Fatty Acids in the Lipid of Food Products from Sea Urchin

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The fatty acid compositions of the lipids from the fermented sea urchin paste, the salted sea urchin gonads, and the raw sea urchin gonads have been investigated with open-tubular gas-liquid chromatography. All the samples contained about 10.1-17.1% of the unusual 5-olefinic acids such as 5-18:1, 5-20:1, 5, 11-20:2, 5, 13-20:2, 5, 11, 14-20:3, and 5, 11, 14, 17-20:4 and about 0.9-1.3% of the unusual 7-olefinic acids such as 7, 13-22:2 and 7, 15-22:2, similar to the previous studies in our laboratory. The major polyunsaturated acids of these samples were 18:4 n-3 (0.2-6.3%), 20:4 n-6 (4.1-7.6%), and 20:5 n-3 (1.9-17.0%). All the samples showed about 40 species of fatty acid components and various fatty acid compositions. Especially, the salted sea urchin gonads B contained 20:5 n-3, ten times as much as the salted sea urchin gonads A. It was inferred that the difference between fatty acid compositions of these samples were related to the fatty acid compositions of the sea urchins which were used as the material.

The processed sea urchin gonads are fermented marine foods in Japan. The lipids of sea urchins and their food products have been investigated in some previous papers.¹⁻¹⁰⁾ Recently, the fatty acid compositions of sea urchin have been elucidated in detail by open-tubular gas-liquid chromatography (GLC) in our laboratory.^{11-13), *2} The occurrence of the unusual 5-olefinic acids such as 5-18:1, 5-20:1, 5, 11-20:2, 5, 13-20:2, 5, 11, 14-20:3, and 5, 11, 14, 17-20:4 was noticeable in the lipids of sea urchins, amounting to as much as 6-20% of fatty acids from the total lipids of whole animals. In this study, fatty acids of lipids from two kinds of food products from sea urchin, the fermented sea urchin paste (Neri-uni) and the salted sea urchin gonads (Shio-uni), have been investigated in greater detail. The 5-olefinic acids were found in the lipids of all the samples. The major polyunsaturated acids of these samples were 20:4 n-6 and 20:5 n-3. Recently, these polyunsaturated acids have been noticeable in biochemistry and pharmacology as precursors of prostaglandins. In this study, the contents of the 5-olefinic and polyunsaturated acids of these two kinds of products and the raw sea urchin gonads were compared one another.

Materials and Methods

Materials

The samples of the food products from sea urchin studied and the contents of the lipids are listed in Table 1. The fermented sea urchin paste (Neri-uni) and the salted sea urchin gonads (Shio-uni) A and B were bought at food markets in Hakodate in February, 1980 (the fermented sea urchin paste and the salted sea urchin gonads A) and October, 1982 (the salted sea urchin gonads B), respectively. The raw gonads were separated from sea urchin *Strongylocentrotus nudus* obtained from subtidal water near Hakodate. They were kept frozen at -20°C for a few months until used.

Preparation of Methyl Esters

The total lipids were extracted by the method of Bilgh and Dyer.¹⁴⁾ Fractionation of the total lipids (TL) into the neutral and polar lipids (NL and PL) was carried out by column chromatography using silicic acid (Kiesel Gel 60, Merck) with chloroform and methanol as the developing solvents. Isolation of triacylglycerols (TG) and free fatty acids (FFA) from NL were carried out by thin-layer chromatography (TLC) with Silica Gel

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Table 1. Samples and lipid contents (wt%)*

Sample	TL	NL	FA	TG	FFA
Fermented sea urchin paste	3.1	—	—	—	—
Salted sea urchin gonads A	6.1	—	—	—	—
Salted sea urchin gonads B	8.2	77.4	74.4	30.2	19.9
Raw sea urchin gonads	5.9	71.9	64.3	—	—

* TL: Total lipids of a wet sample. NL: Neutral lipids of TL. FA: Recovered fatty acids of TL. TG: Triacylglycerols of TL. FFA: Free fatty acids of TL.

