FATTY ACID COMPONENT OF LIPID OF *EUPHAUSIA SUPERBA*

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ABSTRACT

Lipids extracted from the raw frozen and boiled-frozen krill, *Euphausia superba*, harvested in the Antarctic Ocean were identified and quantitated by a combination of thin-layer and gas-liquid chromatography.

The lipid contents of *E. superba* on the wet weight basis were 3.41% of the raw frozen sample and 5.62% of the boiled-frozen sample. There were shown to consist of 0.8% of steryl esters, 27.4% of free fatty acids, 52.3% of triglycerides, 4.7% of sterols, a trace amount of diglycerides, 2.2% of monoglycerides, 6.9% of phospholipids and 4.1% of pigments in the raw frozen sample, and 0.6% of steryl esters, 4.6% of free fatty acids, 76.8% of triglycerides, 4.2% of sterols, 9.2% of phospholipids and 3.5% of pigments in the boiled-frozen sample. The predominant fatty acids contained in total lipids and the fractions of free fatty acids, triglycerides and phospholipids were myristic (5.98-22.16%), palmitic (16.83-30.49%), palmitoleic (5.92-26.31%), oleic (19.31-29.17%), eicosapentaenoic (0.52-12.59%) and docosatrienoic (0.50-11.74%) acids.

INTRODUCTION

Euphausia superba is a shrimp-like crustacean of about 5 cm in body length, and inhabits only in the Antarctic Ocean, though many euphausiid species are found widely over the world oceans. *E. superba* composes one of the most important prey organisms for the inhabitants in the oceanic regions of the Antarctic ecosystem. In addition to utilization by the faunistic inhabitants there, *E. superba* should be regarded as the food resources for mankind in the future. The present study stands on such viewpoint, and intends to give a basic knowledge for utilizing *E. superba* as to be a possible humans' food.

There have been several reports on the chemical properties and the fatty acid composition of *E. superba* lipids (Saiki and Mori, 1953; Saiki *et al.*, 1959; Tsuyuki *et al.*, 1964a and b). So far as these works were carried out by the fractional distillation method, the minute examinations of the fatty acid component have not been reported yet.

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On the other hand, Kayama and Nakagawa (1975), and Kayama and Ikeda (1975) reported on the lipids of some micronektonic shrimps.

The objective of this study is to identify and quantitate the lipids and the fatty acid components of *E. superba* by a combination of thin-layer and gas-liquid chromatography.

MATERIALS AND METHODS

Euphausia superba, used in this study were harvested in the waters off Enderby Land, 10°E in the Antarctic Ocean between December, 1974 and February, 1975 by a expedition of commercial basis Nippon Suisan Co. Ltd. A part of the *E. superba* harvested was quickly frozen as the raw frozen products and another part of them was similarly quick frozen as the boiled-frozen products after flash boiling. Both products were processed as the whole body of *E. superba*. They were in the range from 0.4 to 0.7 g in the body weight and in the range from 3.5 to 5.5 cm in the body length.

The method of Bligh and Dyer (1959) was used to extract and purify the lipids in these samples. The extracted lipids were then weighed and stored at −20°C under nitrogen atmosphere. The chemical properties of the extracted lipids were examined by ordinary methods.

The fractionation of *E. superba* lipids was subsequently separated by thin-layer chromatography using 20×20 cm glass plates coated with activated Silica gel G (Merck Chemical Co.). The solvent systems used were petroleum ether : diethyl ether : acetic acid, 85 : 15 : 1, v/v/v. The pure standards *e. g.* phospholipids, cholesterol, steryl palmitate, mono, di, triglycerides, oleic acid (Nihon Chromato Works Ltd.) were used to identify zones on the thin-layer plates which were removed, dried, sprayed with 50% sulfuric acid and charred at 110°C for 15 min. The fractionated zones were scraped into Toyo filter paper No. 2 cones (Toyo Roshi Kaisha Ltd.) and eluted with chloroform respectively. The eluting solvents were evaporated off with a stream of nitrogen and weighed.

