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Note

Lipid Contents and Fatty Acid Composition of Total Lipid of Sea Cucumber *Stichopus japonicus* and *Konowata* (Salted Sea Cucumber Entrails)

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The fatty acid compositions in the body wall, gonads, and alimentary canal of male and female *Stichopus japonicus* caught in the Sea of Okhotsk off the coast of Abashiri were analyzed to clarify their characteristics. The fatty acid composition was studied also in *konowata* sold at the market. The analysis was performed by capillary gas chromatography. Major fatty acids that constituted the lipids of the body wall were iso-C15, C16:0, C16:1n-7, C18:0, C18:1n-7, C20:4n-6, C20:5n-3, C23:1n-9, and C22:6n-3. The fatty acid compositions in various tissues were nearly identical between males and females. Although the fatty acid compositions in the body wall and gonads were similar, they markedly differed from the fatty acid composition in the alimentary canal. The iso-C15, C16:0, and C16:1n-7 levels were very high in the body wall, and the C20:1n-9, C20:4n-6, and C20:5n-3 levels were high in the alimentary canal. A very high monoenoic acid content and the absence of polyunsaturated fatty acids with the exception of icosapentaenoic acid were characteristics of the fatty acid composition in *konowata*.

Keywords: sea cucumber, body wall, gonads, alimentary canal, fatty acid, *konowata*

Lipids in marine products are important nutrient components and are related to their deliciousness in season. Lipids are also an important factor in the deterioration of quality of raw sea foods and seafood products. Therefore, the lipid contents and fatty acid composition in marine products is a matter of interest in food science and nutrition. Sea cucumbers are seasoned with vinegar and eaten raw or are processed into foods such as "*konowata*" by salting the entrails and gonads, "*iriko*" by drying the muscles, and "*kuchiko*" by drying the gonads.

Some types of sea cucumbers are known to contain considerable quantities of fatty acid C23:1 (n-9), which is absent in other marine animals (Kaneniwa *et al*., 1986). A new fatty acid, 7-methyl-6-octadecenoic acid, has also been discovered (Carballeira *et al*., 1996). The fatty acid compositions of sea cucumbers in markedly different habitats such as coastal regions of the Sea of Okhotsk, which are completely covered by drift ice during the coldest part of the year, are of great interest.

There have been a few reports about the fatty acid composition of lipids in the Holothuroidea of the Tropical and Temperate Zones (Carballira *et al*., 1996; Fredalina *et al*., 1999; Svetashev *et al*., 1991; Isay & Busarova, 1984; Svetashev & Busarova, 1984; Chang-Lee *et al.,* 1989). Also, the fatty acid composition of lipids of the *Stichopus japonicus* caught along the Pacific coast of Southern Hokkaido has been analyzed (Kaneniwa *et al*., 1986), but there are no studies concerning the fatty acid composition of the Holothuroidea caught in the colder Sea of Okhotsk.

Thus, we evaluated the lipid contents and the fatty acid composition in the body wall, alimentary canal, and gonads of male and female sea cucumbers caught in the Sea of Okhotsk. We also studied fatty acid compositions of salted and matured entrails of sea cucumbers (*konowata*), and compared them with those of raw sea cucumbers.

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Materials and Methods

We caught 125 mature green sea cucumbers (*S. japonicus*) in October, 2001 in the Sea of Okhotsk off the shore of Abashiri. They were 20–31.5 cm long and weighed 320–510 g. The body of each one was cut from the anus nearly to the oral organ, and the ovaries and testes were collected. The alimentary canal was separated and, after the contents were removed, washed carefully with distilled water. Lipids were extracted from 20 g each of the body wall, alimentary canal, and gonads, repeating the procedure 5 times each. The samples of *konowata* were products of Company A and Company B purchased at Tsukiji Market. Lipids were extracted by the Bligh and Dyer method (1959), and the total lipid content was determined gravimetrically. Fatty acids were methylated by the method of Metcalfe and Schmitz (1961). Analysis was performed using a Hitachi 263-50 gas chromatograph, to which a simple flow regulator unit (GL Science, Tokyo) and an FID detector were attached, and the results were recorded with a Hitachi D-2500 Chromato Integrator. Helium as a carrier gas was flowed at 0.4 ml/min through a capillary column Rtx-2330® (0.25 mm i.d. '50 m; Hewlett-Packard, USA), and the detector and oven temperatures were set at 250°C and 230°C, respectively. The column temperature was increased from 120°C to 225°C at a rate of 3°C/min, and the split ratio was set at 1 : 10. Fatty acids were identified from their relative retention times and the linear relationship between the numbers of carbon atoms of the homologous series in standard samples and the logarithms of the relative retention times (Ackman, 1969).