G plate of thickness 0.5 mm by developing with *n*-hexane/ether (85:15 v/v). Lipids were saponified by refluxing with 1 N KOH-EtOH for 1 h, and the unsaponifiables were extracted with ether. Following acidification with dilute HCl, fatty acid were recovered by ether extraction. Recovered fatty acids and FFA were converted to methyl esters by heating in a sealed tube at 100°C for 10 min with 7% BF₃-MeOH. TG were converted to methyl esters by direct transesterification; heating in a sealed tube with 5% HCl-MeOH at 80°C for 3 h or 0.5 N CH₃ONa-MeOH at 60°C for 20 min. The methyl esters were separated from other products by TLC under the same conditions as used in isolation of TG and FFA.

Gas-Liquid Chromatography (GLC)

Open-tubular GLC of the methyl esters was done with a Shimadzu GC 6AM and 7A instruments (Shimadzu Seisakusho Co., Kyoto) with a flame-ionization detector, on wall-coated open-tubular glass column coated with SP 2300 (50 m × 0.3 mm id). The column temperature was 180°C and injector and detector were held at 230°C. Peak area percentages were obtained with Shimadzu integrators C-R1A and C-R2AX. The component of each peak on the gas chromatogram was identified on the basis of comparison of the retention data with those of the reference specimen and methyl esters of sea urchin fatty acids^{12,13} and algae fatty acids.¹⁵ The log plot procedures and systematic separation factor procedures¹⁶ were used concurrently for the identification.

Results and Discussion

Major Fatty Acid Components

The fatty acid compositions of the lipids of samples are shown in Table 2. The major components, those percentages to the total fatty acids were above 3% in one or more samples, were 14:0, 16:0, 16:1 n-7, iso-17:0, 18:0, 18:1 n-9, 18:1 n-7, 18:4 n-3, 5-20:1, 20:1 n-9, 5, 11-20:2, 20:4 n-6, 20:3 n-3, 20:5 n-3 and 22:1 n-9. The

total contents of the components above 3% were relatively higher, amounting to 85–90%. There was no significant difference between fatty acid components of each samples.

5-Olefinic Acids

All the samples contained about 10–17% of the 5-olefinic acids. The results were similar to those of the previous study of sea urchins.^{11–13} In this study, the total contents of the 5-olefinic acids were 4–7 mg per gram of the samples. The major 5-olefinic acids of these samples were 5-20:1, 5, 11-20:2, and 5, 13-20:2. Especially, 5-20:1 was concentrated in polar lipids like 20:4 n-6 and 20:5 n-3. The 7-olefinic acid such as 7, 13-22:2 and 7, 15-22:2 were reported in the previous papers.^{11–13} They were presumed to be formed by C₂ elongation of 5, 11-20:2 and 5, 13-20:2. These fatty acids were also found in this study (0.8–1.3%).

Polyunsaturated Acids

The major polyunsaturated acids of these samples were 18:4 n-3, 20:4 n-6, and 20:5 n-3. The contents of these acids were 6–30% of total fatty acids. Especially, the salted sea urchin gonads B contained 8% of 20:4 n-6 and 17% of 20:5 n-3. These acids are precursors of prostaglandins, and biological activity of prostaglandins has been noticeable in biochemistry and pharmacology. The contents of 20:4 n-6 and 20:5 n-3 were 5 mg and 10 mg per gram of this sample, respectively.

Fatty Acids from Each Lipid

Compositions of fatty acids from PL, NL, TG, and FFA of the salted sea urchin gonads B and raw sea urchin gonads are shown in Table 2. The fatty acids from PL were generally rich in the 5-20:1, 20:4 n-6, and 20:5 n-3. 20:4 n-6 and 20:5 n-3 in NL were concentrated in FFA. Fujino *et al.*⁹ reported that the salted sea urchin egg contained a smaller amount of TG, phosphatidyl ethanolamine, and phosphatidyl choline and a larger amount of FFA than the raw sea urchin