The three major fractions were recovered triglycerides, free fatty acids and phospholipids for analyzed the fatty acid components. The other minor fractions were noted on the plates but were not in sufficient quantity to be recovered.

The fatty acid methyl esters from total lipids, triglycerides, free fatty acids and phospholipids of *E. superba* lipids esterified by the saponification-transesterification method as described by Metcalfe *et al.* (1966). 5 ml of a 0.5 N methanolic KOH solution were added to approximately 30 mg of total lipids, triglycerides, free fatty acids and phospholipids, and heated over a steam bath for 5 min. Then, 5 ml of 12.5% boron trifluoride in methanol were added to these mixtures and boiled for 3 min. Thin-layer chromatography was used to determine the completeness of the transesterification. Silica gel G plates were spotted with the reacted products and known standards, and developped with petroleum ether : diethyl ether : acetic acid as previously described. A comparison of the R_f values of the reacted products, triolein, methyl oleate and

oleic acid (Nihon Chromato Works Ltd.) indicated that the conversion of total lipids, triglycerides, free fatty acids and phospholipids fractions to methyl esters was complete.

The methyl esters were analyzed weth a Shimadzu Gas Chromatograph Model 5A (Shimadzu Seisakusho Co.) equipped with a dual flame ionization detector. The columns used were 3 m×3.0 mm I. D. glass coil tubing packed with Diasolid ZF on 80/100 mesh, and with 3% SE-30 on 80/100 mesh Chromosorb W (Nihon Chromato Works Ltd.). The carrier gas was nitrogen at flow rate of 40 ml per min. The column were operated isothermally at 185°C for Diasolid ZF and 225°C for SE-30. The injector block and detector were at 215°C for Diasolid ZF and 245°C for SE-30, respectively.

Some of the gas-liquid chromatographic peaks were identified by comparison with standard peaks obtained from pure methyl ester mixture (Nihon Chromato Works Ltd.). Also, equivalent chain-length values were determined according to the method of Miwa (1963) and were compared with those reported by Hofstetter *et al.* (1965) for identifying peaks for which no pure methyl esters were available. A portion of each sample esterified was hydrogenated to confirm the correctness of identification of the unsaturated acids. Approximately 20 mg of methyl esters, 1 ml of methanol and a pinch of platinum black were added to a screw cap vial. Hydrogen was bubbled in for 5 min, the vial sealed and then reacted for 15 min with frequent shaking. After reaction, the methyl esters were transferred to *n*-hexane, dried, then taken up and injected into the gas-liquid chromatograph with the same condition as above mentioned. The area of each chromatographic peak representing a fatty acid present was obtained by multiplication of the height of each peak by the width at half-height. The areas of each peak was then compared with the total combined area at all of the peaks to obtained the percentage of each specific fatty acid.

RESULTS AND DISCUSSION

The chemical properties of the total lipids extracted from *Euphausia superba* are shown in Table 1. The lipid contents are comparatively low *i. e.*, 3.41% of the raw frozen sample and 5.62% of the boiled-frozen sample on the wet

TABLE 1. PROPERTIES OF TOTAL LIPIDS FROM *EUPHAUSIA SUPERBA*

	Lipids	
	Raw frozen sample	Boiled-frozen sample
Appearance (20°C)	Reddish brown liquid	Reddish brown liquid
Oil content (%)	3.41	5.62
Refractive index (40°C)	1.4788	1.4820
Acid value	74.1	18.2
Iodine value	142.3	127.9
Saponification value	181.3	197.6
Unsaponifiables (%)	5.6	5.8

weight basis. The total lipid of the raw frozen sample was higher in iodine and acid values, and lower in saponification value than those of the boiled-frozen sample. These are seemed to be autoxidized during frozen storage or thawing.