Results and Discussion

Table 1 shows the lipid contents of the body wall, alimentary canal, and gonads of the sea cucumber and *konowata*. The lipid content of the body wall was not significantly different between males and females; however that of the alimentary canal was significantly higher in females than in males ($p<0.05$), and that of the gonads was significantly higher in males than in females ($p<0.05$). The lipid contents of the alimentary canal and testes in males were about twice that in the body wall. The lipid content of the alimentary canal in females was 4.3 times that in the body wall and 5.2 times that in the ovary. No marked difference was observed in the lipid content of *konowata* between the products of Company A and Company B.

Fatty acids contained in the body wall at 5% or more of the total fatty acid were iso-C15, C16:0, C16:1n-7, C18:0, 18:1n-7, C20:4n-6, C20:5n-3, C23:1n-9, and C22:6n-3 in males and females, respectively (Table 2). There was little difference in the contents of various fatty acids that constituted the lipids of the body wall between the sexes. The fatty acid with the highest content was C20:4n-6, accounting for 11%. Branched fatty acids accounted for about 10% of the total fatty acid. Sea-bed sediment is known to contain branched fatty acids derived from microorganisms (Leo & Parker, 1966). Detritus is a major article of the diet of sea cucumbers (Kinoshita & Tanaka, 1939), and branched fatty acids, which were detected in a relatively large quantity in this study, are believed to have been derived from this diet.

Kaneniwa *et al*. (1986) reported that major fatty acids in *S. japonicus* collected in the Pacific Ocean along the Mori Coast of

Table 1. Total lipid content (wt%) extracted from sea cucumber and *Konowata*. (g/100 g±SD)

Sample	Body wall	Alimentary canal	Gonad
Male	0.63±0.15	1.11±0.12*	1.15±0.15*
Female	0.58±0.11	2.49±0.33*	0.48±0.08*
Konowata A	—	1.73±0.28	
Konowata B	—	1.62±0.31	

Mean±standard deviation, Significant difference from male and female sea cucumbers at $p<0.05$ with alimentary canal and gonad.

Table 2. Fatty acid composition of total lipids extracted from sea cucumber *Stichopus japonicus* and *Konowata*. (%)