Table 2. Fatty acid compositions of the lipids from the samples (wt%)*

Fatty Acid	RRT	ECL	Paste TL	A-TL	B-TL	B-PL	B-NL	B- TGNa	B- TGHCl	B- FFA	Raw- TL	Raw- PL	Raw- NL
14:0	0.263	14.00	11.44	8.23	6.86	5.95	7.57	8.85	9.24	6.46	16.45	11.21	18.77
iso-15:0	0.309	14.47	0.21	0.30	0.63	0.41	0.67	0.85	0.90	0.62	1.61	1.56	1.76
15:0	0.364	15.00	0.72	0.61	0.23	0.16	0.24	0.28	0.32	0.24	0.37	0.26	0.40
16:0	0.525	16.00	26.39	27.02	15.31	17.73	14.32	19.59	20.23	14.39	17.62	14.57	19.37
16:1 n-7	0.591	16.37	2.68	4.98	4.84	4.27	5.33	6.84	7.00	4.22	3.88	2.59	4.17
iso-17:0	0.609	16.46	2.37	1.83	1.81	0.50	2.00	2.27	2.30	1.90	4.26	1.85	4.42
17:0	0.717	17.00	0.52	0.20	0.36	0.26	0.38	0.30	0.37	0.42	—	—	—
iso-18:0	0.880	17.60	0.10	—	0.37	0.20	0.39	0.28	0.29	0.54	0.15	—	0.19
18:0	1.000	18.00	3.50	4.57	2.48	3.96	2.06	1.86	1.99	2.24	1.40	2.57	1.33
18:1 5	1.055	18.16	1.03	1.02	0.71	0.35	0.81	0.56	0.64	0.67	0.43	0.25	0.48
18:1 n-9	1.113	18.33	2.27	2.64	1.63	1.48	1.74	2.54	2.70	1.37	3.04	1.49	3.30
18:1 n-7	1.143	18.41	4.84	3.76	2.62	1.12	2.76	2.98	2.97	2.84	3.86	1.87	3.92
18:1 n-5	1.174	18.49	0.31	0.30	0.19	0.08	0.22	0.15	0.21	0.22	0.43	0.22	0.49
18:2 n-9	1.279	18.75	0.52	0.20	0.41	0.16	0.45	0.55	0.56	0.41	1.35	0.63	1.55
18:2 n-6	1.314	18.83	0.72	1.32	0.96	0.62	1.07	1.25	1.16	1.01	1.24	0.91	1.27
18:3 n-6	1.450	19.13	0.62	0.41	0.49	0.40	0.52	0.52	0.51	0.54	0.85	0.51	1.04
18:3 n-3	1.616	19.46	0.82	0.81	1.99	1.12	2.33	2.29	1.92	2.37	1.23	0.67	1.30
18:4 n-3	1.799	19.79	0.21	0.20	6.27	2.36	7.56	6.37	5.08	8.76	2.78	1.44	2.86
20:0	1.928	20.00	1.03	1.02	0.66	0.94	0.55	0.72	0.78	0.62	0.57	0.88	0.48
20:1 5	2.056	20.19	5.77	7.11	4.57	8.74	3.53	3.49	3.80	3.55	3.09	5.63	2.43
20:1 n-11	2.091	20.24	1.03	0.20	0.69	1.01	0.58	0.91	1.03	0.57	0.92	0.86	0.97
20:1 n-9	2.150	20.32	5.15	4.77	2.75	3.17	2.63	3.11	3.19	2.45	4.67	3.88	4.85
20:1 n-7	2.201	20.39	—	—	0.84	0.62	0.94	1.02	1.08	0.96	1.34	1.04	1.42
20:2 5, 11	2.292	20.51	7.73	6.81	4.50	1.84	5.21	4.60	4.81	4.05	4.55	4.81	4.42
20:2 5, 13	2.320	20.54	1.75	1.63	1.33	0.56	1.40	1.44	1.56	1.30	1.81	1.17	1.96

Table 2. (Continued)