The lipid composition of each extract as determined by thin-layer chromatography is markedly different between the samples of the raw frozen and boiled-frozen. By visual evaluation of these chromatograph as shown in Fig. 1, the zones of steryl esters, triglycerides, free fatty acids, monoglycerides, sterols, diglycerides, phospholipids and pigments are appearently present in the raw frozen sample while the boiled-frozen sample is not found the presence of diglycerides and monoglycerides. There are also appearent differences in the compositions of triglycerides and free fatty acids between two samples. The lipid compositions of these fractions are reported in Table 2. In the extracts of the boiled-frozen sample, triglycerides are the preponderant fraction comprising 76.8% of the total lipid fractions, whereas in the raw frozen sample, triglycerides fraction is much lower. On the other hand, the composition of free fatty acids fraction is much higher in the raw frozen sample than in the boiled-frozen sample. These appearently explained to the difference of acid value in Table 1. Also, the raw frozen sample had 2.2% of monoglycerides

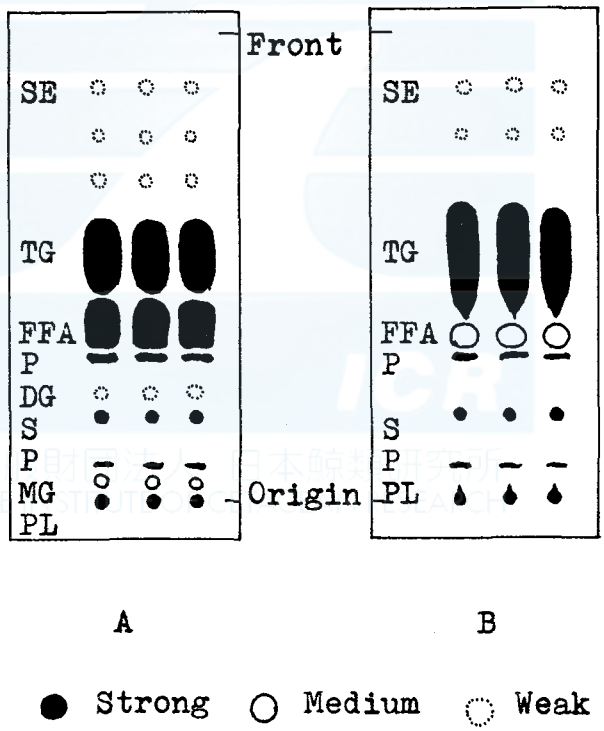

Fig. 1. Thin-layer chromatograms of total lipids extracted from *E. superba*. A: the raw frozen sample. B: the boiled-frozen sample. SE: steryl esters. TG: triglycerides. FFA: free fatty acids. P: pigments. DG: diglycerides. S: sterols, MG: monoglycerides. PL: phospholipids.

and a trace of diglycerides fractions while the boiled-frozen sample was not detected them.

The fatty acid methyl esters of the total lipids and the main three fractions contained fatty acids *i. e.*, triglycerides, free fatty acids and phospholipids were analyzed by gas-liquid chromatography, but no attempt was made to further study the component of other fractions. The presence of the fatty acids contained in *E. superba* lipid is a very wide variety as reported in Table 3, plus trace quantities of unidentified fatty acid methyl esters. With a few exceptions, the fatty acids of the total lipids and the three main fractions in both samples had nearly similar chromatographic patterns and distributions with each other. The major fatty acid compositions of both total lipids were 14 : 0, 16 : 0, 16 : 1, 18 : 1, 20 : 5 and 22 : 3 acids and contained more than 87% of the total fatty acids. Both triglyceride fractions contained 14 : 0, 16 : 0, 16 : 1 and 18 : 1 acids as the predominate fatty acids whereas were not detected the fatty acids of 22 : 5, 22 : 6 and 24 : 1 which contained in the total lipids. The total composition of 14 : 0, 16 : 0, 16 : 1 and 18 : 1 acids accounted for more than 85% of the total fatty acids. The free fatty acid fractions of both samples were mainly consisted of 14 : 0, 16 : 0, 16 : 1, 18 : 1, 20 : 5 and 22 : 3 acids similar to the total lipids. The total composition of the six main fatty acids were more than 77% of the total fatty acids. The fatty acids of 22 : 5 and 22 : 6 which contained in the total lipids were not found in this fraction of the boiled-frozen sample. In both phospholipid fractions, the fatty acids of 14 : 0, 16 : 0, 16 : 1, 18 : 1 and 20 : 5 as the predominant fatty acids comprised approximately 76% of the total fatty acids.

