CUTTLE-FISH OIL AND MACKEREL OIL

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(Received May 26, 1953)

Cuttle-fish oil is obtained as a byproduct of the manufacture of dried cuttle-fish, for which the species *Ommastrephes sloani pacificus* Steenstrup is chiefly used. This oil is prepared from cuttle-fish viscera by rendering, but the resulting oil is derived mainly from livers. Production of this oil has so rapidly increased after the war with Hokkaido as the production center that this oil has recently replaced sardine and herring oils which were formerly the most important fish oils in Japan. Mackerel oil is prepared either from the offals or sometimes from the whole body of mackerel, *Scomber japonicus* Houttuyn. Production of this oil has also greatly increased after the war chiefly in Hokkaido.

Cuttle-fish oil and mackerel oil were formerly studied by Tsujimoto and his co-workers, but literatures relating to the characteristics of these oils are very scanty up to the present. The cuttle-fish oil examined by Tsujimoto[1] showed d_4^{15} 0.9316, n_D^{20} 1.4806, A.V. 3.9, S.V. 189.6, I.V. 177.0, Unsap. matter 1.14%, and polybromides of fatty acids 57.45% with Br content 70.91%. Unsaponifiable matter of another sample examined by the same author contained 48% of cholesterol together with cetyl, oleyl, batyl, chimyl, and selachyl alcohols. The oil reported by Tsujimoto and Kimura[2] had d_4^{15} 0.9200, n_D^{20} 1.4833, A.V. 18.2, S.V. 176.9, I.V. (pyridine sulfate dibromide method) 179.5, Unsap. matter 4.20%, solid acids by lead salt alcohol method 22.6% with I.V. 14.1, and highly unsaturated acids by lithium salt acetone method 41.3% with I.V. 372.1. Saturated acids contained palmitic acid, and unsaponifiable matter contained 56% of cholesterol. The oil contained also vitamin A. Kitabayashi, Nakamura, and Shuto[3] have recently reported 3 samples of cuttle-fish oil having d_4^{15} 0.9365–0.9393, A.V. 10.0–15.8, S.V. 180.5–186.2, I.V. 175.0–182.6, and Unsap. matter 3.5–5.5%. One sample contained 25.4% of solid acids with I.V. 8.8. Tsujimoto[4] examined also an oil obtained from another kind of cuttle-fish, *Watasenia scintillans* (Berry). André and Canal[5] reported the properties of European cuttle-fish oil.

The mackerel oil reported by Tsujimoto[6] showed d_4^{15} 0.9301, n_D^{20} 1.4811, A.V. 1.7, S.V. 191.6, I.V. 167.4, and brominated fatty acids insoluble in acetic acid 36.18% with Br content 69.38%. Spanish mackerel oils[7] are reported to have S.V. 182.5–204.9 and I.V. 115.3–136.7. Characteristics of mackerel liver oils[8] are recorded as Sp. Gr. 0.928–0.969, Refr. index 1.4810–1.4969, F.F.A. 24.3–36.8%, S.V. 166.8–177.6,

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I.V. 129.1–158.2, Upsap. matter 5.78–15.63%, vitamin A (U.S.P. units per g) 30,000–200,000, and vitamin D (I.U. per g) 1,400–5,400.

The present paper records the more important properties of the cuttle-fish and mackerel oils which were produced in 1951 and examined by the authors.

1. Cuttle-Fish Oil

Properties of 26 oils examined are given in Table 1. All the oils were prepared by rendering under the condition which facilitated much autolysis in the course of rendering. They have a reddish orange color, though the color of each oil varies somewhat in depth. They are liquid at ordinary temperature, and deposit a very small amount of stearine even in winter. Since a few oils were turbid due to the presence of moisture, they were filtered through a dry filter paper before they were used for the determination of characteristics. It is known for some fish oils that there is a definite relationship between the season of fish catching and the properties of fish oil, but no such relationship is found in the case of cuttle-fish oil, so far as the results of the present experiments recorded in Table 1 are con-

