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Fatty Acid Details for Bivalves, *Tapes philippinarum* and *Corbicula japonica*, and Marine Types of Algae, *Nannochloropsis* sp. and *Chlorella* sp.

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Abstract

Gas-liquid chromatography (GLC) on an open tubular column (a fused silica WCOT CP-Sil 88) was conducted to investigate the fatty acid compositions of lipids from 2 species of bivalves, *Tapes philippinarum* (Japanese name, Asari) and *Corbicula japonica* (Japanese name, Yamatoshijimi), and marine types of algae, Japanese *Nannochloropsis* sp. (NC) and Malaysian *Chlorella* sp. (CR). The prominent fatty acids of the bivalves were 16:1n-7, 18:1n-9, 18:1n-7, 18:3n-3, 20:5n-3, and 22:6n-3. The n-3 highly unsaturated fatty acid (HUFA) levels in *C. japonica* considerably differed with their habitats, gravel or muddy regions. The brackish bivalve, *C. japonica*, obtained from the gravel region contained n-3 HUFA such as 22:6n-3 as much as the marine bivalve *T. philippinarum*. Whereas the *C. japonica* obtained from the muddy region contained lesser proportion of 22:6n-3 than that from the gravel region. The dominant fatty acids of the algae were 16:0 (NC 8.6%, CR 8.6%), 16:1n-7 (NC 13.2%), 16:1n-9 (CR 4.9%), 18:2n-6 (CR 11.4%), 18:3n-6 (CR 8.2%), 18:3n-3(CR 26.3%), 20:4n-6 (NC 5.6%), and 20:5n-3 (NC 39.3%). The Malaysian CR possessed 18:2n-6, 18:3n-6, and 18:3n-3 and only very small amounts of n-3 HUFA in contrast to the Japanese NC containing large amounts of n-3 HUFA such as 20:5n-3 and 20:4n-6. This suspects that the rotifer cultured with Malaysian CR have a low nutritive value for marine fishes which strictly require n-3 HUFA as an essential fatty acid.

Gas-liquid chromatography (GLC) is often used to determine fatty acid compositions in studies on lipid metabolism of aquatic organisms which contain complex fatty acid mixtures.[1-4] GLC using packed columns, however, is not powerful for the separation of some positional and geometrical isomers of unsaturated fatty acids. This has prompted the development of capillary GLC.[5-16] The detailed fatty acid compositions of fish have being accumulated in the field of food science.[16-23]

In the area of fish nutrition, although much research has been conducted to investigate the nutritional role of dietary lipids,[24,25] little studies[12,13,26,27] have dealt with the detailed

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compositions of fatty acids which were analysed by open-tubular GLC. The rigorous identification and quantification of fatty acid isomers are necessary to clarify the metabolism of lipids, especially exogenous (dietary n-3 and n-6 fatty acids) and endogenous fatty acids in fish and invertebrates. In the present study, we attempted to analyse the fatty acid compositions of the bivalves, which contain a complex mixtures of fatty acids, and marine types of algae by using capillary GLC on a fused silica WCOT CP-Sil 88. This paper presents the fatty acid compositions of these organisms and the characteristics of WCOT CP-Sil 88 column in the separation of fatty acid methylesters (FAME).

Materials and Methods

Reference Fatty Acid Methylesters

FAME standards were obtained from several commercial sources. The following analytical FAME were obtained from Nippon-Chromato-Kogyo Co., Japan. FAME mixture L-203; methyl pentadecanoate (15:0), methyl palmitate (16:0), methyl heptadecanoate (17:0), methyl stearate (18:0), and methyl nonadecanoate (19:0). FAME mixture L-207; methyl 18:0, methyl oleate (18:1n-9), methyl arachidate (20:0), methyl behenate (22:0), methyl erucate (22:1n-9), methyl lignocerate (24:0), and methyl nervonate (24:1n-9). FAME mixture L-209; methyl 20:0, methyl *cis*-eicos-5-enoate (20:1n-15), methyl arachidonate (20:4n-6), methyl 22:1n-9, and docosahexaenoate (22:6n-3). Branched chain fatty acids; isomyristate (iso-14:0), anteisopentadecanoate (anteiso-15:0), isopalmitate (iso-16:0), and isostearate (iso-18:0). Other branched chain fatty acids such as 14-methylhexadecanoate (anteiso-17:0), 16-methyloctadecanoate (anteiso-19:0), and 18-methylnonadecanoate (iso-20:0) were obtained as FAME mixtures (Qualimix BR 1, Qualimix BR 4, and Qualimix M) from the Larodan Fine Chemicals, Sweden. Tokyo-Kasei Co., Japan, was the source of the fatty acids containing a *trans* double bond, elaidic acid (18:1: *trans*-9-octadecenoic acid) and brassidic acid (22:1; *trans*-13-docosenoic acid). Methyl palmitoleate (16:1n-7), methyl linoleate (18:2n-6), methyl γ -linolenate (18:3n-6), methyl α -linolenate (18:3n-3), and icosapentaenoate (20:5n-3) were purchased from Gaschro-Kogyo Co. Ltd, Japan. FAME prepared from cod liver oil was also used as a secondary reference.[5,28)]

