Composition and Distribution of Lipids in Different Tissues of the Arrow Squid *Loligo bleekeri*

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Lipid class, fatty acid, and glyceryl ether composition in different tissues of the male and female arrow squids *Loligo bleekeri* was determined.

In both sexes of this species, the livers had a relatively high amount of lipids, which were characterized by high quantities of triglyceride class. On the contrary, the lesser lipids from the muscles and gonads were low in the above lipid class but high in phospholipids. The unsaponifiable matters isolated from the tissue lipids contained remarkably high levels of sterols and minor amounts of glyceryl ethers and alkenyl types. The component fatty acids of the muscle and/or liver lipids were very similar in composition between both sexes, respectively. Few differences were found in the percentage of the component fatty acids between the lipids from the testes and ovaries. While the fatty acid composition of the muscle lipids was markedly different from that of the liver lipids, the former tending to be more polyenoic with abundant 22:6n-3 acid. Glyceryl ethers of the male and female liver lipids showed similar components: the most predominant constituent was chimyl alcohol.

Key words: arrow squid, lipid, fatty acid, glyceryl ether

Cephalopod species, such as the Newfoundland squid *Illex illecebrosus*,¹⁾ the Japanese common squid *Todarodes pacificus*,²⁾ and the firefly squid *Watasenia scintillans*,³⁾ have been found to possess large amounts of triglycerides in their livers. However, some peculiar species of the deep-sea nektonic squids, Oegopsidae sp.⁴⁾ and Cranchiidae sp.⁵⁾ are reported to contain wax esters in a relatively large proportion within their lipids. On the other hand, the authors have demonstrated previously that three species of the gonatid squids belonging to the family Gonatidae: *Beryteuthis magister*,^{2,6-8)} *Gonatopsis makko*,^{2,9)} and *G. borealis*,¹⁰⁾ and the giant squid belonging to the family Onychoteuthidae: *Moroteuthis robusta*,¹¹⁾ all contained in their livers significant amounts of glyceryl ethers in the form of diacyl glyceryl ethers.

The present study deals with the lipid level and composition of the livers, muscles, and gonads of both sexes of the pelagic species of the arrow squid *Loligo bleekeri*. Special attention was paid to glyceryl ether concentration and composition. The fatty acid composition of total lipids of the tissues was also determined and compared.

Materials and Methods

Materials

Ten male and ten female specimens of the arrow squids *L. bleekeri*, ranging from 59.3 to 132.9 g (mean $\pm$ standard deviation: 86.8 ± 23.8 g) and from 75.8 to 118.2 g (96.1 ± 12.1 g) in body weight, respectively, were caught in the Sea of Japan in April 1993 and kept frozen at -20°C for a few months until analysis. The pooled samples of the muscles, livers, and gonads of both sexes of this species were used individually for lipid extraction.

Lipid Extraction and Analysis

The methods used for extraction of tissue lipids, qualitative and quantitative determination of constituent lipids by thin-layer chromatography (TLC) and an Iatroscan TH-10 flame ionization detector (Iatron Lab. Inc., Japan), isolation of glyceryl ethers and fatty acids from tissue lipids, preparation of isopropylidene derivatives of glyceryl ethers and methyl esters of fatty acids, and determination of the above compounds by gas-liquid chromatography, were performed as described in previous reports.^{10,12)} Identification of alkenyl glyceryl ethers was accomplished by TLC, before and after hydrogenation, with a known available standard.¹³⁾

Results and Discussion

Content and Composition of Lipids

Lipid content and class composition of the muscles, livers, and gonads of the male and female arrow squids *L. bleekeri* are listed in Table 1. Also in Table 1, there are the content and composition of unsaponifiable matters isolated from tissue lipids of the above samples. Irrespective of sex of this species, the livers yielded 5.8–8.7% lipids in wet weight basis, being relatively higher than those from the muscles (1.8–1.9%), testes (1.6%), and ovaries (3.6%). The liver lipids were characterized by a high level of triglycerides (45.8–63.0%). In comparison, the lipids of the muscles and gonads of both sexes had low levels of triglycerides (0.3–6.9%), but were high in phospholipids (80.3–84.3%). Appreciable quantities of free fatty acids (8.1–9.3%) were found in the liver lipids of both sexes. Nonesterified fatty acids do not occur in nature in high concentration in normal tissues. Therefore, the relatively high levels of free fatty acids suggested that they may result from lipolysis of lipids of the squid samples during frozen