TABLE 1. Cuttle-Fish Oil

Sample No.	Catching		d_4^{20}	n_D^{20}	A.V.	S.V.	I.V. (Wijs)	Unsap. matter (%)	Ether-insol. brominated fatty acids (%)
	Locality	Date (1951)							
1	Matsumae	Dec. 4	0.9289	1.4838	11.3	182.4	206.5	4.04	79.27
2	Ohata	Late Oct.	0.9273	1.4838	7.0	187.2	203.2	2.45	75.27
3	Tokai District	Late Nov.	0.9277	1.4835	22.8	187.3	197.9	3.62	74.68
4	Kayabe	Late Aug.	0.9267	1.4820	16.8	184.9	195.9	4.28	77.81
5	Ohata	Early Oct.	0.9274	1.4832	10.2	185.0	193.3	3.01	76.19
6	Muroran	Aug. 30	0.9263	1.4817	12.5	186.8	193.2	4.43	75.00
7	Hakodate	Dec. 1	0.9279	1.4832	7.3	185.1	193.0	2.59	73.90
8	Hachinohe	Late Oct.	0.9263	1.4832	17.8	185.2	192.9	3.45	76.07
9	Hakodate	Sept. 19	0.9257	1.4834	14.2	186.7	191.8	4.47	76.32
10	Hakodate	Aug. 11	0.9263	1.4820	5.3	187.7	190.8	3.72	75.60
11	Fukushima	Aug. 17	0.9259	1.4820	12.2	183.1	190.2	4.83	72.34
12	Fukushima	Aug. 17	0.9267	1.4820	12.4	183.6	189.4	4.84	75.51
13	Aonae	Aug. 10	0.9268	1.4830	12.3	183.0	188.5	5.31	74.29
14	Hachinohe	Middle Oct.	0.9270	1.4822	16.3	179.8	188.5	3.64	74.36
15	Hakodate	Dec. 15	0.9272	1.4831	8.3	181.5	188.1	6.94	72.52
16	Mitsuishi	—	0.9248	1.4820	17.3	182.8	186.7	4.87	74.88
17	Matsumae	Oct. 23	0.9267	1.4825	5.2	179.1	186.4	4.02	73.79
18	Hakodate	Oct. 3	0.9249	1.4820	10.3	186.2	185.1	3.65	71.94
19	Hakodate	Sept. 20	0.9244	1.4828	6.4	187.0	185 0	2.94	71.99
20	Urakawa	—	0.9254	1.4821	14.9	183.8	183.8	4.32	73.32
21	Urakawa	—	0.9253	1.4820	11.5	185.4	183.8	3.26	72.99
22	Hakodate	Aug. 2	0.9240	1.4809	14.4	186.8	183.2	4.16	70.33
23	Urakawa	—	0.9247	1.4818	11.2	185.3	181.5	3.56	71.39
24	Urakawa	—	0.9249	1.4820	12.5	186.7	180.1	3.79	74.37
25	Urakawa	—	0.9254	1.4820	13.9	186.9	179.9	4.11	73.32
26	Hakodate	Jul. 24	0.9230	1.4800	17.0	184.9	178.8	4.58	64.71
Highest			0.9289	1.4838	22.8	187.7	206.5	6.94	79.27
Lowest			0.9230	1.4800	5.2	179.1	178.8	2.45	64.71
Average			0.9261	1.4824	12.4	184.8	189.1	4.03	73.93

Notes: The localities of catching of Nos. 2, 5, 8, and 14 belong to Aomori Prefecture, and that of No. 3 to Tokai District while those of other samples belong to Hokkaido. Oils from Nos. 1, 3, 7, and 15 were prepared in our laboratories.

cerned. The yields of ether-insoluble brominated fatty acids are very high, exceeding 70% except oil No. 26, and hence cuttle-fish oils are found to contain a very large amount of highly unsaturated acids.

