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Composition of Lipid and Amino Acid in *Semisulcospira gottschei* Tissues

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다슬기중 지방질 및 아미노산 조성

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ABSTRACT—This study was performed to investigate the detailed lipid content, lipid composition and amino acid composition of *Semisulcospira gottschei* tissues. Lipids of *Semisulcospira gottschei* tissues were extracted by the mixture of chlololform-methanol, fractionated into neutral lipids, glycolipids and phospholipids by silicic acid column chromatography and the composition of these lipid classes were determined by TLC and GLC. The amino acids in *Semisulcospira gottschei* tissues was analyzed by the amino acid auto analyzer. The total lipids content was 1.4% and the main components of the total lipids were neutral lipids 67.9%, glycolipids 19.3% and phospholipids 12.8%, respectively. The main fatty acids of total lipids were palmitic acid (20.5%), palmitoleic acid (16.4%) and linolenic acid+eicosenoic acid (13.3 %). The main fatty acids of neutral lipids were palmitic acid (24.8%), linolenic acid+eicosenoic acid (15.0%) and linoleic acid (13.1%), the main fatty acids of glycolipids were palmitic acid (41.9%), palmitoleic acid (19.7%) and oleic acid (11.7%), and the main fatty acids of phospholipids were linolenic acid+eicosenoic acid (55.1%), oleic acid (17.3%) and palmitic acid (11.4%). The main amino acids were glutamic acid (16.0%) and aspartic acid (11.1%).

Keywords □ Lipid, Amino Acid, *Semisulcospira gottschei*

The lipid content, fatty acid composition and amino acid composition of shellfish were influenced by condition of growing environment; depth of inhabitation and kinds of feed[1-3]. There are many papers on the lipid composition and amino acid composition of seawater shellfish[4-10] and freshwater fish[11,12]. However, there are little reports on lipid composition, fatty acid composition and amino acid composition of freshwater shellfish[4,11-14].

Semisulcospira gottschei, belongs to *pleuroceridae*[1-3], has used for nutrition and health food because of special flavor, irrespective of age and sex since old times.

The freshwater snail genus *Semisulcospira* is widespread in Korea, Japan, Taiwan and China[1]. The number of whorls and adult shell length are subject to environmental control. This shellfish inhabit very popular in rivers and lakes of Korea, especially in Chuncheon[2,3] and this study was performed to investigate the detailed lipid and amino acid composition of *Semisulcospira gottschei*.

Therefore, in this paper, the authors report the results of composition and content of lipids and amino acids from *Semisulcospira gottschei* tissues.

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Materials and Methods

Materials

Semisulcospira gottschei were obtained from Anbori, Chuncheongun, Kangweon-do, Korea, on June 18, 1993 and was kept frozen after casting off the shell and operculum. These snails were about 2 cm long.

All solvents were used guaranteed grade. 10% Boron triflouride-methanol was obtained from Fluka, A.G. Fatty acid methyl esters used as standards were purchased from Sigma Chemicals and Kiesegel 60 for TLC were the products of Merck. All other reagents were analytical grade.

Perkin-Elmer 900 gas chromatograph was used for fatty acid analysis and LKB 4150 α Analyzer was used for amino acid.

Chemical composition analysis

Moisture and ash contents were determined by the usual gravimetrical methods. Crude protein was determined by the Kjeldahl method and crude lipid was measured by the Soxhlet method. The carbohydrate content was calculated by substracting the other component from the total.

Total lipid extraction and purification

Total lipids were extracted from *Semisulcospira gottschei* tissues with a mixture of chloroform-methanol according to the procedure of Bligh and Dyer[15]. The tissues (100 g) were homogenized in a Waring Blender for 2 min. with a mixture of 100 ml chloroform and 200 ml methanol. The mixture was then added 100 ml chloroform and after blending for 30 seconds, 100 ml distilled water was added and blending continued for another 30 sec. The homogenate was filtered through Advantec No. 2 (Toyo No.2) filter paper on a Büchner funnel with slight suction. The filtrate was transferred to a 500 ml separatory funnel and after allowing a few minutes for complete separation and clarification. The chloroform layer was evaporated to small volume under reduced pressure in an atmosphere of nitrogen, transferred to tared vials, and finally taken to complete dryness under high vacuum, and weighed.

Crude total lipid was purified according to the method of Folch, *et al*[16,17]. The dried lipid was dissolved in a small volume of chloroform-methanol, transferred to separatory funnel, and shaken with one fifth of its volume of air-free distilled water (boiled out and cooled in a current of nitrogen) to remove non-lipid substance in the upper layer. The chloroform layer was transferred to tared vials, evaporated in a stream of nitrogen at 40℃ and weighed.