Bivalves and Algae

The short-necked clam *Tapes philippinarum* (Japanese name, Asari) habits in gravel regions from intertidal zone to 10 m depth in sea. The brackish bivalve, *Corbicula japonica* (Japanese name, Yamatoshijimi) was collected from two habitats with gravel or muddy bottoms. Specimens of *Tapes* and *Corbicula* were obtained from fish markets in Kagoshima in April and June, 1990, respectively.

The marine types of algae, Japanese *Nannochloropsis* sp. and Malaysian *Chlorella* sp. were isolated by the members of the Faculty of Fisheries, Kagoshima University, and the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, respectively. The algae were cultured indoors in a polycarbonate tank set up near the window. The incubation medium was the same as that reported previously[29)] and contained the following fertilizers

(g/ton of sea water; salinity 34‰): $(NH_4)_2SO_4$, 150; super-phosphate, 15; urea, 10; Clewat-32, 30. Japanese *Nannochloropsis* and Malaysian *Chlorella* were incubated at 25℃ and 35℃, respectively, and harvested when the cell concentrations reached 5-6 times the innoculation levels.

Extraction of Lipids, Preparation of FAME, and GLC on Packed Columns

Lipids were extracted from alive bivalves by the method of Bligh and Dyer.[30] FAME were prepared by transmethylation with BF_3/methanol, and impurities were removed from the FAME by thin-layer chromatography (TLC) on silicagel G with petroleum ether-ether (95:5). GLC on packed columns using 10% DEGS or 5% Shinchrom E-71 was conducted as described previously.[31,32]

Silver Ion Chromatography

Silver ion chromatography was conducted by the method of Christie[33] using solid-phase extraction columns packed with a bonded-sulfonic acid. Bond Elut™ SCX (ρ -propylbenzene sulfonic acid) and PRS (Na-propylsulfonate) solid-phase extraction columns were purchased from Analytichem International, U.S.A. For better separation of FAME, however, the solvent elution scheme recommended by Christie[33] were slightly modified as follows: When Bond Elute (500 mg) was used, the elution was conducted with 5 m*l* dichloromethane (Fr. 1), 5m*l* dichloromethane-acetone (92:8)(Fr. 2), 5 m*l* dichrolomethane-acetone (10:90)(Fr. 3), 10 m*l* acetone (Fr. 4), 10 m*l* acetone-acetonitrile (97:3)(Fr. 4), 5 m*l* acetone-acetonitrile (92:8)(Fr. 6), and 5 m*l* acetone-acetonitrile (60:40)(Fr. 7). Fractions 1 to 7 gave saturates, monoenes, dienes, trienes, tetraenes, pentaenes, and hexaenes, respectively. When the FAME of Japanese short-necked clam lipids was analysed by this method, there were less than about 5-10% cross-contamination of tetraenes and hexaenes fractions.

Capillary GLC

A Shimadzu Model GC-9AF gas-chromatograph (Shimadzu, Kyoto, Japan) was fitted with a capillary column and equipped with a dual flame-ionization detector and data processor Chromatopak C-R4A. The column is a fused silica wall coated (WCOT) open tubular column coated with CP-Sil 88 (100% cyanopropyl-polysiloxane) which has the almost same polarity as Silar10CP. The Tailor-made column, 50 m x 0.25mm FS-WCOT CP-Sil 88 (0.2 μm), was obtained from Chrompack International B.V. (The Netherlands) and has a high theoretical plate number, more than 225,000. GLC conditions are listed in Table 1.

For the identification of each peak, GLC was isothermally carried out at 170℃. Retention times (RT) from the front of solvent peak (hexane) to peak tops were determined[9] and relative retention times (RRT, relative to 18:0 methylester) were calculated. Equivalent chain length (ECL) values were read directly from the graph or obtained from the linear equation which was determined using reference 14:0, 16:0, 18:0, 20:0, and 22:0 methylesters. In this text, fatty acids were analysed in the methylester derivatives, unless speficied otherwise. In addition, attempt was carried out to separate the complex FAME mixtures within a short time in a single run by GLC. For this purpose, GLC was carried out under the prog-

Table 1. GLC conditions for analysis of FAME

Condition	Remark
Gas–chromatograph :	Shimadzu GC–9A (Shimadzu, Japan)
	(Split sampling was used)
Data processor	Chromatopak C–R4A (Shimadzu, Japan)
Column :	50 m x 0.25 mm i.d.,
	WCOT fused silica CP–Sil 88 (0.2 μm)
	Theoretical plate number = 225,000
Column temperature :	Programmed ; 170°C (40 min), 170°C → 220°C
	(2.5°C/min), 220°C (20 min)
	Isothermal ; 170°C
Injector temperature :	250°C
Detector temperature :	250°C
Carrier gas :	
	Helium, flow rate (ml/min) = 0.94
	Inlet pressure = 1.02 kg/cm^2
	Splitting ratio = 1/100
Make–up gas :	Helium (60 ml/min)
Sample size :	0.2–0.4 μl of a hexane solution of FAME
Detector FID	Attenuations 5 x10^{-22}

rammed temperatures (Table 1) or isothermally at a high temperature (200°C).