Table 1. Content and class composition of lipids from different tissues of arrow squid

	Males			Females		
	Muscle	Liver	Testis	Muscle	Liver	Ovary
Lipid content % ^{*1}	1.9	5.8	1.6	1.8	8.7	3.6
Lipid component % ^{*2}						
Steryl esters	nd	0.7	0.8	nd	0.7	0.7
Triglycerides	0.3	45.8	2.7	0.3	63.0	6.0
Free fatty acids	1.1	8.1	4.7	1.2	9.3	1.6
Sterols	14.8	5.7	11.3	14.2	4.5	8.1
Partial glycerides	nd	0.4	0.2	nd	0.7	0.3
Phospholipids	83.8	39.3	80.3	84.3	21.8	83.3
USM content % ^{*2}	16.1	9.5	14.6	15.6	7.3	11.1
USM component % ^{*3}						
Hydrocarbons	0.4	1.3	3.1	0.4	1.9	0.7
Sterols	98.2	92.6	93.4	98.1	87.9	96.1
Alkenyl glyceryl ethers	1.1	3.5	2.7	1.2	3.2	2.8
Glyceryl ethers	0.1	1.3	0.3	0.1	4.7	0.1
Polar compounds	0.2	1.3	0.5	0.2	2.3	0.3

*¹ % to wet weight basis of tissue.

*² % to total lipid.

*³ % to unsaponifiable matter.

Abbreviation: USM, unsaponifiable matters; nd, not detected.

storage and thawing. On the other hand, the unsaponifiable matters isolated from the tissue lipids studied, accounting for 7.3 to 16.1% of their lipids, contained remarkably high levels of sterols (87.9–98.2%) and minor amounts of glyceryl ethers (0.1–4.7%) and alkenyl types (1.1–3.5%).

The content of the liver lipids (mean: 7.3%) of the arrow squid *L. bleekeri* examined in this study, similar to those of the diamondback squid *Thysanoteuthis rhombus* (3.9%)¹⁴ and the cuttlefish *Sepia officinalis* (7.8%),¹⁵ showed a lower level than those of the Newfoundland squid *I. illecebrosus* (22.0%),¹ the Japanese common squid *T. pacificus* (37.8%),² the firefly squid *W. scintillans* (36.1%),³ the gonatid squids *B. magister* (30.7%),^{2,6–8} *G. makko* (36.0%)^{2,9} and *G. borealis* (26.5%),¹⁰ and the giant squid *M. robusta* (17.7%).¹¹ The lipid content of the ovaries of *L. bleekeri* was relatively higher percentage than that of the testes. This was very similar to that in the ovary lipids from the mature females and males of *S. officinalis*.¹⁵

The lipid class composition of the liver lipids from *L. bleekeri* studied, being rich in triglycerides (mean: 54.4%), were analogous to those of *I. illecebrosus* (70.0%),¹ *T. pacificus* (77.7%),² and *W. scintillans* (69.8%).³ This differed from the liver lipids, consisting large quantities of diacyl glyceryl ethers, in the deep-sea squid species of *B. magister* (mean: 37.6%),^{2,6–8} *G. makko* (77.1%),^{2,9} *G. borealis* (16.3%),¹⁰ and *M. robusta* (16.2%).¹¹ On the other hand, small amounts of the above compounds were also found in the liver lipids of the pelagic squid species of *T. pacificus* (1.0%)² and *W. scintillans* (2.8%).³ Diacyl glyceryl ethers in *L. bleekeri* studied were not easy to detect in TLC, but glyceryl ethers (3.0%) occurred in minor amounts in the unsaponifiable matters of the liver lipids. The biological role of diacyl glyceryl ethers in marine organisms is not well known. Malins and Barone¹⁶ have postulated that ether-linked lipids are deposited at the expense of triglycerides to offset the increase in body weight.

