Seasonal Variations of Lipid Content and Composition in Starfish *Asterias amurensis* Lütken

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Abstract

The total lipid content, lipid classes and fatty acid composition in the internal organs of starfish (*Asterias amurensis*) were analyzed to determine the effects of seasons (winter and spring). The non-polar and polar lipid fractions obtained from starfish internal organs were analyzed through two seasons using thin layer chromatography (TLC) and gas liquid chromatography. Total lipid content of internal organs was 10.18% in spring and 8.21% in winter as wet weight basis. The predominant lipid class in spring was triglyceride whereas free fatty acids were the main lipid class in winter. The most abundant fatty acid of non-polar lipid fraction was eicosamonoenoic acid (C20:1) in spring having the highest proportion (29.2% of total fatty acid) as compared to the winter. Eicosapentaenoic acid (EPA) was also found significantly ($P < 0.05$) higher in spring compared to winter in the non-polar lipid fraction. On the other hand, comparatively lower amount of EPA was observed in spring than winter in the polar lipid fraction. Proportions of other fatty acids in non-polar and polar lipid fractions were also varied seasonally. This result might be useful for commercial production of lipid from internal organ of starfish with a view to potential use in food, pharmaceutical, cosmetics and other non-food industries.

Introduction

The starfish *Asterias amurensis* Lütken (family asteroidea, phylum Echinodermata), inhabits in the North Pacific Ocean. In recent years, the number of starfish has increased and its large outbreak has been observed in Hokkaido, northern part of the Japanese Islands. Any outbreak of this starfish causes serious damage to the fishery and aquacultural grounds for benthic shellfish [1, 2]. In Hokkaido, large amounts (~15,000 tons/year) of starfish are collected and disposed as waste. Most of the starfish waste is dumped at a huge expense and remains unutilized. Thus, efficient use of starfish waste as a biological resource is desired as part of an ecological recycling system.

The starfish are considered an extremely rich source of biologically active compounds, for example, steroidal glycosides, steroids, anthraquinones, alkaloids, glycolipids and phospholipids [3-5]. Generally, marine organisms reflect, to varying degrees, exogenous sources in their fatty acid components. Fatty acids are characteristic for primary producers (algae and microorganisms) and those which cannot be synthesized by the animals, are found in invertebrates when they consume different foods [6]. The diversity of fatty acids in terms of chain length, degree of unsaturation and position of the double bonds is responsible for the ultimate characteristics of lipids among different organisms.

It is known that, sea organisms are sources of the n-3 fatty acids and especially of the essential eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). Moreover, n-3 fatty acids are considered as essential fatty acids for marine invertebrates because of their inability to synthesize them endogenously [7-10]. It has been reported that *A. amurensis* powder is rich in eicosamonoenoic acid and EPA bound phosphatidylcholine [11]. EPA is useful in the maintenance of the correct skin hydration and in the prevention and/or the treatment of the effects of skin aging, in particular wrinkles because it helps to pile up the horny layer of the epidermis [12]. Moreover, EPA and DHA have been recognized as beneficial factors in human health and nutrition as documented in numerous investigations [13-14]. Therefore, there is a growing demand for marine oils, the main source of n-3 polyunsaturated fatty acids (PUFA) and very long chain monoenoic

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acids, for the use in human food, pharmaceuticals and as industrial intermediates. Identification of sustainable sources rich in n-3 PUFA, to meet the growing demand is very important.

The fat content and fatty acid composition of sea organisms is not constant. They are related to the life cycle and external factors, such as temperature, salinity, and nutrition [15-17]. The geographical origin of the organisms is an additional factor that affects the lipid content and fatty acid composition of sea organisms [18]. To the best of our knowledge, influence of season on the lipid and fatty acid compositions of *A. amurensis* have not been investigated to date. Thus, the fatty acid dynamics of this species are not yet well known. The objective of this study was to determine the seasonal variations of the lipid content, lipid classes and fatty acid composition in the internal organs of starfish.

Experimental

Sampling

Asterias amurensis was collected at the coast of Kushiro city, Hokkaido, Japan, during 2002-2003 (Spring, April 11, 2002; Winter, January 15, 2003). Starfish samples were pooled into three groups. Internal organs were carefully removed from starfish, and stored at - 40 °C until use.