Identification of FAME by GLC

Individual FAME were identified on the basis of RRT or ECL obtained under the isothermal condations in GLC, mobilities in silver ion chromatography,[33] products of hydrogenation, and possible biosynthetic relationships. For instance, the FAME from *Tapes* lipids gave at least 90 peaks with relative abundance of 0.03% or more in GLC on CP-Sil 88 (Table 2 and Fig. 1). The individual FAME were provisionally identified by the following methods: Comparison of RRTs with (1) known standards and (2) secondary standards (cod liver oil), (3) comparison of equivalent chain lengths (ECL) with published data,[6,9,33] and (4) graphical techniques and systematic separation factors (plot of carbon number *vs.* log RRT). ECL values in GLC on packed column are known to be similar to those in GLC on open tubular columns.[1,33] Next, aliquots of *Tapes* FAME were subjected to silver ion chromatography to fractionate the FAME according to the number of double bonds in the molecule, and each fration was analysed by capillary GLC to ensure the accuracy of identification of peaks. Finally, each FAME fraction from silver ion chromatography was hydrogenated and rechromatographed on the capillary column to confirm the identification of major components with regard to carbon numbers and the stability of polyunsaturates during GLC. The presence of positional isomers with double bonds at n-15, n-13, or n-4, of which standards were not analysed, were tentatively identified in consideration of reported elution patterns. In GLC using polar liquid phases, DEGS etc., n-16 isomers are generally eluted first, followed by n-14, n-15, n-13, n-11, n-9, n-7, n-6, n-5, n-4, and n-3 isomers, in decreasing order

of elution.[1,33,34] Itabashi and Takagi[9] have also observed a similar elution pattern of isomers in GLC using glass WCOT Silar-5CP or WCOT Silar-10CP column.

Results and Discussion

Fatty Acid Compositions of Bivalves and Algae

In the present study, the capillary GLC on WCOT CP Sil-88 was used for the analysis of fatty acid compositions of bivalves, *Tapes* and *Corbicula*, and marine types of algae, *Nannochloropsis* and *Chlorella*. The results are given in Tables 2 to 5 and Figs. 1 to 4. Many peaks were detected in GLC, but attempts to identify the peaks were conducted on the components present at a level of 0.05% or more in a sample in GLC under the isothermal conditions (170℃).

The short-necked clam *Tapes* gave the complex fatty acid profiles as also observed in other bivalve species, reflecting the diversity of food organisms. The major fatty acids of *Tapes* were similar to those reported by Ueda,[35,36] who demonstrated by using GLC on packed column of 10% DEGS that the short-necked clam contained 16:0 (11.2-19.1%), 16:1 (3.4-8.4%), 18:0 (6.3-9.8%), 18:1 (5.9-9.0%), 20:1 (5.1-7.8%), 20:5 (11.3-14.8%), and 22:6 (8.4-17.9%) as the major fatty acids. The present study showed that 16:1n-7, 18:1n-9, and 20:1n-9 were the predominant monoene isomers in *Tapes* lipids. Salient aspects of *Tapes* fatty acid composition determined in the present study were the occurrence of high proportions of n-3 highly unsaturated fatty acids (HUFA) such as 20:5n-3, 22:6n-3, etc. Ueda[35] reported the occurrence of relatively large amounts of C_{16}-branched fatty acid (1.1-4.5%), 17:0 (2.1-3.9%), and C_{18}-branched fatty acid (3.1-6.7%) in *T. philippinarum*. But, these fatty acids were present at lesser levels in the short-necked clam analysed in the present study.