Also, there might have been a regulatory mechanism in the dogfish body system, involving the selective metabolism of diacyl glyceryl ethers and triglycerides for the delicate attenuation of near neutral buoyancy. It is of interest to note from viewpoint of physiological or ecological role that the deep-sea organisms are abundant in diacyl glyceryl ethers.

Composition of Fatty Acids

Table 2 shows the major fatty acid composition of total lipids in each different tissue of both sexes of *L. bleekeri*. As for the examined tissues of this species, 16:0, 18:1n-9, 20:5n-3, and 22:6n-3 acids were characteristic, and their contents ranged from 1.3 to 33.3%, from 3.2 to 10.5%, from 14.7 to 22.3%, and from 9.7 to 27.8% of the total fatty acids, respectively. In both the female and male liver lipids, the component fatty acid percentage was similar in composition. However, some differences were noted. For example, the fatty acid composition of the muscle lipids was markedly different from that of the liver lipids, the former tending to be more polyenoic (43.6–45.5%) with abundant 22:6n-3 acid (25.8–27.8%). On the other hand, the component fatty acid percentage of the testis lipids was characterized by a relatively high proportion of polyenes with abundant 20:5n-3 and 22:6n-3 acids when compared with that of the ovary lipids.

The component fatty acids of the liver lipids of *L. bleekeri* contained significant amounts of polyenes (38.2%) with 20:5n-3 and 22:6n-3 as the main components. This was quite similar to those in the pelagic squid species, *I. illecebrosus* (36.6%),¹ *T. pacificus* (33.3%),² *W. scintillans* (34.3%),³ and *T. rhombus* (27.9%),¹³ undoubtedly reflecting the dietary intake of these carnivorous pelagic animals. However, this was different in the component fatty acid percentage from that of the liver lipids of the deep-sea squid species of *G. makko*,² *B. magister*,⁸ and *G. borealis*,¹⁰ which consisted pre-

Table 2. Fatty acid composition of total lipids from different tissues of arrow squid

	Males			Females		
	Muscle	Liver	Testis	Muscle	Liver	Ovary
14:0	3.5	4.9	3.5	3.2	5.8	5.2
15:0	0.5	0.3	0.2	0.5	0.5	0.2
16:0	33.3	22.2	23.0	33.6	21.3	28.1
17:0	0.6	1.1	0.4	0.7	1.2	0.3
18:0	3.1	4.4	5.4	3.4	3.7	7.2
16:1n-7	1.1	5.0	2.8	0.9	6.5	2.5
18:1n-9	3.7	8.6	6.4	3.2	10.5	6.3
18:1n-7	3.4	5.1	4.6	2.9	5.9	4.2
18:1n-5	0.3	0.5	0.9	0.3	0.4	0.4
20:1n-11	0.3	0.8	0.6	0.2	0.7	1.8
20:1n-9	3.3	2.8	3.5	3.5	2.8	2.8
22:1n-9	0.2	0.6	0.6	tr	0.4	0.3
24:1n-9	0.2	0.6	0.2	0.2	0.3	0.2
16:2n-6	tr	0.7	0.4	tr	1.0	0.3
18:2n-6	0.3	0.7	0.8	0.2	0.9	0.8
18:3n-3	tr	0.4	tr	tr	0.5	0.2
18:4n-3	tr	0.5	0.4	tr	0.7	0.4
20:4n-6	1.4	3.1	1.2	1.2	2.7	1.7
20:5n-3	14.9	20.0	22.3	15.2	20.5	14.7
22:5n-3	0.5	0.5	0.4	0.4	0.4	0.4
22:6n-3	25.8	13.3	17.8	27.8	9.7	18.8
A16:0	0.6	0.4	0.2	0.4	0.4	0.4
A18:0	0.2	0.2	1.1	tr	0.2	0.2
Saturates	41.0	32.9	32.5	41.4	32.5	41.0
Monoenes	13.2	25.3	20.6	11.8	29.3	19.4
Polyenes	43.6	39.7	44.4	45.5	36.7	38.0
Branched	1.2	1.1	1.3	0.8	0.6	0.6
Unknowns	1.0	1.0	1.2	0.5	0.9	1.0

Abbreviation: A, anteiso; tr, trace (less than 0.05%).