Lipid Extraction

The lipids were extracted from internal organs of starfish samples with a solvent combination of chloroform:methanol:distilled water (10:5:3, v/v/v) according to the described method [19] with slight modification. The extracted lipid was redissolved in chloroform and stored on argon gas and kept in dark condition at –20 ºC till further analysis.

Fractionation of Total Lipids

Non-polar and polar lipid were separated from the total lipid by using Sep-Pak Vac 12cc silica cartridges (Waters Corporation, Milford, MA, USA) as described by Juaneda and Rocquelin [20]. The lipid samples (200 mg of total lipid) were loaded on the top of the cartridges. Then non-polar and polar lipids were eluted with chloroform and methanol respectively, in sequential order.

Analysis of Lipid Class Composition

The lipid class compositions of non-polar lipid were determined by using a commercial silica gel $60F_{254}$ (Merck KGaA, Darmstadt, Germany) thin layer chromatographic plate with a solvent system consisting of *n*-hexane, diethyl ether and acetic acid (80:20:1, v/v/v). The plate was sprayed with 50% H_2SO_4 and heated at 150-160 ºC for 15 min. Each spot was identified by authentic lipid standards and then lipid compositions were analyzed by using scanner and Scion Image software (Scion Corporation, Frederick, Maryland, USA). Monoglycerides were also determined by TLC with a combined solvent system of n-hexane, diethyl ether and acetic acid (50:50:1, v/v/v) and a spray reagent (50% H_2SO_4).

Lipid composition of polar lipid was determined by TLC with the solvent system of chloroform:methanol:water (65:25:4, v/v/v) and 50% H_2SO_4 as a detection reagent. Phosphatidylserine (PS) and lysophosphatidylethanolamine (LPE) were determined by using the solvent system of chloroform:methanol:25% ammonia (65:25:5, v/v/v) and ninhydrin as a detection reagent.

Analysis of Fatty Acid Composition

Fatty acids obtained from non-polar and polar lipids of starfish internal organs were converted to corresponding methyl esters following the method described by Prevot and Mordret [21]. In brief, dried lipid samples were dissolved in 1 mL *n*-hexane and then added 0.2 mL of 2N NaOH solution in MeOH. The mixture was shaken and kept at 50ºC for 20 seconds and then 0.2 mL of 2N HCl solution in MeOH was added. The n-hexane layer was collected, concentrated and subjected to gas chromatographic analysis with a Hitachi 163 Gas Chromatograph (Hitachi, Ltd. Tokyo, Japan) connected with a PEG-20M liquid phase-coated G-300 column (1.2 mm i.d. × 40 m, 0.5 μm; Chemicals Evaluation and Research Institute, Saitama, Japan) with flame ionization detection. The temperature of the column, detector and injector were 170, 250 and 240 ºC, respectively. The fatty acids were identified by comparing the peak retention times with authentic standards (GL Sciences Inc. Tokyo, Japan) and theoretical linear relationship between the carbon number unit or the number of double bonds of fatty acid and logarithm of the corresponding retention times.

Statistical Analysis

The Q value examination proposed by Dean and Dixon [22] was used to minimize the error. Student's *t*-test was used to determine significant differences between samples. Trends were considered significant when the mean of compared sets differed at $P < 0.05$.

Results and Discussion

Morphometric Data and Lipid Content

The morphometric data and lipid contents of starfish internal organs obtained in spring and winter are shown in Table 1. Body length, arm length and body weight of starfish were not significantly ($P > 0.05$) different during the spring and the winter. The weight of internal organs of starfish were significantly ($P < 0.05$) higher in winter than in spring. The mean lipid content of starfish internal organs in spring and winter were 10.18% and 8.21%, respectively, in wet weight basis, which was higher than that of gonad (3.3%) and viscera (6.6%) lipid content of *Asterias amurensis* [23]. Lipid content in the internal organs of starfish was found to be significantly ($P < 0.05$) higher in spring than in winter. In winter, the total lipid content decreased to its lowest level, being related to spawning and feeding activity [24]. The non-polar lipid content was significantly higher in spring than in winter (83.25% and 68.79% of total lipid). On the other hand, polar lipid content was found to be significantly higher in winter than in spring (29.76% and 15.45% of total lipid).