The brackish bivalve *Corbicula* contained lesser proportions of n-3 HUFA regardless of

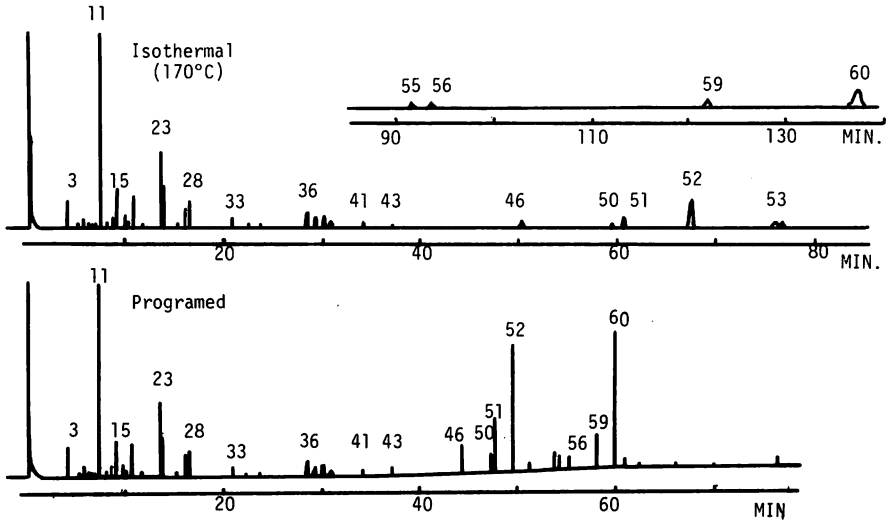

Fig. 1. GLC on WCOT CP-Sil 88 of fatty acid methylesters from the short-necked clam oil. Isothermal conditions at 170℃ and programmed temperatures. Letters indicate peak No. (see Table 2).

Table 2. Fatty acids of some bivalves and algae analysed by GLC on WCOT CP-Sil 88

Peak	RRT	ECL*1	Ag^+ col.*2	Fatty acid*3	PLO	*Tapes*	*Corbicula* (Gravel)	*Corbicula* (Muddy)	*Chlorella* (Malaysia)	*Nannochloropsis*
1	0.180	12.00		12 : 0	0.05	—	—	—	2.24	1.45
2	0.235	13.00	0	13 : 0	—	0.45	0.13	0.05	0.41	0.30
3	0.303	14.00	0	14 : 0	3.84	1.38	1.88	1.92	0.07	3.07
4	0.352	14.51		16 : 0(ip) *4	0.14	—	—	—	—	—
5	0.380	14.77		14 : 1(n−7)	0.05	—	0.17	0.15	0.75	0.75
6	0.393	14.88		14 : 1(n−5)	0.13	—	—	—	—	—
7	0.407	15.00	0	15 : 0	0.29	0.43	0.33	0.58	0.05	0.20
8	0.439	15.25	0	?	—	0.23	0.50	0.31	0.05	0.06
9	0.460	15.40	0	iso−16 : 0	0.29	0.23	0.22	0.34	0.06	0.09
10	0.506	15.72		15 : 1n−6	—	0.36	2.71	2.70	—	—
11	0.551	16.00	0	16 : 0	10.49	18.41	16.91	16.25	8.63	8.62
12	0.583	16.19	0	iso−17 : 0	—	0.17	0.34	0.14	0.09	—
13	0.638	16.49	1	16 : 1(n−11)	0.14	0.87	1.38	2.14	0.47	—
14	0.648	16.54		16 : 1(n−9)	0.15	0.66	0.65	0.25	4.89	1.10
15	0.677	16.69	1	16 : 1(n−7)	7.25	3.21	5.38	9.47	0.40	13.24
16	0.689	16.75	1	16 : 1(n−5)	0.95	0.35	0.84	0.44	0.01	0.15
17	0.740	16.98	1	?	0.15	0.95	1.83	1.10	1.12	0.05
18	0.753	17.05	0	17 : 0	—	0.18	0.62	0.28	0.06	0.25
19	0.792	17.22	0	iso−18 : 0	—	2.49	1.81	1.17	0.04	—
20	0.866	17.51	2	16 : 2(n−4)	0.50	0.51	0.21	0.11	2.45	0.82
21	0.887	17.60		17 : 1(n−8)	—	—	0.10	0.19	0.12	0.15
22	0.966	17.88		16 : 2(n−3)	0.09	—	0.19	0.27	0.07	0.18
23	1.000	18.00	0	18 : 0	0.18	7.51	4.80	4.69	0.52	0.14
24	1.011	18.04	0	iso−19 : 0	—	3.71	0.41	0.07	0.06	—
25	1.118	18.37	1	18 : 1(n−11)	0.05	0.42	0.28	0.36	0.27	0.05
26	1.164	18.51		16 : 3(n−3)	0.43	—	0.11	0.09	6.27	0.11
27	1.183	18.56	1	18 : 1(n−9)	11.88	2.09	2.69	6.04	2.28	1.86
28	1.213	18.65	1	18 : 1(n−7)	4.65	2.68	2.65	5.00	1.93	0.20
29	1.239	18.72	4	16 : 4(n−3)	—	—	—	—	6.19	—
30	1.261	18.78		18 : 1(n−5)	0.33	—	0.10	0.16	—	—
31	1.350	19.00		19 : 0	0.05	—	—	—	—	—
32	1.486	19.32		16 : 4(n−1, Δ^6)	0.64	—	—	—	—	—
33	1.532	19.42		18 : 2(n−6)	0.87	1.26	1.51	4.38	6.52	2.98
34	1.634	19.64	2	18 : 2(n−3)	0.16	0.44	0.21	0.14	0.07	—
35	1.845	20.05		18 : 3(n−6)	—	—	—	—	11.38	—
36	2.069	20.43	3	18 : 3(n−3)	0.48	2.64	6.52	3.04	26.29	—
37	2.071	20.43		20 : 1(n−11)						
38	2.121	20.52	1	20 : 1(n−9)	10.15	1.31	1.09	3.11	—	—
39	2.189	20.62	1	20 : 1(n−7)	2.79	2.15	0.40	0.82	0.14	0.41
40	2.251	20.71	1	20 : 1(n−5)	0.27	0.88	—	0.36	0.27	—
41	2.494	21.05	4	18 : 4(n−3)	1.99	0.96	1.31	1.87	0.66	0.11
42	2.545	21.12	2	20 : 2(n−9)	—	—	—	—	1.70	0.79
43	2.727	21.35		20 : 2(n−6)	0.28	1.28	1.00	0.72	0.05	0.23
44	3.083	21.76		20 : 3(n−6)	0.05	—	0.17	0.28	—	—
45	3.340	22.03		20 : 3(n−3)	0.19	—	0.17	0.07	—	—
46	3.686	22.36	4	20 : 4(n−6)	0.50	2.10	3.29	2.01	0.26	5.89
47	3.830	22.45		22 : 1(n−11)	12.36	—	—	—	0.11	—
48	3.894	22.54		22 : 1(n−9)	1.75	—	—	—	0.09	0.09
49	4.066	22.69		20 : 4(n−3)	0.62	—	—	—	—	—
50	4.356	22.91	2	22 : 2(n− ?)	—	1.21	2.17	1.70	0.17	0.28
51	4.457	22.99	2	22 : 2(n− ?)	—	4.96	—	—	0.45	0.39
52	4.961	23.35	5	20 : 5(n−3)	10.66	9.93	7.84	9.34	0.22	39.34
53	5.581	23.75	3	22 : 3(n−6)	—	1.26	1.00	0.83	—	1.10
54	5.649	23.78		22 : 3(n− ?)	—	—	1.16	—	—	—
55	6.679	24.35	4	22 : 4(n−6)	0.08	1.41	—	—	—	1.10
56	6.832	24.42	1	24 : 1(n−9)	0.57	0.90	0.44	0.57	0.33	0.35
57	7.213	24.60		22 : 4(n−3)	0.13	—	1.23	1.29	0.37	0.26
58	7.538	24.75		22 : 5(n−6)	0.05	—	—	—	—	—
59	8.871	25.29	5	22 : 5(n−3)	1.02	2.19	3.35	3.30	0.09	0.06
60	10.025	25.70	6	22 : 6(n−3)	6.95	14.40	13.50	6.68	0.47	1.08