The purified total lipid was dissolved in chloroform, transferred to vials and stored at refrigerator until analysis.

Column chromatography of neutral lipids, glycolipids and phospholipids

The purified total lipid was fractionated by silicic acid column chromatography (they are refered to hereafter as SACC) according to Rouser, *et al*.[18,19] Silicic acid was rinsed with distilled water and methanol. The residue was activated for overnight at 100℃. Prepare a slurry of 15~20 g activated silicic acid in a 100 ml beaker in about 30~50 ml of chloroform and pour into a 20 mm×30 cm column equipped with Teflon stopcock and sintered glass at the bottom[19].

The purified lipid (200~400 mg) was taken up with successive small volume (2~5 ml) of chloroform, which were transferred to a medium porosity sintered glass column. Chloroform (10 column volumes), acetone (40 column volumes) and methanol (10 column volumes) were sequentially to elute the neutral lipids (NL), glycolipids (GL) and phospholipids (PL), respectively[19]. The flow rate (about 3 ml/min) was controlled by nitrogen gas pressure (2~3 psi) on the column. Each lipid class was evaporated in a stream of nitrogen at the low temperature (35℃), and weighed. Each lipid stored under nitrogen at −15℃ until analysis. Lipids were dissolved in a small volume (1~3 ml) of chloroform-methanol (2 : 1) and examined by TLC for identification of components[16,17,19-21].

Thin layer chromatography

Thin layer chromatography was performed on standard 20×20 cm chromatoplates, precoated with a 0.25 mm layer of Silica Gel G (E. Merck).

Small volume of each lipid classes in chloroform-methanol were spotted at the origin and the plates were developed until the front went up to the level 3 cm below the top.

Solvent system containing petroleum ether-diethyl ether-acetic acid (80 : 20 : 1, v/v/v) for first development and hexane-diethyl ether-acetic acid (70 : 30 : 1, v/v/v) for second development were used for separation of NL[19-21].

The spots were checked by iodine vapor and identified by spraying 50% sulfuric acid. The lipids separated by TLC were identified by comparing R_f values with those reported previously[16,17,19-21] and with those pure compounds.

Analysis and preparation of fatty acid methyl ester

Purified lipids were saponified with the methanolic NaOH (0.5 N) in a hot water bath (60~70℃) for 1 hour. After saponification, fatty acids were methylated following the method of Metcalfe[22,23]. The fatty acid methyl esters were analyzed using a Perkin-Elmer 900 gas chromatograph equipped with flame ionization detector. Operating conditions for GLC are described in Table 1.

The relative concentration of each fatty acid was calculated by triangulation of the peak areas on the chromatogram and was expressed as percentage of total peak area. Each analysis was repeated 3 times.

Analysis of amino acid composition

The total amino acid concentrations were determined by the column chromatographic method based on the Spackman method[24], using a LKB model 4150 α auto amino acid analyzer. The hydrolysis of protein was made as described by Zumwalt *et al*[25] and Cavins *et al*[26].

Weigh 5~10 mg sample into long-necked ampoule (tear drying bulb). Add 2 ml of 6 N HCl, constrict neck of ampoule (but do not seal), and freeze sample in dry-ice bath. Attach ampoule to vacuum line and apply vacuum of about 1 mmHg with mechanical pump. Let sample melt while vacuum is maintained and after air bubbles have been removed (1~2 min). Seal ampoule at constriction, still maintaining vacuum. Heat sample 24 hours in 110 ℃ dry oven. Dilute hydrolysate with H_2O (sealed ampoule) and evaporate to near dryness in rotary evaporator at 45℃. Add water and evaporate sample to dryness two more times. Dissolve residue

Table 1. Operating condition of GLC analysis of fatty acid methyl ester

−Instrument	·Perkin-Elmer 900 gas chromatograph
−Column	·15% DEGS+1% H_3PO_4 on Chromosorb W, AW, DMCS, 80~100 mesh, 1.8 m×2.0 mm (I.D.) Stainless steel column
−Column temperature	
Initial temperature	·170℃
Initial time	·4 min
Program rate	·3℃/min
Final temperature	·200℃
−Injector temperature	·250℃
−Detector(FID) temperature	·250℃
−Carrier gas	·Nitrogen, 40 ml/min
−Chart speed	·0.5 cm/min
−Attenuation	·×16
−Amplifier A range	·×100
−Recorder	·QPD_{74} Hitachi two-pen recorder

Table 2. Operating conditions of auto amino acid analyzer

−Instrument	LKB 4150-α analyzer
−Column size	6×200 mm(L)
−Analysis time	90 min
−Buffer flow rate	45 ml/hr
−Ninhydrin flow rate	35 ml/hr
−Buffer pressure	22 bar
−Ninhydrine pressure	16 bar
−Column temperature	50~80℃
−Reaction bath temperature	130℃
−pH range	3.2~10