Table 3. Glycerol ether composition of liver lipids of arrow squid (%)

	Males	Females
Component* ¹		
14:0	10.3	12.2
15:0	1.9	2.1
16:0	46.2	47.5
17:0	1.0	0.9
18:0	3.7	3.6
14:1	1.5	1.4
16:1	14.0	13.9
18:1	10.0	8.7
20:1	0.7	0.2
Br15:0	2.8	3.0
Br16:0	1.3	0.9
Br17:0	0.7	0.7
Br18:0	3.7	3.6
Br19:0	1.1	1.2
Br20:0	1.1	0.1
Saturates	63.1	66.3
Monoenes	26.2	24.2
Branched	10.7	9.5

*¹ Indicated by chain length and double bond of alkyl moiety.

Abbreviation: Br, branched compounds.

dominantly of monoenes (67.8%, 55.0%, and 71.5%, respectively) with 18:1, 20:1, and 22:1 acids as the main components.

Composition of Glycerol Ethers

The quantitative distribution of glycerol ether components of the liver lipids from the males and females of *L. Bleekeri* are given in Table 3. Between both sexes, the com-

ponent glycerol ethers were very similar in composition. Even, as opposed odd, numbers of carbon atoms, ranging from C₁₄ to C₂₀, prevailed for the alkyl moiety. The most predominant component found was 16:0 (chimy alcohol, 46.2–46.5%) followed by 16:1 (13.9–14.0%), 14:0 (10.3–12.2%), 18:1 (selachyl alcohol, 8.7–10.0%), and 18:0 (batyl alcohol, 3.6–3.7%), indicating a high level of saturated (63.1–66.3%). The branched glycerol ethers also occurred in considerable amounts (9.5–10.7%).

The component glycerol ethers of the liver lipids of *L. bleekeri* examined in this study were characteristically rich in chimyl alcohol (mean: 46.9%). This result was in agreement in composition to those of the squid species of *T. pacificus* (50.3%),^{2,9)} *G. makko* (62.9%),²⁾ *B. magister* (64.6%),^{2,6–8)} *G. borealis* (45.9%),¹⁰⁾ and *M. robusta* (55.4%).¹¹⁾ Also, similar findings have been reported for the squid *Loligo* sp., in which phosphorylated glycerol ethers (7.9% of the phospholipid content) contained chimyl alcohol at a high percentage (59%).¹⁷⁾ It should be noted that the component glycerol ethers in the liver lipids of these squid species were extremely different in composition from those of the liver lipids of ratfish and sharks. That is, the component glycerol ethers for sharks and ratfish are rich in monoenes with abundant selachyl alcohol: *Squalus acanthias* (59.3%),¹⁸⁾ *Apristurus platyrhynchus* (53.4%),¹⁸⁾ *Centroscyllium ritteri* (48.3%),¹⁸⁾ *Centrophorus* sp. (45.6%),¹⁸⁾ *Dalatis licha* (51.8%),¹⁹⁾ *Hydrolagus novaezealandiae* (51.2%),²⁰⁾ *H. barbouri* (57.4%),²¹⁾ and *Rhinochimaera pacifica* (49.1%).²¹⁾