Determination of saturated acids. When the lead salt alcohol method is applied for the separation of solid acids from mixed fatty acids of fish oils, it gives solid acids having a relatively high iodine value in most cases, while lower members of saturated acids, such as lauric and myristic acids, are not completely precipitated as lead salts. If the procedure is carried out so as to obtain solid acids having a very low iodine value, a considerable proportion of saturated acids tends to remain in liquid acids. and the estimation of saturated acids in mixed fatty acids becomes difficult. In these experiments, mixed fatty acids were converted into methyl esters which were then subjected to the oxidation with potassium permanganate in acetone. The acidic scission product from unsaturated esters was then separated from neutral saturated esters. The acetone permanganate method was first developed by Hilditch[9] for the purpose of determining saturated glycerides in oils and has been applied to various kinds of oil, but it appears to have been little used for the determination of saturated acids in mixed fatty acids. The procedures taken in these experiments are as follows: Mixed fatty acids freed from unsaponifiable matter were esterified with methanol in the usual way. Two g of methyl esters (A.V. below 2) were dissolved in 40 cc of acetone, and 20 g of powdered potassium permanganate was added in small portions. After the mixture was refluxed for 1-1.5 hours in order to complete the oxidation, the acetone was distilled off. The residue was added with 120-150 cc of water and then treated with a concentrated solution of sodium bisulfite in order to reduce the oxides of manganese and the excess of potassium permanganate. The solution was then extracted with 700 cc of ether. Extraction was repeated using 400-500 cc of ether. The ether solution was washed several times with water and then with a solution of sodium carbonate in order to remove acidic substances as sodium salts. After washing with water and dehydration with anhydrous sodium sulfate, the ether solution was distilled, and the residue consisting of saturated esters was weighed.

Four oils were examined by this method with the results given in Table 2. Thus the contents of saturated acids in mixed fatty acids of these oils are estimated at 25-27%.

TABLE 2. Saturated Acids

Oil No.	6	16	25	26
Saturated methyl esters (%)	26.53	25.79	25.54	27.41
I.V. of sat. methyl esters	0.82	1.4	0.73	0.92

Determination of tri-saturated glycerides. The same four oils which were used for the determination of saturated acids were subjected to the acetone permanganate oxidation for the determination of tri-saturated glycerides. The oxidation of glycerides was performed in a similar way as described above for the oxidation of methyl esters. The oxidation product was extracted with ether, and the ether solution was washed with a solution of sodium carbonate and then with water.

After dehydration and distillation of ether, the residue obtained was again oxidized with potassium permanganate in acetone. The oxidation product was treated as before. On distilling off ether from the ether solution, there was obtained a solid residue. It had, however, an exceedingly high acid value indicating the presence of a considerably large proportion of some acidic snbstances, possibly the oxidation product from di-saturated glycerides, besides tri-saturated glycerides. By washing a petroleum ether solution of the residue obtained above with a solution of sodium carbonate, the acid value of the substances remained in the petroleum ether solution was decreased, but the substances obtained finally from the petroleum ether solution, designated as tri-saturated glycerides in Table 3, still showed relatively high acid values, and the complete removal of acidic oxidation product was not attained in these experiments. Based on the data given in Table 3, the amount of tri-saturated glycerides in the oils examined are estimated at about 1-2%.

TABLE 3. Tri-Saturated Glycerides

Oil No.	6	16	25	26
Tri-saturated glycerides (%)	2.1	2.5	2.6	2.5
A.V. of tri-sat. glycerides	19.0	9.1	7.6	15.7

Sterols. The amounts of sterols in the unsaponifiable matter of oils No. 19 and No. 21 were found to be 70.55% and 63.41%, respectively, by the digitonid method. The unsaponifiable matter from several oils recorded in Table 1 was combined, and recrystallized from alcohol. The product obtained after three recrystallizations had M.P. 142–143°C, which was raised to 145°C by further two recrystallizations. These results indicate that the main component of sterols is cholesterol.

Vitamin A. Oils No. 6, No. 25, and No. 26 in hexane were examined by the spectrophotometric method, and the values $E^{1\%}_{1cm}$ at 328 mμ were found to be 0.418, 0.585, and 0.511, respectively.

2. Mackerel Oil

Sixteen samples of mackerel oil prepared by the boiling out process were examined. The characteristics of these oils are given in Table 4. Oils No. 8, No. 13, and No. 15 are light orange yellow, while the other oils are reddish orange; especially oils No. 4 and No. 12 are dark in color. All the oils deposit more or less stearine at ordinary temperature. In winter, they deposit large amounts of stearine, and oils No. 3, No. 4, and No. 8 are not mobile.