The compositions of the total saturated acids in the raw frozen sample were 39.96% of the total lipids, 56.17% of the triglyceride fraction, 37.96% of the free fatty acid fraction and 27.14% of the phospholipid fraction, and those of unsaturated acids were 58.95%, 42.83% 60.84% and 71.56% respectively. On the other hand, those of the total saturated acids in the boiled-frozen sample

TABLE 2. LIPID COMPOSITION OF *EUPHAUSIA SUPERBA* BY THIN-LAYER CHROMATOGRAPHY.

Composition	Lipids	
	Raw frozen sample (%)	Boiled-frozen sample (%)
Triglycerides	52.3	76.8
Diglycerides	trace	——
Monoglycerides	2.2	——
Free fatty acids	27.4	4.6
Phospholipids	6.9	9.2
Sterols	4.7	4.2
Steryl esters	0.8	0.6
Pigments	4.1	3.5
Unknowns	1.6	1.1

TABLE 3. FATTY ACID COMPOSITION OF *EUPHAUSIA SUPERBA* LIPIDS.
(percentage of total lipids)

Fatty acids	Lipids in raw frozen sample				Lipids in boiled-frozen sample			
	Total lipids	Triglycerides	Free fatty acids	Phospholipids	Total lipids	Triglycerides	Free fatty acids	Phospholipids
12 : 0	0.21	0.27	0.16	0.54	0.22	0.32	0.47	0.85
12 : 1	0.02	0.05	0.02	0.03	0.05	0.01	0.03	0.12
13 : 0	0.05	0.08	0.03	0.08	0.07	0.11	0.09	0.19
Iso-14 : 0	0.01	0.03	0.01	0.04	0.02	0.02	0.02	0.19
14 : 0	8.71	22.16	9.07	5.98	11.54	21.35	8.66	9.62
14 : 1	0.14	0.15	0.13	0.10	0.09	0.15	0.19	0.24
Iso-15 : 0	0.07	0.05	0.03	0.01	0.08	0.04	0.04	0.06
15 : 0	0.37	0.84	0.52	0.46	0.59	0.73	0.33	1.08
15 : 1	0.06	0.13	0.07	0.15	0.12	0.10	0.09	0.28
Iso-16 : 0	0.04	0.10	0.04	0.12	0.05	0.06	0.06	0.12
16 : 0	28.94	30.49	26.01	16.83	29.20	24.04	24.82	25.22
16 : 1	5.92	8.86	7.96	26.31	6.27	13.37	6.49	9.53
16 : 2	0.14	0.53	0.71	0.58	0.25	0.83	0.41	0.45
Iso-17 : 0	0.02	0.02	0.01	0.01	0.02	0.03	0.02	0.02
17 : 0	0.69	0.55	0.57	0.88	0.64	0.68	0.73	0.90
17 : 1	0.09	0.17	0.07	0.06	0.09	0.06	0.11	0.10
Iso-18 : 0	0.04	0.05	0.04	0.01	0.07	0.05	0.02	0.09
18 : 0	0.40	1.18	0.96	1.78	0.78	1.02	1.60	4.52
18 : 1	19.84	28.25	29.17	28.49	19.31	25.87	21.82	26.24
18 : 2	2.79	1.87	3.47	2.53	2.43	2.03	3.03	2.42
18 : 3	0.12	0.12	0.80	0.54	0.06	0.49	4.10	0.41
19 : 0	0.15	0.20	0.20	0.07	0.16	0.13	0.39	0.11
20 : 0	0.10	0.09	0.13	0.20	0.04	0.55	0.24	0.15
20 : 1	1.02	0.76	0.52	0.52	0.65	1.47	0.77	0.53
20 : 2	0.28	0.13	0.12	0.47	0.24	0.74	1.46	1.37
20 : 3	1.45	0.35	0.95	0.83	0.82	0.60	4.61	0.29
20 : 4	0.94	0.26	1.15	0.47	0.55	0.87	1.64	0.63
20 : 5	12.59	0.52	7.04	6.59	10.96	1.40	8.84	6.12
21 : 0	0.21	0.06	0.18	0.13	0.07	0.10	0.22	0.12
22 : 1	0.34	0.08	0.92	0.43	0.41	0.11	0.51	0.37
22 : 2	0.56	0.10	0.31	0.25	0.30	0.07	0.06	0.53
22 : 3	11.29	0.50	6.89	2.66	11.74	1.20	6.38	4.12
22 : 5	0.48	——	0.13	0.33	0.23	——	——	0.12
22 : 6	0.67	——	0.13	——	0.41	——	——	——
24 : 1	0.37	——	0.27	0.22	0.36	——	0.59	0.78