Table 1

The morphometric data and lipid contents of starfish internal organs in spring and winter

	Spring	Winter
Morphological parameters[a]		
Body length (cm)	16.09 ± 2.51	14.33 ± 1.04
Arm length (cm)	4.12 ± 0.23	4.67 ± 0.29
Body weight (g)	317.64 ± 120.28	499.58 ± 133.73
Internal organs weight (g)	92.27 ± 28.57	220.93 ± 57.13*
Lipid content[b]		
Total lipid (% wet weight basis)	10.18 ± 1.25	8.21 ± 0.68*
Non-polar lipid (% total lipid)	83.25 ± 5.91	68.79 ± 7.22*
Polar lipid (% total lipid)	15.45 ± 2.35	29.76 ± 7.29*

[a] Each value is expressed as mean ± SD (n = 11).
[b] Pairs of mean corresponding to internal organs of spring and winter starfish were compared and those that were significantly different ($P < 0.05$) are indicated by (*). Results represent mean ± SD; n = 3 as samples were pooled into three groups.

Lipid Class Composition

The lipid class composition (% of total lipid) in internal organs of starfish in spring and winter are presented in Table 2. Triglyceride (TG) was the main lipid component followed by free fatty acids (FFA), sterol esters (SE) plus hydrocarbons (HC) and ceramide monohexoside (CMH) in spring, whereas the major lipid component in winter was FFA followed by phosphatidylcholine (PC), TG, and CMH. TG and CMH content of starfish internal organs lipid were found significantly ($P < 0.05$) higher in spring. On the other hand, FFA and PC content were found to be significantly higher in winter.

Table 2

The lipid class composition (% of total lipid) of the internal organs of starfish in spring and winter[1]

Lipid component	Spring	Winter
Non-polar lipid		
Monoglyceride	1.35 ± 0.32	0.75 ± 0.28
1,2- diglyceride	6.05 ± 0.69	3.88 ± 1.06*
1,3- diglyceride	4.98 ± 1.15	0.51 ± 0.34*
Sterols	n.d.	1.60 ± 0.69
Triglyceride	38.69 ± 3.84	11.25 ± 0.77*
Free fatty acid	20.41 ± 1.98	47.77 ± 5.09*
Sterol esters + hydrocarbon	11.77 ± 2.28	3.03 ± 1.26*
Polar lipid		
Lysophosphatidylcholine	1.06 ± 0.25	0.39 ± 0.09*
Sphingomyelin	0.11 ± 0.03	0.82 ± 0.28*
Phosphatidylserine	0.33 ± 0.12	0.73 ± 0.25*
Lysophosphatidylethanolamine	0.58 ± 0.28	0.47 ± 0.35
Phosphatidylcholine (PC)	4.05 ± 1.18	14.56 ± 3.41*
Phosphatidylethanolamine	0.15 ± 0.08	3.43 ± 0.53*
Ceramide monohexoside	6.20 ± 0.45	4.92 ± 0.29*
PL others	2.97 ± 0.68	4.44 ± 1.06

[1] Pairs of mean corresponding to internal organs of spring and winter starfish were compared and those that were significantly different ($P < 0.05$) are indicated by (*). Results represent mean ± SD (n = 6); n.d., not detected.

Triglycerides are known as major reserve lipids in many marine organisms [25]. Our result suggests that the starfish store lipids in the form of TG when food resources are abundant and used to maintain body energy during the food-limited winter period. Low-density lipids (1,2-DG, 1,3-DG, SE + HC) of the internal organs showed a clear inverse relation-

ship between the seasons. It is known that the major function of sterols is to maintain the structural integrity of cell membranes [26]. CMH content of starfish internal organs in the spring and winter were 6.20% and 4.92%, respectively, of total lipid. However, the amount of cerebrosides found in starfish internal organs was much higher than that of cerebrosides found in some plant sources (1-40 mg/100 g dry weight) [27]. Cerebrosides are used as food and cosmetic ingredients [27, 28]; therefore, cerebrosides from starfish internal organs have the potential to be utilized in food and pharmaceutical industries. Moreover, cerebrosides have been reported to exhibit various pharmacological effects including antitumor and neuritogenic activities [29-31].