*1 The separation could be achieved on the isomers differing in ECL (equivalent chain length) value by about 0.05

*2 Letters indicate the number of double bond which was estimated by the elution order in Ag^+ column chromatography

*3 RRT of standards : 20:0 (1.797), 22:0 (3.300), 23:0 (4.652).

*4 ip, isoprenoid fatty acid.

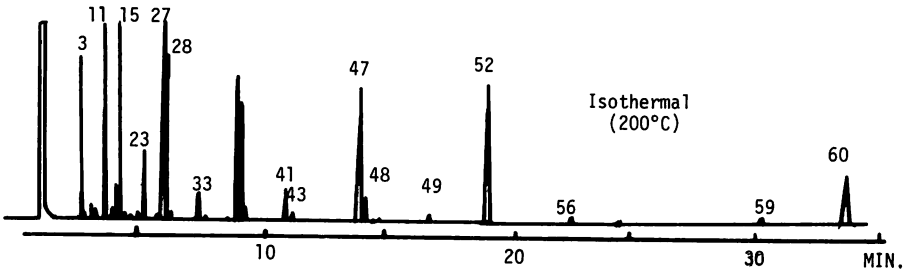

Fig. 2. GLC on WCOT CP-Sil 88 of fatty acid methylesters from pollack liver oil (PLO). Isothermal conditions at 200℃. Letters indicate peaks No. (see Table 2).