	Male			Female			*Konowata*	
Fatty acid	B.w	Testis	Alim. ca.	B.w	Ovary	Alim. ca.	A	B
C14:0	1.45	1.33	0.68	1.41	1.05	0.53	2.26	2.05
iso-C15:0	6.27	5.78	1.91	5.85	5.56	1.76	1.18	1.19
C14:1n-7	3.97	3.72	1.09	3.11	3.56	1.14	0.94	0.81
C15:0	0.61	0.72	0.43	0.76	0.65	0.09	—	—
iso-C16:0	0.74	0.59	0.72	0.45	0.85	0.62	—	—
anteiso-C16:0	0.17	0.06	0.04	0.13	0.64	0.06	—	—
C16:0	8.48	6.65	3.76	7.35	6.57	3.63	8.28	8.57
C16:1n-9	1.77	1.30	1.05	2.01	1.29	1.13	0.42	0.22
C16:1n-7	6.12	5.05	1.77	5.19	4.90	2.07	6.84	6.45
iso-C17:0	0.24	2.44	1.39	0.18	2.19	0.95	0.14	0.23
anteiso-C17:0	2.84	3.03	0.21	2.54	2.87	1.25	—	—
C17:0	1.42	1.77	1.55	1.51	1.46	1.32	2.31	2.29
iso-C18:0	0.27	0.45	2.30	0.19	1.85	3.39	1.65	2.02
anteiso-C18:0	0.35	0.22	0.18	0.26	0.26	0.34	—	—
C18:0	7.67	5.98	4.27	7.58	6.33	4.26	12.91	12.40
C18:1n-9	2.20	1.69	0.87	2.23	1.81	0.70	2.32	2.36
C18:1n-7	6.83	7.97	7.18	6.98	7.85	7.34	9.89	10.27
C18:1n-5	0.93	1.05	0.56	1.16	0.45	0.43	1.53	1.57
C19:0	0.36	0.54	1.08	0.21	0.83	1.18	0.13	0.09
C18:2n-9	0.09	0.22	0.26	0.09	0.45	0.19	—	—
C18:2n-6	0.34	0.45	0.56	0.21	0.19	0.22	—	—
C19:1n-8	0.12	0.16	0.23	0.24	0.12	0.13	—	—
C20:0	1.19	0.77	0.68	1.47	0.08	0.66	1.21	0.92
C18:3n-3	0.06	0.88	1.69	0.79	1.88	1.57	—	—
C18:4n-3	0.01	0.08	0.10	0.25	0.22	0.34	—	—
C20:1n-9	1.62	3.61	5.41	1.77	3.80	5.34	7.16	7.15
C20:1n-11	2.62	1.95	1.75	2.61	1.35	1.59	4.58	4.80
C21:0	1.16	0.82	1.38	0.76	1.01	1.73	2.03	1.68
C20:2n-6	0.74	0.83	1.37	0.83	0.95	1.46	0.95	0.70
C22:0	0.79	0.99	1.36	1.16	0.87	1.38	3.26	3.89
C20:4n-6	10.79	8.91	14.91	11.01	8.68	15.20	—	—
C22:1n-9	0.63	0.93	0.71	0.99	0.85	0.86	2.14	2.05
C22:1n-7	2.51	4.20	4.32	2.23	3.63	4.89	6.78	7.24
C23:0	0.72	0.86	1.19	0.79	0.43	0.24	2.01	1.52
C20:5n-3	7.46	9.82	12.55	8.01	9.36	12.47	3.29	1.98
C24:0	0.05	0.06	0.07	0.05	0.10	0.50	1.53	1.64
C23:1n-9	5.52	3.26	5.34	6.36	3.61	5.67	8.63	8.78
C22:4n-6	1.74	1.58	2.10	1.89	1.79	1.98	—	—
C24:1n-9	1.07	1.48	1.56	1.29	1.90	1.93	4.24	4.70
C22:5n-3	0.70	0.93	1.44	0.72	0.91	0.89	—	—
C22:6n-3	5.18	4.76	7.70	5.14	4.86	6.25	—	—
Total unkn.	2.20	2.11	2.38	2.24	1.99	1.18	1.39	2.43
Total Sat.	34.78	33.06	23.20	32.65	33.60	25.03	38.90	38.49
Total Mono.	35.91	36.37	31.84	36.17	35.12	33.22	55.47	56.40
Total PUFA	27.11	28.46	42.58	28.94	29.29	40.57	4.24	2.68

Mean of five samples (each standard error of mean was less than 5% of data); B.w, body wall; Alim. ca., alimentary canal; Sat., Saturated fatty acids; Mono., Monounsaturated fatty acids; PUFA, Poly-unsaturated fatty acids. A dash in the column indicates either the absence of concrete acid or its trace amount.

Southern Hokkaido were C16:0 (10.83%), C16:1n-7 (12.44%), C18:0 (6.73%), C18:1n-7(5.07%), C20:4n-6 (13.31%), and C20:5n-3 (8.38%). They also reported that major fatty acids in the samples caught in the Pacific Ocean off the Shikabe Coast in Southern Hokkaido were C16:0 (8.2%), C16:1n-7 (16.96%), C18:0 (15.98%), C18:1n-7 (9.26%), and C20:1n-9 (5.92%). The content of DHA (C22:6n-3) was markedly different compared with these reports and was more than 10 times higher in our study. In contrast, C16:1n-7 content in our samples was low at about 6%. Also, Kaneniwa *et al.* (1986) identified and assayed the specific fatty acid C23:1n-9 in addition to those usually detected in marine animals. This fatty acid was detected in a relatively large quantity in all samples of this study.

Svetashev *et al.* (1991) reported that major fatty acids in *S. japonicus* caught in the Peter the Great Bay in the Japan Sea, which is temperate water, were C16:0 (11.6%), C16:1n-7 (16.5%), C18:0 (6.4%), C20:4n-6 (8.0%), and C20:5n-3(15.4%). The C16:1n-7 and C20:5n-3 contents were higher than our results. They further reported fatty acid composition in 10 species of sea cucumbers collected in the Tropical Zone and reported that no branched fatty acid was contained and that the C20:4n-6 content was very high at 11–27.2%. These values differed markedly from the results of our study using samples caught in a cold water area.