Fatty Acid Composition of Non-Polar Lipid

Seasonal variation of the fatty acid compositions (% of total fatty acids) of non-polar lipid of starfish internal organs are presented in Table 3. During the months of spring and winter, total monounsaturated fatty acids (MUFA) accounted for 42.7-43.0% of the total fatty acid composition. Total PUFA accounted for 26.2-36.5%, whereas saturated fatty acids (SFA) made up to 18.8-23.1% of the total fatty acid composition. No significant difference could be observed in total MUFA in both seasons; however, total PUFA were significantly ($P < 0.05$) higher in spring than in winter. The predominant fatty acids were C20:1 (29.2%) followed by C20:5 (20.0%), C18:1 (9.0%) and C14:0 (8.8%) in spring. In winter, the most abundant fatty acids were C20:1 (24.5%) followed by C22:6 (14.3%), C18:1 (11.1%) and C16:0 (9.8%). In general, echinoderms contain moderate amounts of C18:1, but high proportions of C20:1 [32, 33]. The major monoenoic fatty acids of echinoderms are isomers of C20:1 and echinoderms may have the metabolic capability to synthesize *de novo* various moieties of C20:1 including C20:1 (n-9) [34, 35]. The presence of eicosamonoenoic acid (C20:1) as a major component of starfish internal organs' lipid, indicating its potential for use in cosmetic industries.

The source of both C20:5 and C22:6 in the diet of asteroid is likely to be photosynthetically derived and seasonally available phytodetritus [36, 37]. In this study, DHA content of starfish internal organs in winter was found significantly ($P < 0.05$) higher than in spring, whereas EPA content was found to be significantly higher in spring than in winter. It has been reported that the variations in the fatty acid composition might be related to the changes in nutritional habits of the deep-sea seastar [38]. The variations in PUFA contents of starfish internal organs may be attributed to the season. Apart from that, reproductive status of starfish, environmental conditions, and especially water temperature, influence lipid content and fatty acid composition of starfish to a certain extent [39, 40].

Table 3

Seasonal variation of fatty acid composition (% of total fatty acids) of non-polar lipid in starfish internal organs[1]

Fatty acid	Spring	Winter
C14:0	8.8 ± 0.65	5.7 ± 0.17*
C16:0	5.2 ± 0.13	9.8 ± 1.37*
C18:0	4.8 ± 0.29	4.1 ± 0.75
C20:0	n.d.	1.1 ± 0.12
C22:0	n.d.	2.4 ± 1.20
Σ saturated	18.8 ± 1.07	23.1 ± 0.76*
C16:1	4.5 ± 0.26	5.8 ± 0.21
C18:1	9.0 ± 0.55	11.1 ± 0.46*
C20:1	29.2 ± 3.42	24.5 ± 2.12
C22:1	n.d.	1.6 ± 0.15
Σ monounsaturated	42.7 ± 1.71	43.0 ± 1.0
C16:2	1.7 ± 0.16	1.7 ± 0.15
C20:3	1.5 ± 0.12	1.7 ± 0.40
C20:5	20.0 ± 1.06	8.4 ± 2.14*
C22:3	1.6 ± 0.15	n.d.
C22:5	3.2 ± 0.45	n.d.
C22:6	8.5 ± 0.99	14.3 ± 1.37*
Σ polyunsaturated	36.5 ± 0.49	26.2 ± 1.14*
Others	2.0 ± 0.15	7.7 ± 1.25*

[1] Pairs of mean corresponding to internal organs of spring and winter starfish were compared and those that were significantly different ($P < 0.05$) are indicated by (*). Results represent mean ± SD (n = 6); n.d., not detected