Fatty Acid	RRT	ECL	Paste TL	A-TL	B-TL	B-PL	B-NL	B- TGNa	B- TGHCl	B- FFA	Raw- TL	Raw- PL	Raw- NL
20: 2 n-6	2.537	20.81	1.44	2.84	2.12	1.72	2.21	1.90	1.94	2.04	1.98	2.26	1.60
20: 3 5, 11, 14	2.673	20.96	0.31	0.41	0.23	0.12	0.26	0.20	0.21	0.30	0.15	0.18	0.14
20: 3 n-6	2.775	21.07	0.62	0.51	0.58	0.19	0.69	0.77	0.76	0.68	1.20	0.44	1.23
20: 4 n-6	2.982	21.28	4.74	4.06	7.63	9.45	6.24	4.14	4.01	7.01	5.92	15.42	3.57
20: 3 n-3	3.111	21.41	2.68	3.86	2.24	2.57	2.11	2.27	2.04	2.42	0.85	0.92	0.84
20: 4 5, 11, 14, 17	3.273	21.56	0.11	0.15	0.26	0.07	0.27	0.21	0.18	0.31	0.08	0.03	0.09
20: 4 n-3	3.413	21.68	0.62	0.41	2.15	0.51	2.53	2.26	1.95	2.79	1.10	0.47	1.25
20: 5 n-3	3.691	21.91	2.27	1.93	17.01	18.48	16.31	9.41	8.63	18.42	6.14	12.10	4.11
22: 1 n-9	4.099	22.22	3.81	4.16	2.60	6.31	2.49	3.35	3.63	1.80	3.11	5.34	2.45
22: 2 7, 13	4.384	22.41	0.21	—	0.23	0.48	0.19	0.24	0.24	0.19	0.29	0.38	0.29
22: 2 7, 15	4.481	22.47	0.93	1.32	0.65	1.43	0.55	0.70	0.80	0.40	0.57	0.99	0.54
22: 5 n-3	6.935	23.75	0.21	0.20	0.34	0.37	0.33	0.35	0.34	0.34	0.12	0.11	0.13
22: 6 n-3	7.301	23.88	0.31	0.20	0.47	0.26	0.55	0.56	0.62	0.58	0.59	0.49	0.62
Total Sat.			46.28	43.78	28.70	30.12	28.19	35.00	36.42	27.42	42.43	32.90	46.73
Total Mono.			26.90	28.95	21.44	27.15	21.03	24.96	26.25	18.67	24.78	23.17	24.47
Total Poly.			26.81	27.27	49.86	42.73	50.77	40.03	37.33	53.90	32.79	43.93	28.80
Total 5-Olefin			16.71	17.12	11.59	11.69	11.47	10.50	11.22	10.19	10.10	12.08	9.52
AA+EPA			7.01	5.99	24.64	27.93	22.55	13.55	12.63	25.42	12.06	27.52	7.68

* RRT: Relative retention time, ECL: Equivalent chain length, Paste: Fermented sea urchin paste, A: Salted sea urchin gonads A, B: Salted sea urchin gonads B, Raw: Raw sea urchin gonads, TL: Total lipids, PL: Polar lipids, NL: Neutral lipids, TG: Triacylglycerols, FFA: Free fatty acids, Na: Methylation by CH_3ONa , HCl: Methylation by HCl, Sat.: Saturated acids, Mono.: Monoenoic acids, Poly.: Polyunsaturated acids, 5-Olefin: 5-Olefinic acids, AA: 20: 4 n-6, EPA: 20: 5 n-3.

egg. Further, they reported that unsaturated fatty acids of C_{18} – C_{22} such as 18:2, 18:4, 20:0 (or 18:3), 20:1, 20:2, 20:4 (or 22:0), 20:5, and 22:1 seemed to be liberated rather easily than the other kinds of fatty acid during curing with salt. In this study, high contents of 20:4 n-6 and 20:5 n-3 in FFA were also caused by liberation of esterified fatty acids by activity of the enzyme.

TG were converted to methyl esters by acid catalyst and alkaline catalyst. There were no significant difference between the fatty acid compositions obtained with the two catalytic methylation methods. This result suggests that cyclic fatty acids which were unstable for acid catalyst, were not exist in the lipids of sea urchins.

Comparison of Fatty Acid Compositions among Products

The fatty acid composition of the salted sea urchin gonads A was similar to that of the fermented sea urchin paste rather than the salted sea urchin gonads B. The materials of the salted sea urchin gonads A and B were obtained from the same place (Rishiri Island), but their harvest times were different (A: November, 1979, B: July, 1982). Furthermore, fatty acid composition of the raw sea urchin gonads was different from other samples. Fujino *et al.*⁹⁾ reported that there was no significant difference between the total fatty acid compositions of raw and salted sea urchin eggs. Then, it is inferred that the difference between fatty acid compositions of the samples in this study are related to the fatty acid compositions of the sea urchins which are their materials.

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