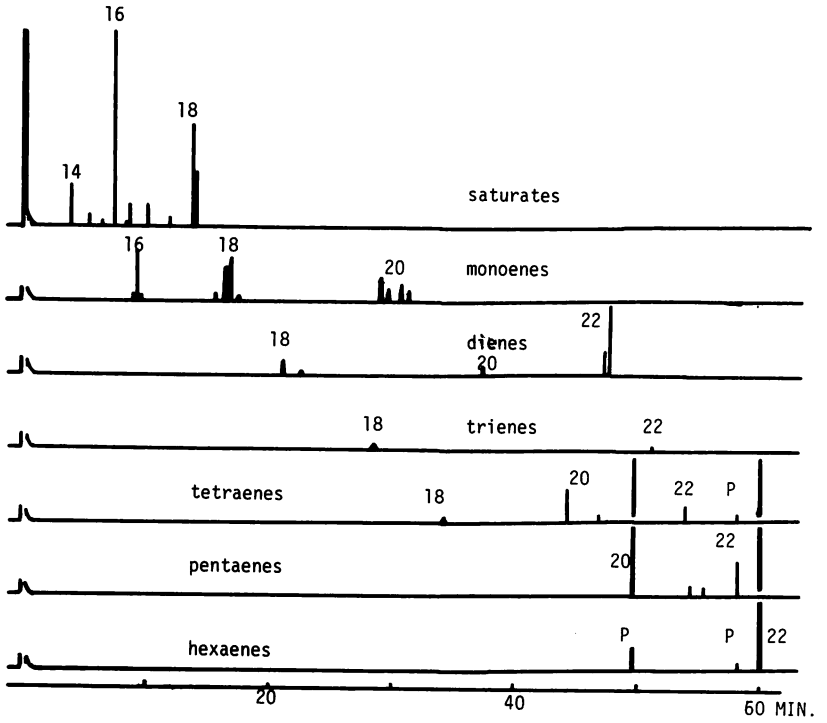

Fig. 3. GLC of the fractions obtained by $AgNO_3$ column chromatography of fatty acid methylesters from the short-necked clam lipids. Programmed temperatures. Letters, 14, 16, 18, 20, and 22, indicate the carbon numbers of the components.

the habitats than the marine bivalve, *Tapes*. But, the *Corbicula* from the gravel region had a higher proportion of 22:6n-3 than that from the muddy region. This suggests that the fatty acid composition of *Corbicula* varies with the habitats. Ueda[35] has thought that the variation in fatty acids of *Tapes* with environmental temperature is probably due to the direct influence of temperature on the fatty acid metabolism rather than an indirect influence through the food-chain.

Table 3. Separation factors of isomers with same chain lengths

Monoenes	Separation factor						
(isomers)	16 : 1	18 : 1	20 : 1	22 : 1	18 : 2	20 : 3	20 : 4
n−9/n−11	1.02	1.03	1.03	1.03			
n−7/n−9	1.04	1.03	1.03	−			
n−5/n−7	1.05	1.04	1.03	−			
n−6/n−9	−	−	−	−	1.73	1.72	
n−3/n−6	−	−	−	−	1.12	1.08	1.10
n−9/Sat*	1.18	1.18	1.18	1.18			

* Saturated FAME

Table 4. Separation factors of dienes and polyenes

Fatty acids	n−3/n−6	n−6/n−9	n−3/n−9
18 : 3 (n−3)			
18 : 2 (n−6)	1.35		
18 : 1 (n−9)		1.30	1.75
18 : 4 (n−3)			
18 : 3 (n−6)	1.33		
20 : 4 (n−3)			
20 : 3 (n−6)	1.33		
20 : 2 (n−9)		1.21	1.71
20 : 5 (n−3)			
20 : 4 (n−6)	1.35		
22 : 5 (n−3)			
22 : 4 (n−6)	1.33		

Table 5. Separation factors of the same chain lengths with varying numbers of double bonds and the same end carbon chains

Fatty acid	Separation factor	Fatty acid	Separation factor
22 : 6 (n−3)		20 : 4 (n−3)	
22 : 5 (n−3)	1.13	20 : 3 (n−3)	1.21
22 : 5 (n−6)		20 : 3 (n−6)	
22 : 4 (n−6)	1.13	20 : 2 (n−6)	1.20
22 : 5 (n−3)		20 : 2 (n−9)	
22 : 4 (n−3)	1.22	20 : 1 (n−9)	1.20
22 : 4 (n−6)		18 : 4 (n−3)	
22 : 3 (n−6)	1.20	18 : 3 (n−3)	1.20
20 : 5 (n−3)		18 : 3 (n−6)	
20 : 4 (n−3)	1.21	18 : 2 (n−6)	1.20
20 : 4 (n−6)			
20 : 3 (n−6)	1.20		