Table 3. Chemical composition of *Semisulcospira gottschei* tissues (wt%)

Sample	Moisture	Ash	Crude protein	Crude lipid	Carbohydrates
Semisulcospira gottschei	80.7	2.9	10.6	1.6	4.2

Table 4. Lipid contents of *Semisulcospira gottschei* tissues (wt%)

Sample	Crude lipid	Percentage in total lipid		
		NL	GL	PL[a]
Semisulcospira gottschei	1.4± 0.25	67.9± 0.82	19.3± 0.34	12.8± 0.30

[a] NL: Neutral Lipid, GL: Glycolipid, PL: Phospholipid

in suitable volume of pH 2.2 citrate buffer. The color intensity of the amino acid-ninhydrin complex was measured at 570 nm except proline which was measured at 440 nm. Concentrations of the amino acids were calculated by the peak area of the standard amino acid chromatogram. The peaks were integrated electronically with LKB 2220 recording integrator according to the operating conditions for analysis of amino acid described in Table 2.

Results and Discussion

Chemical composition

The chemical composition of *Semisulcospira gottschei* tissues is shown in Table 3. The tissues contained about 1.6% lipid and 10.6% protein.

Content of total lipids

Crude total lipid content of *Semisulcospira gottschei* tissues (moisture content: 80.7%) was 1.4% and purified total lipid by Folch method was 92.8% of the crude total lipid. This result showed a very resemblance to that of *Crassostrea gigas* (1.8%)[4].

Contents of neutral lipid, glycolipid and phospholipid in total lipid

The results of lipid fraction by using of silicic acid column chromatography are showed in Table 4. In total lipid, neutral lipid was the predominant component (67.9%), followed by glycolipid (19.3%) and phospholipid (12.8%). However, Lee *et al.*[13] reported that neutral lipid was the predominant component (66.0%), next phospholipid (18.0%) and glycolipid (16.0%). According to Son *et al.*[14], neutral lipid was the predominant component (61.4%), secondly glycolipid (15.5%) and phospholipid (9.5%). This results were found to be quite similar to those of given in the literature. There were no significant differences in the lipid compositions of the seawater shellfish and freshwater shellfish.

Composition of neutral lipids

Neutral lipids of total lipids extracted from sample were quantitatively fractionated by SACC. Neutral lipids were spotted on the Kieselgel 60 precoated plate. The neutral lipid from *Semisulcospira gottschei* tissues were resolved by TLC as shown in Fig. 1.

The neutral lipid compositions of the *Semisulcospira gottschei* were found to be quite similar to those of given in the report[13], but fairly differ to those of other[14]. This may have been due to condition of growing environment during growth. The contents of NL fractioned from chloroform by Lee *et al*[13] were similar to our results.

By TLC, the compositions of neutral lipid were separated into triglyceride (TG), free sterol (FS), monoglyceride (MG) and diglyceride (DG), etc. The TG was the predominant component, followed by FS and MG.

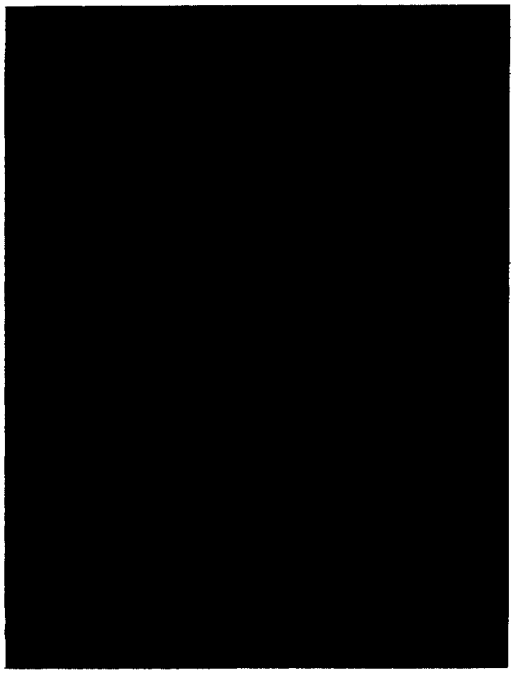

Fig. 1. Thin layer chromatogram on neutral lipid (NL) in *Semisulcospira gottschei* tissues.

Plate: Kieselgel 60 (0.2 mm precoated, Merck Co.)

