleic acids. In both ling and "English" hake the liver predominates as a fat depot, but in groper the liver is subordinate as a fat depot to the main fat depots of the head and body. Groper head fat [Shorland & Hilditch, 1938] has been shown to conform approximately to the "average" marine type [cf. Lovern, 1937] of the North Sea and it is therefore possible that the highly abnormal composition of the liver oil may be a result of selective deposition.

Ling liver oil is shown to conform essentially to the "average" marine type as regards fatty acid composition, with slightly increased proportions of C_{18} unsaturated acids which have been a consistent feature of all New Zealand fish oils so far examined. This suggests that the conception of an "average" marine type of fat based on analyses of specimens taken mainly from the North Sea may not be applicable to other localities, and in this connexion the presence of increased proportions of C_{18} acids in Antarctic whale oil as compared with Arctic samples [Hilditch & Terleski, 1937] may be significant.

The roe glyceride somewhat resembles the liver fat as regards fatty acid composition, while the increased proportions of C_{20} and of C_{22} unsaturated acids and the diminished proportions of hexadecenoic acid of the roe phosphatide as compared with the corresponding glyceride are in accordance with previous observations made on animal liver phosphatides [cf. Klenk, 1933; 1935; Hilditch & Shorland, 1937; Shorland & Hilditch, 1938]. Although the visceral fat contains a considerably smaller proportion of palmitic acid than the roe phosphatide, it resembles the latter as regards the proportions of the total groups of acids. It has been generally found that roe lipins are more unsaturated than the corresponding depot fats [Channon & El Saby, 1932; Lovern, 1934], especially just prior to spawning. The higher iodine number of the roe lipins does not necessarily indicate an increased content of C_{20} and C_{22} highly unsaturated acids as compared with the depot fats. The greater degree of unsaturation of the fat of salmon ova

as compared with the body fat is due to the higher mean unsaturation of the C_{20} and C_{22} acids, which are present in smaller proportions than in the body fat. In the case of the ling, however, the higher iodine value of the roe lipins as compared with the liver oil may be attributed both to increased proportions of C_{20} and of C_{22} unsaturated acids and to the higher mean unsaturation of the C_{20} acids.

In view of the low proportions of fat in the viscera and roe as compared with the liver it would be expected on the basis of Lovern's hypothesis that the former depots would be relatively impermeable to the fatty acids of higher molecular weight. Contrary to previous observations on fish oils, however, these relatively impermeable depots show no appreciable increase in the proportions of C_{16} or lower fatty acids as compared with the liver fat but a marked increase in their content of C_{20} and to a lesser extent of C_{22} highly unsaturated acids.

Summary

Analyses of 7 samples of ling (*Genypterus blacodes*) liver oil taken at different periods showed no significant seasonal variation in fatty acid composition. The accuracy of the ester fractionation procedure was tested by various methods, all of which returned similar values for the proportions of component fatty acids. In the case of fractionation by a heated and packed column the calculated mean unsaturation of the C_{18} acids was much less than the value obtained by use of the less efficient Willstätter bulb. The composition of the liver oils was found to be generally similar to the "average" marine type with increased proportions of C_{18} unsaturated acids.

The iodine value and bromination data of highly purified C_{18} unsaturated acids, prepared from the corresponding methyl esters which had been repeatedly fractionated in a heated and packed column, showed that at least 96·5 % of the acids consisted of octadecenoic acid together with traces of octadecatetraenoic and octadecatrienoic acids. There was no indication of the presence of either linoleic or linolenic acid.

The roe glyceride was found to contain similar proportions of component fatty acids to the liver fat and contained more hexadecenoic and less C_{20} and C_{22} highly unsaturated acids than the corresponding phosphatide.

Contrary to Lovern's hypothesis of molecular filtration, the viscera and roe, which are relatively insignificant fat depots of the ling as compared with the liver, were characterized by containing fatty acids with higher proportions of C_{20} and C_{22} unsaturated acids than the liver oil.

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