Isay and Busarov (1984) also analyzed polyunsaturated fatty acids in *S. japonicus* collected in the Peter the Great Bay and reported that the C20:4n-6 content was high but that the C20:5n-3 and C22:6n-3 contents were trace levels, showing marked differences from our results.

Major fatty acids that constitute the lipids in the testis and ovary were iso-C15, C16:0, C18:0, C18:1n-7, C20:4n-6, and C20:5n-3, and the fatty acid profile was nearly identical between these two organs. Also, major fatty acids that constituted the lipids of the alimentary canal were C18:1n-7, C20:1n-9, C20:4n-6, C20:5n-3, C23:1n-9, and C22:6n-3 and were differed little between males and females.

Generally, the diet, degree of maturation, water temperature, and species characteristics are known to be included in the factors that affect the properties of lipids and fatty acid composition in marine animals (Deng *et al.*, 1976; Morris & Culkin, 1989). The fatty acid composition differs greatly between males and females in some fish species (Kasai *et al.*, 1997; Kusaka *et al.*, 1985).

Distinct differences have also been noted in the fatty acid composition between the testis and ovary (Satue & Lopez, 1996; Yamada & Hayashi, 1975; Hayashi & Yamada, 1975; Kojima *et al.*, 1986). In this study, there was little difference in the results of analysis of various tissues between males and females. However, while the fatty acid compositions in the body wall and gonads were similar between them, those in the body wall and gonads were markedly different from that in the alimentary canal. The iso-C15, C16:0, and C16:1n-7 levels were notably higher in the body wall, but the C20:1n-9, C20:4n-6, and C20:5n-3 levels were high in the alimentary canal.

Major fatty acids in *konowata* were C16:0, C16:1n-7, C18:0, C18:1n-7, C20:1n-9, C22:1n-7, and C23:1n-9, and these fatty acids accounted for 60% of the total fatty acid. The fatty acid profile was similar between the products of Company A and Company B. No polyunsaturated fatty acid was contained with the exception of C20:5n-3. Also, the total monoenoic acid content was very high at 55% and 56% in the products of Company A and Company B, respectively.

Among the fatty acids, the contents of C16:0, C16:1, C20:4, C20:5, and C22:6 differed markedly between raw entrails of sea cucumbers and *konowata*. In *konowata*, C16:0, C16:1, and C18:0 were increased, but C20:5 was reduced, and C22:4 and C22:6 were not detected.

Although there is no reference to the fatty acid composition of *konowata*, there are a few reports concerning the fatty acid composition of the salted gonads of sea urchins, which are Echinodermata similar to sea cucumbers (Ohshima *et al.*, 1986). Shimada and Ogura (1990) reported that the fatty acids contained in salted sea urchin gonads were mainly C16:0, C18:0, C20:1, C20:2, C20:4, C20:5, and C22:1, which were nearly identical to the fatty acid composition of the raw sea urchin, and that the percentage of highly polyunsaturated fatty acids in the total fatty acids was 33.2%. Kaneniwa and Takagi (1986) reported a similar fatty acid composition and a percentage of polyunsaturated fatty acids, i.e. C20:4 and C20:5, of 27.8%.

According to the results of our study, the fatty acid composition differed markedly between raw entrails of sea cucumbers and *konowata*. Also, arachidonic acid (C20:4) was not detected in *konowata*. Moreover, while the percentage of total monoenoic acids in salted sea urchin gonads was 27.2% and 28.0% in the above reports, it was very high at 55–56% in *konowata*.

These results suggest that auto-digestive enzymes act primarily in an early period of preparation of *konowata*, but that specific microbial enzymes act in the later maturing process, and produce the characteristic fatty acid composition through fermentation. Further studies are needed on changes in the fatty acid composition in the maturing process of *konowata*.

References

Ackman, R.G. (1969). Gas-liquid chromatography of fatty acids and esters. In "Methods in Enzymology," ed. by J.M. Lowenstein, vol. 14, Academic Press New York. pp. 329–381.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

Carballeira, N.M., Cruz, C. and Sostre, A. (1996). Identification of the novel 7-methyl-6-Octadecenoic acid in *holothuria mexicana*. *J. Nat. Prod.*, **59**,1076–1078.