Fatty Acid Composition of Polar Lipid

The fatty acid compositions of polar lipid of starfish internal organs in spring and winter are presented in Table 4. The major fatty acids in the starfish internal organs were C18:0, C20:1, C20:5 and C22:6, and they comprised more than 73% in spring and 63% in winter of the total fatty acid composition. Among monounsaturated fatty acids, C20:1 was most dominant fatty acids in both seasons. The PUFA content of starfish internal organs showed significantly higher levels in winter (61.9%) compared to spring (49.6%). However, total MUFA showed an inverse relationship with total PUFA, i.e. starfish internal organs contain 14.9% in winter, was signifi-

cantly ($P < 0.05$) lower than in spring (28.0%). The major fatty acids of PUFA were C20:5 and C22:6, and the total amount of EPA and DHA in the spring and winter were 41.5% and 50.1%, respectively. On the other hand, arachidonic acid (C20:4) is represented in smaller quantities in spring, which is more characteristic of sea urchins, and starfish [41]. This result suggest that diatoms and dinoflagellates contribute less to the diets of starfish than do other phytoplanktonic groups [38, 42]. This fatty acid is essential for the synthesis of some neurotransmitters such as the series two prostaglandins [43].

Table 4
Seasonal variation of fatty acid composition (% of total fatty acids) of polar lipids in starfish internal organs[1]

Fatty acid	Spring	Winter
C14:0	1.7 ± 0.15	n.d.
C16:0	6.6 ± 0.81	3.7 ± 0.10*
C18:0	8.4 ± 1.05	5.3 ± 0.80*
C20:0	n.d.	10.0 ± 0.50
C22:0	2.2 ± 0.16	n.d.
Σ saturated	18.9 ± 2.17	19.0 ± 0.40
C17:1	n.d.	2.1 ± 0.10
C18:1	4.9 ± 0.55	3.8 ± 0.05
C20:1	23.1 ± 1.84	7.9 ± 0.70*
C22:1	n.d.	1.1 ± 0.05
Σ monounsaturated	28.0 ± 2.39	14.9 ± 0.80*
C17:2	n.d.	4.9 ± 0.10
C18:2	2.2 ± 0.65	n.d.
C20:3	n.d.	6.9 ± 0.35
C20:4	3.8 ± 0.58	n.d.
C20:5	28.8 ± 2.19	36.6 ± 0.65*
C22:4	2.1 ± 0.13	n.d.
C22:6	12.7 ± 1.38	13.5 ± 0.25
Σ polyunsaturated	49.6 ± 4.93	61.9 ± 1.35*
Others	3.5 ± 0.75	4.2 ± 0.15

[1] Pairs of mean corresponding to internal organs of spring and winter starfish were compared and those that were significantly different ($P < 0.05$) are indicated by (*). Results represent mean ± SD (n = 6); n.d., not detected.

In the internal organs of starfish, EPA was found in more significant amount than DHA in both seasons. Significantly ($P < 0.05$) higher amount of EPA was observed in winter compared to spring. However, no significant ($P > 0.05$) difference was observed in DHA content in spring and winter month. EPA is shown to be characteristic fatty acid in most of the Japanese sea invertebrates and the echinoderms comprise the richest source of both quality and quantity of polyunsaturated fatty acids [41]. Moreover, several n-3 and n-6 fatty acids have been used as biomarkers to assess relationships between primary producers and invertebrate consumers [10, 44, 45]. The bulk of *de novo* biosynthesis of EPA and DHA is thought to take place among phototrophic algae at the base of the marine food web. From there, they are transferred through trophic levels and accumulated as major constituents of the lipids of all marine animals [46]. Generally, marine invertebrates store lipids for convenient energy source to sustain the reproductive cycle or as a response to physical factors such as oxygen levels and temperature [47]. In this study, the differences in lipid content and composition, and fatty acid composition in different lipid fractions may be caused by multiple factors such as life cycle, sex, variation of plankton in different seasons and temperature, which could influence physiological activities and metabolism of starfish.

Conclusions

This study has shown that the lipid content and fatty acid composition of starfish internal organs vary with the seasons. A higher proportion of PUFA was found in winter (61.9%) compared to spring (49.6%) in the polar lipid fraction. Moreover, starfish lipid is rich in glucosylceramide (approximately 630 mg/100 g internal organs), eicosamonoenoic acid (C20:1) and EPA bound phospholipids (approximately 1200 mg/100 g internal organs), indicating their potential use as cosmetic or pharmaceutical ingredients.

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