The algae, *Nannochloropsis* sp. and *Chrolella* sp., are often used as algal diets for the rotifer *Brachionus plicatilis* in the seed production of fish.[24,37-39] The Japanese *Nannochloropsis* contained 16:0, 16:1n-7, and 20:4n-6 as prominent fatty acids in addition to large amounts of 20:5n-3 (39.3% of total fatty acids) as demonstrated in earlier studies using GLC on packed columns.[29] *Nannochloropsis* sp. is generally thought to be good algal diets due to the inclusion of large amounts of 20:5n-3 as essential fatty acids.[24,39] It is, therefore, noteworthy that the *Nannochloropsis* contained substantial amounts of 20:4n-6 (5.8%), which is often overlapped with 22:1 in GLC on DEGS packed column. The Malaysian *Chlorella* contained 16:0, 16:1n-9, 16:4n-3, 16:3n-3, 18:3n-6, and 18:3n-3 as the prominent fatty acids. In contrast to the Japanees *Nannochloropsis*, the Malaysian *Chlorella*, nevertheless the marine species, contained only small amount of n-3 and n-6 HUFA, the major EFA being 18:2n-6, 18:3n-6, and 18:3n-3. The Japanese *Nannochloropsis* and Malaysian *Chlorella* are closely related algae, but the biosynthesis of fatty acids in two the algae is conceived to be different each other. Further elongation and desaturation of 18:3n-3 and 18:3n-6 to HUFA may be supressed in the Malaysia *Chlorella* growing at high water temperatures as indicated in the Japanese *Nannochloropsis* (formerly, marine *Chlorella*) cultured at a high temperature.[29] The fatty acid profile of the Malaysian *Chlorella* suspects that the rotifer cultured with Malaysian *Chlorella* have a low nutritive value for marine fishes which strictly require n-3 HUFA as an essential fatty acid.

Separation of FAME by Capillary GLC on WCOT CP-Sil 88

In GLC on packed columns, three types of polar liquid phases are often used[1-4]: (1) high polarity phases such as DEGS (diethyleneglycol succinate), EGS (polyethyleneglycol succinate), EGSS-X (a copolymer of EGS with a methylsilicone), and Silar-10CP (cyanoalkylpolysiloxane), (2) medium polarity phases such as BDS (buthanediol succinate), EGSS-Y (a copolymer of EGS with a higher proportion of the methylsilicone than in EGSS-X), PEGA (polyethyleneglycol adipate), Shinchrom E71 (polyester containing nitrile groups) and Silar-5CP, and (3) low polarity phases such as NPGS (neopentylglycol succinate). Cyanoalkylpolysiloxane liquid phases (Silar-5CP, Silar-10CP, SP-2340, OV-275, etc.) are useful because of a higher thermal stability. In GLC on packed columns such as DEGS, however, the separation of some fatty acids are generally impossible or incomplete, and also the elution pattern changed with the degree of degration of column after preparation.[1,11] For instance, Hibino *et al.*[11] have shown in GLC on DEGS that the pairs of 18:3n-3/20:1 and 20:4n-6/22:1 were not separated and those of 16:3/18:1 and 16:4/18:2 partially overlapped, and also that the separation of 16:2/18:0 and 18:0/16:3 were incomplete. We[32] also observed that 5% Shinchrom E-71 packed column gave no separation of the following pairs of fatty acids; 16:2n-6/16:2n-7, 16:3n-6/17:0; 16:3n-3/iso-18:0, 18:2n-6/18:2n-9.

In the present study, the fatty acids of the bivalves and algae were analysed by GLC using WCOT CP Sil-88. As shown in Fig. 4, there was an almost linear relationship between log (RRT) and carbon length of analogous FAME, although several workers showed that the plots of log (RRT) *vs.* carbon numbers on the capillary columns with high polarities such as DEGS,[40] OV-1,[11] and Silar-10CP[11] are approximated to be quadratic rather than linear.

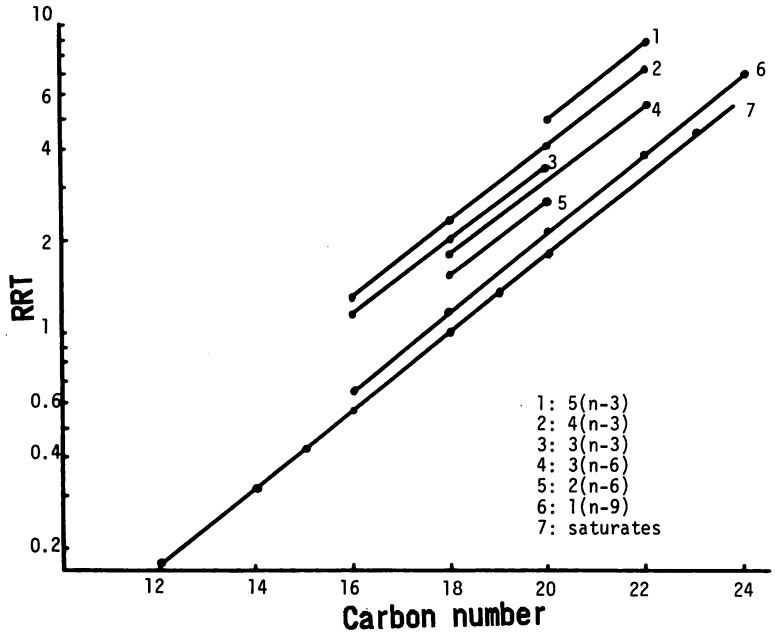

Fig. 4. Relationship between log (RRT) and carbon numbers for saturated and unsaturated fatty acid methylesters. S, saturated; 1(n-9), monoenes(n-9); 2(n-6), dienes(n-6); 3(n-3), trienes (n-3); 4(n-3), tetraenes (n-3); 5(n-3), pentaenes(n-3).