Chang-Lee, M.V., Price, R.J. and Lampila, L.E. (1989). Effect of processing on proximate composition and mineral content of sea cucumbers (*Parastichopus* ssp.) *J. Food Sci.,* **54**, 567–568.

Deng, J.C, Othoefer, F.T., Dennison, R.A. and Watson, M. (1976). Lipids and fatty acids in Mullet (Mugli cephalus): seasonal and locational variations. *J. Food Sci.*, **41**,1479–1483.

Fredalina, B.D., Ridzwan, B.H., Zainal Abidin, A.A., Kaswandi, M.A., Zaiton, H., Zali, I., Kittakoop, P. and Mat Jais, A.M. (1999). Fatty acid composition in local sea cucumber, *Stichopus chloronotus*, for wound healing. *Gen. Pharmacol.*, **33**, 337–340.

Hayashi, K and Yamada, M. (1975). The lipids of marine animals from various habitat depths-II. On the fatty acid composition of the neutral lipids in six species of gadiformes. *Nippon Suisan Gakkaishi*, **41**, 1153–1160 (in Japanese).

Isay, S.V. and Busarova, N.G. (1984). Study of fatty acid composition of marine organisms-I. Unsaturated fatty acids of Japan Sea invertebrates. *Comp. Biochem. Physiol.*, **77B**, 803–810.

Kaneniwa, M., Itabashi, Y., Endo, S. and Takagi, T. (1986). Fatty acids in holothuroidea: Occurrence of *cis*-14-tricosenic acid. *Comp. Biochem. Physiol.*, **84B**, 451–455.

Kaneniwa, M and Takagi, T. (1986). Fatty acids in the lipid of food products from sea urchin. *Bull. Jpn. Soc. Sci. Fish.*, **52**, 1681–1685.

Kasai, T., Ohosawa, A., Higasa, T., Tsujimura, M. and Hasegawa, T. (1997). Proximate composition of dorsal muscles of chum salmon *Onchorhyncus keta*. *Nippon Shokuhin Kagaku Kogakukaishi,* **44**, 724–730 (in Japanese).
Kinoshita, T. and Tanaka, S. (1939). The food of *Stichopus japonecus* Selenka in Hokkaido. *Suisan Kenkyuushi,* **34**, 32–35 (in Japanese).
Kojima, T., Sato, M., Yoshinaka, R. and Ikeda, S. (1986). Chemical composition and fatty acid composition in Lake Biwa. *Nippon Suisan Gakkaishi,* **52**, 1779–1785 (in Japanese).
Kusaka, H., Takaikawa, M., Tsubouchi, T., Ohta, S. and Kamimura, S. (1985). Fatty acid composition of lipids in dorsal muscles of chum salmon. *Yukagaku*, **34**, 54–58 (in Japanese).
Leo, R.F. and Parker, P.L. (1966). Branched-chain fatty acids in sediment. *Science,* **152**, 649–650.
Metcalfe, L.D. and Schmitz, A.A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem*., **33**, 363–364.
Morris, R.J. and Culkin, F. (1989). In "Marine Biogenic Lipids, Fats and Oils" vo.l II, ed. by R.G. Ackmann, CRC Press, Florida, U.S.A. pp.145–178.
Ohshima, T., Wada, S. and Koizumi, C. (1986). Lipid deterioration of salted gonads of sea urchin during storage at 5°C. *Bull. Jpn. Soc. Sci. Fish*., **52,** 511–517.
Satue, M.T. and Lopez, M.C. (1996). Sex-linked differences in fatty acid composition of rainbow trout (*Onchorhynchus mykiss*) liver oil. *Food Chem*., **57**, 359–363.
Shimada, K. and Ogura, N. (1990). Lipid changes in sea urchin gonads storage. *J. Food. Sci*., **55**, 967–971.
Svetashev, V.I., Levin, V.S., Cham Ngok Lam and Do Tuet Nga. (1991). Lipid and fatty acid composition of holothurians from tropical and temperate waters. *Comp. Biochem. Physiol*., **96B**, 489–494.
Svetashev, V.I. and Busarova, N.G. (1984). Study on fatty acid composition of marine organisms-1. Unsaturated fatty acids of Japan Sea inverrerates. *Comp. Biochem. Physiol*., **77B**, 803–810.
Yamada, M and Hayashi, K. (1975). Fatty acid composition from 22 species of fish and mollusk. *Nippon Suisan Gakkaishi*, **41**, 1143–1152 (in Japanese).