TABLE 4. Mackerel Oil

Sample No.	Catching		d^{20}_4	n^{20}_D	A.V.	S.V.	I.V.	Unsap. m. (%)	Ether-insol. brominated fatty acids (%)	Source of oil
	Locality	Date (1951)								
1	Kushiro	Early Sept.	0.9246	1.4794	3.5	188.9	166.1	1.45	58.11	Whole body
2	Hiro-o	Aug. 21	0.9217	1.4780	3.2	190.4	157.9	0.41	44.30	Viscera and heads
3	Hiro-o	Aug. 29	0.9216	1.4780	6.6	191.8	157.9	1.24	52.74	Viscera

TABLEL 4. (*Continued*)

4	Kushiro	Aug. 5	0.9215	1.4782	26.8	187.0	156.2	3.02	50.46	Whole body
5	Kushiro	—	0.9220	1.4766	7.7	187.6	155.2	1.53	45.40	Whole body
6	Kushiro	Early Sept.	0.9208	1.4782	0.38	193.4	153.0	1.11	43.12	Viscera
7	Kushiro	—	0.9199	1.4770	26.7	196.6	153.0	0.64	48.92	—
8	Hiro-o	Aug. 26	0.9212	1.4780	4.0	192.3	152.2	1.06	49.67	—
9	Kushiro	—	0.9210	1.4780	7.7	196.0	152.2	1.43	49.66	—
10	Kushiro	Early Sept.	0.9217	1.4781	0.31	189.1	152.0	1.31	46.91	Viscera
11	Kushiro	Middle Aug.	0.9219	1.4772	24.8	191.5	151.1	1.32	40.49	Viscera and heads
12	Otaru	Early Jun.	0.9226	1.4783	9.3	193.7	148.9	1.30	48.67	—
13	Hiro-o	Aug. 26	0.9206	1.4780	0.64	193.7	148.6	0.47	49.14	—
14	Hiro-o	Aug. 27	0.9215	1.4780	7.7	190.9	148.4	0.88	49.56	—
15	Otaru	Early Jun.	0.9210	1.4780	1.0	191.7	143.9	1.17	49.71	—
16	Otaru	Early Jul.	0.9178	1.4764	10.3	194.4	139.7	1.38	39.84	—
Highest			0.9246	1.4794	26.8	196.6	166.1	3.02	58.11	
Lowest			0.9178	1.4764	0.31	187.0	139.7	0.41	39.84	
Average			0.9213	1.4778	8.79	191.8	152.3	1.23	47.92	

Notes: All localities of catching belong to Hokkaido.

Determinations of saturated acids, tri-saturated glycerides, and vitamin A were carried out with several oils in the same way as described in the case of cuttle-fish oil. The results are shown in Table 5, from which saturated acids in mixed fatty acids are found to be 22–25%, while tri-saturated glycerides in oils are estimated at roughly 1–3%.

TABLE 5. Saturated Acids and Others

Oil No.	3	4	12	15	16
Saturated methyl esters (%)	24.85	24.11	22.39	22.82	22.17
I.V. of sat. methyl esters	0.41	0.60	2.0	0.32	1.0
Tri-saturated glycerides (%)	2.84	1.87	—	—	3.26
A.V. of tri-sat. glycerides	7.4	14.8	—	—	8.3
$E^{1\%}_{1cm}$ 328 mμ	0.144	—	—	0.288	0.328

3. Summary

1. Characteristics of 26 samples of cuttle-fish oil show the following ranges, the figures in parentheses showing average values: d_4^{20} 0.9230–0.9289 (0.9261), n_D^{20} 1.4800–1.4838 (1.4824), A.V. 5.2–22.8 (12.4), S.V. 179.1–187.7 (184.8), I.V. (Wijs method) 178.8–206.5 (189.1), unsap. matter 2.45–6.94% (4.03%), ether-insoluble brominated

fatty acids 64.71-79.27% (73.93%). Several oils were subjected to a further experiment with the following results: saturated acids in mixed fatty acids 25-27%, tri-saturated glycerides in oils roughly 1-2%, sterols in unsap. matter 63.41-70.55%, and $E^{1\%}_{1cm}$ at 328 mμ 0.418-0.585.

2. Characteristics of 16 samples of mackerel oil are found to be d^{20}_4 0.9178-0.9246 (0.9213), n^{20}_D 1.4764-1.4794 (1.4778), A.V. 0.31-26.8 (8.79), S.V. 187.0-196.6 (191.8), I.V. 139.7-166.1 (152.3), unsap. matter 0.41-3.02% (1.23%), ether-insoluble brominated fatty acids 39.84-58.11% (47.92%). Examination of several oils showed the following results: saturated acids in mixed fatty acids 22-25%, tri-saturated glycerides in oils roughly 1-3%, and $E^{1\%}_{1cm}$ at 328 mμ 0.144-0.328.

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