were 43.60%, 49.20%, 38.71% and 43.28% respectively, and 55.34%, 49.40%, 61.19% and 55.69% in the total unsaturated acids of them, respectively. Among the saturated acids, 14 : 0 and 16 : 0 acids were the most constituents in the

total lipids and all fractions of both sample lipids. Although the fatty acids of 16 : 1, 18 : 1, 20 : 5 and 22 : 3 among the unsaturated acids were the main constituents in the total lipids, free fatty acid and phospholipid fractions of both samples, these were the least of amount in both triglyceride fractions. The most pronounced difference between the trigylceride fractions and other lipids was in the higher level of 14 : 0 acid and the lower level of the fatty acids more than 20 carbon atoms. Moreover, the phospholipid fraction of the raw frozen sample contained very higher level (26.31%) of 16 : 1 acid as compared to one of other lipids whereas 14 : 0 acid comprised slightly lower level of 5.98%. The fatty acids of 18 : 3 (4.10%) and 20 : 3 (4.61%) in the free fatty acid fraction and 18 : 0 acid (4.52%) in the phospholipid fraction of the boiled-frozen sample were slightly higher levels compared to others. Finally, there were little differences in the fatty acid compositions and distribution patterns of both samples except of the phospholipid fraction in the raw frozen sample.

Saiki *et al.* (1959) reported that the fatty acid composition of *E. superba* lipid were 16.0% of 14, 33.0% of 16, 36.1% of 18 and 13.6% of 20 carbon fatty acids. Also, Tsuyuki *et al.* (1964a) reported that these were 6.2% of 14, 25.9% of 16, 35.2% of 18, 24.5% of 20 and 8.2% of 22 carbon fatty acids. We have found that these were 8.86–11.65% of 14, 35.04–35.77% of 16, 22.65–23.19% of 18, 13.26–16.38% of 20 and 13.09–13.34% of 22 carbon fatty acids in both total lipids.

Although the fatty acid components analyzed by gas-liquid chromatography between the raw frozen and boiled-frozen euphausiacea lipids was very low difference, the total lipid thin-layer chromatograms indicated that the free fatty acid fractions had appearently greater difference with each other. The results obtained with the free fatty acid fractions fractionated by thin-layer chromatography in this study indicated that the boiling procedure supported considerably in the maintenance of the *E. superba* lipid quality during handling and frozen storage.

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SUMMARY

1. The chemical properties of lipid contained in *Euphausia superba*, were studied.

2. The lipid fractions of the euphausiacea were quantitated by thin-layer chromatography.

3. The main lipid compositions were triglycerides, phospholipids and free fatty acids fractions.

4. The component fatty acids of the total lipids and each fraction fractionated by thin-layer chromatography were analyzed by gas-liquid chromatography.

5. The predominant fatty acids were myristic, palmitic, palmitoleic, oleic, eicosapentaenoic and docosatrienoic acids.

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