References

- 1) P. M. Jangaard and R. G. Ackman: Lipids and component fatty

- acids of the Newfoundland squid, *Illex illecebrosus* (Le Sueur). *J. Fish. Bd. Canada*, **22**, 131–137 (1965).
- 2) K. Hayashi and K. Kawasaki: Unusual occurrence of diacyl glyceryl ethers in liver lipids from two species of gonatid squids. *Nippon Suisan Gakkaishi*, **51**, 593–597 (1985).
 - 3) K. Kawasaki, T. Oozumi, S. Hayashi, and K. Hayashi: Lipid class and fatty acid composition of firefly squid *Watasenia scintillans*. *Nippon Suisan Gakkaishi*, **60**, 247–251 (1994) (in Japanese).
 - 4) R. F. Lee, J. Hirota, and A. M. Barnett: Distribution and importance of wax esters in marine copepods and other zooplankton. *Deep-Sea Res.*, **18**, 1147–1165 (1971).
 - 5) R. F. Lee and J. Hirota: Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. *Limnol. Oceanogr.*, **18**, 227–239 (1973).
 - 6) K. Hayashi, Y. Okawa, and K. Kawasaki: Liver lipids of gonatid squid *Berryteuthis magister*: a rich source of alkyl glyceryl ethers. *Nippon Suisan Gakkaishi*, **51**, 1523–1526 (1985).
 - 7) K. Hayashi and S. Yamamoto: Content and composition of alkyl glyceryl ethers in liver of gonatid squid *Berryteuthis magister* from the northwestern Pacific. *Nippon Suisan Gakkaishi*, **53**, 137–140 (1987).
 - 8) K. Hayashi and S. Yamamoto: Distribution of diacyl glyceryl ethers in the different tissues and stomach contents of gonatid squid *Berryteuthis magister*. *Nippon Suisan Gakkaishi*, **53**, 1057–1063 (1987).
 - 9) K. Hayashi and K. Kawasaki: Diacyl glyceryl ethers in the flesh and liver of gonatid squid *Gonatopsis makko*. *Nippon Suisan Gakkaishi*, **56**, 1354 (1990).
 - 10) K. Hayashi: Occurrence of diacyl glyceryl ethers in liver lipids of gonatid squid *Gonatopsis borealis*. *Nippon Suisan Gakkaishi*, **55**, 1383–1387 (1989).
 - 11) K. Hayashi, H. Kishimura, and Y. Sakurai: Level and composition of diacyl glyceryl ethers in the different tissues and stomach contents of giant squid *Moroteuthis robusta*. *Nippon Suisan Gakkaishi*, **56**, 1636–1639 (1990).
 - 12) K. Hayashi and H. Kishimura: Preparation of n-3 PUPA ethyl ester concentrates from fish oil by column chromatography on silicic acid. *Nippon Suisan Gakkaishi*, **59**, 1429 (1993).
 - 13) K. Hayashi and T. Takagi: Occurrence of neutral plasmalogens in the liver lipids of ratfish, *Hydrolagus novaezealandiae*. *Nippon Suisan Gakkaishi*, **46**, 1043–1049 (1980).
 - 14) M. Yamada and K. Hayashi: Fatty acid composition of lipids from 22 species of fish and mollusk. *Nippon Suisan Gakkaishi*, **41**, 1143–1152 (1975) (in Japanese).
 - 15) B. Balanchier and E. Boucaud-Camou: Lipid in the digestive gland and the gonad of immature and mature *Sepia officinalis* (Mollusca: Cephalopoda). *Mar. Biol.*, **80**, 39–43 (1984).
 - 16) D. C. Malins and A. Barone: Glyceryl ether metabolism; Regulation of buoyancy in dogfish *Squalus acanthias*. *Science*, **167**, 79–80 (1970).
 - 17) R. W. Lewis: Studies of the glyceryl ethers of the stomach oil of leach's petrel *Oceanodroma leucorhoa* (Viellot). *Comp. Biochem. Physiol.*, **19**, 363–377 (1966).
 - 18) M. Kayama, Y. Tsuchiya, and J. C. Nevenzel: The glyceryl ethers of some shark liver oils. *Nippon Suisan Gakkaishi*, **37**, 111–118 (1971).
 - 19) K. Hayashi and T. Takagi: Distribution of squalene and diacyl glyceryl ethers in the different tissues of deep-sea shark *Dalatias licha*. *Nippon Suisan Gakkaishi*, **47**, 281–288 (1981).
 - 20) K. Hayashi and T. Takagi: Composition of diacyl glyceryl ethers in the liver lipids of ratfish *Hydrolagus novaezealandiae*. *Nippon Suisan Gakkaishi*, **46**, 855–861 (1980).
 - 21) K. Hayashi, T. Takagi, and M. Kitagawa: Compositions of ether-linked lipids in livers of two species of ratfish. *Nippon Suisan Gakkaishi*, **49**, 777–782 (1983).