$Y = 0.129X - 2.313$ ($r = 0.999$)

$Y = 0.001X^2 + 0.096X - 2.036$ ($r = 1.00$)

The WCOT CP Sil-88 column sufficiently separated the pairs of 20:3n-9/20:2n-6 (RRT: 2.868/2.727) and 20:5n-3/22:1 which are not separable on the DEGS packed column. There were no overlap between the pairs of 18:3n-3/20:1n-9, 20:4n-6/22:1, 16:3n-3/18:1n-7, 16:4n-3/18:2n-6, 16:2n-4/18:0 and 18:0/16:3n-3. Moreover, the capillary column gave a good separation of the probable positional isomers of monoenes such as 14:1 (n-7, n-5), 16:1 (n-9, n-7, n-5), 18:1 (n-11, n-9, n-7, n-5), 20:1 (n-11, n-9, n-7), and 22:1 (n-11, n-9, n-7), polyenes such as 18:2 (n-9, n-6, n-3), 18:3 (n-9, n-6, n-3), 20:3 (n-6, n-3), 20:4 (n-6, n-3), 20:5 (n-6, n-3), and 22:6 (n-6, n-3). However, 18:3n-3 were not separated from 20:1n-11 in this column. Possible occurrence of 15:0 and 17:0 branched fatty acids and separation of iso- and anteiso-fatty acids were also suggested. C_{18}-and C_{20}-monoenes with shortest RRTs were assumed to be 18:1n-13 and 20:1n-15 with a double bond at C-5. C_{22}-dienes (RRT 4.356, 4.457) with shorter RRT than 20:2n-9 and 22:2n-6, respectively, were tentatively speculated to be non-methylene interrupted (NMID) fatty acids (possibly 22:2 $\Delta^{7,13}$, and 22:2 $\Delta^{7,15}$) which were present in molluscs[40] and sea urchins.[41]

The GLC under the programmed temperatures (Fig. 1) or the isothermal temperature of 200℃ (Fig. 2) lost something of the resolution of fatty acids but gave the chromatograms

with much of essential information in the reduced analytical time. Earlier studies have shown that there is some loss of polyunsaturated fatty acids within capillary columns, Itabashi and Takagi[11], however, found that such a loss of polyunsaturated fatty acids could be markedly reduced the shortening of RT. They[11] revealed that the ratios of peak areas correspond to those of weight ratios in GLC on the capillary columns, OV-1 and Silar-5CP, as well as on packed columns when GLC is conducted under the conditions that 22:6 elutes within 100 minutes. Therefore, we believe that the GLC on WCOT CP-Sil 88 give sufficient information on fatty acid compositions, in the viewpoint of fish and nutrition, with short analytical times when conducted at a high temperature of 200℃ (isothermal) or the programmed conditions. In this study, however, some of small peaks with longer RRTs than 20:5n-3 peak seemed to be lost when analyzed under the isothermal conditions (170℃).

There are applications of the capillary column to the fatty acid analysis of marine lipids. In these studies, fatty acid compositions have been analysed by using a variety of types of columns with different polarity liquid phases; Silar-10CP (glass, WCOT),[9] Silar-5CP (glass, WCOT),[9,10] SP-2340,[4] Carbowax 20M (fused silica, WCOT),[11] FAAP (chemical bonded silica),[12] and Supelcowax-10 (bonded carbowax 20M)[13] or non-polar columns, OV-1 (glass, WCOT),[9] SE-30 (glass, SCOT),[14] and SP-2100 (fused silica, WCOT).[15,16] In the present study, we attempted to determine the components of complex fatty acid mixtures by GLC on a fused silica WCOT CP-Sil 88 in conjunction with silver ion chromatography. As a result, an excellent separation of isomers with the same number of double bonds was achieved by this column. The identification of each compounds could be achieved in a relatively short analytical times when complex mixtures of FAME are analysed by GLC on WCOT CP Sil-88 after fractionation by Ag^+ chromatography using a commercially prepacked solid phase (Bond Elut SCX). However, there were the chain length overlap of different carbon chain lengths as also observed in GLC on other high polarity columns.[33,42] For simplification of analysis of fatty acids in marine lipids, Ackman[42] and other several workers[11,33] have proposed the use of the liquid phase of the Carbowax 20M or Silar-5CP because of the absence of the confusing overlap of important fatty acids with different chain lengths.

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