Solvent system

1st development: Petroleum ether/diethyl ether/acetic acid (80 : 20 : 1, v/v/v)

2nd development: Hexane/diethyl ether/acetic acid (70 : 30 : 1, v/v/v)

MG: monoglyceride, FS: free sterol, DG: diglyceride, FFA: free fatty acid, /TG: triglyceride, ES: esterified sterol, TGE: triglyceride ester, WX: wax, HC: hydrocarbon.

Fatty acid composition

Fatty acid compositions of lipid in *Semisulcospira gottschei* tissues are shown in Table 5.

Fatty acid compositions of total lipids, nonpolar lipids and polar lipids of the *Semisulcospira gottschei* tissues are characterized by a high level of saturated fatty acids. In total lipid, Palmitic acid ($C_{16:0}$) was the predominant component (20.5%), followed by palmitoleic acid ($C_{16:1}$, 16.4%) and linolenic acid ($C_{18:3}$)+eicosenoic acid ($C_{20:1}$) (13.3%).

Fatty acid compositions of NL were that palmitic acid ($C_{16:0}$, 24.8%) was present as major components, and secondly linolenic acid ($C_{18:3}$)+eicosenoic acid ($C_{20:1}$) (15.0%), and linoleic acid ($C_{18:2}$, 13.1%).

Table 5. Fatty acid composition of total lipid and three lipid classes in *Semisulcospira gottschei* tissues (The content is expressed as peak area percentage)

Fatty acid	Tissues in *Semisulcospira gottschei*			
	TL	NL	GL	PL
14 : 0	4.13	2.68	6.94	0.60
16 : 0	20.51	24.83	41.88	11.40
16 : 1	16.36	11.41	19.74	0.58
18 : 0	4.55	0.22	7.70	4.03
18 : 1	11.15	9.26	11.66	17.30
18 : 2	3.09	13.05	2.31	11.03
18 : 3+20 : 1	13.29	14.99	7.00	55.06
22 : 0	–	2.35	–	–
22 : 1	4.89	3.13	2.78	–
Un 1	0.46	1.30	–	–
Un 2	–	1.79	–	–
Un 3	7.22	14.09	–	–
Un 4	10.92	–	–	–
Un 5	3.44	0.89	–	–
Saturated	29.19	30.38	56.52	16.03
Unsaturated	48.78	51.84	43.49	83.97

TL: total lipids, NL: neutral lipids, GL: glycolipids, PL: phospholipids, Un: Unknown

The major fatty acid of GL were that the content of palmitic acid was the highest (41.9%), and then palmitoleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$) acid were 19.7% and 11.7%.

On the other hand, fatty acid of phospholipids are specified by predominance of polyunsaturated, monounsaturated fatty acids such as linolenic acid+eicosenoic acid (55.1%), follwed by oleic acid (17.3%) and palmitic acid (11.4%).

Palmitic acid was found to constitute 11.4~41.9% of each lipid. This result showed a difference from that of Bugbangjohgae (*Spisula sachalinensis*)[5], Purple Shell, Abalone[10] and Cockle clam[6]. This result showed a very resemblance to that of neutral lipid in *Hurumi zecheob*[4].

Amino acid composition

Table 6. Amino acid composition of tissues in *Semisulcospira gottschei*

Amino acid	Tissues in *Semisulcospira gottschei* mg%	% to total amino acid
Asp	1178.6	11.1
Thr	576.6	5.4
Ser	505.2	4.8
Glu	1703.4	16.0
Pro	463.8	4.4
Gly	690.2	6.5
Ala	625.2	5.9
Cys	45.2	0.4
Val	593.4	5.6
Met	207.9	2.0
Ile	486.3	4.6
Leu	928.7	8.7
Tyr	384.5	3.6
Phe	530.0	5.0
His	257.0	2.4
Lys	797.7	7.5
Arg	658.3	6.2
Total	10632.0	100.0

Table 6 shows the total amino acid of *Semisulcospira gottschei* tissues.

Semisulcospira gottschei tissues have contained the amino acids of 17 kinds. With the exception of taurine and tryptophan, all the common amino acids were detected together with a considerable large amount of glutamic acid. Glutamic acid was the most predominant acid amounting to more than 1700 mg%. The contents of glutamic acid, aspartic acid, leucine, lysine, glycine, arginine and alanine were fairly high in the tissues. Glutamic acid, aspartic acid, leucine, lysine, glycine, arginine and alanine accounted for 61.9% of the total amino acids in *Semisulcospira gottschei* tissues. Amino acid compositions of the *Semisulcospira gottschei* tissues are characterized by a high level of glutamic acid and aspartic acid. These have been the key flavor components. Glycine can contribute to sweetness and histidine to meaty character[14].

On the other hand, studies on the free amino acid compositions of shellfish and fishes in freshwater have been carried out by several scientists, such as Lee *et al*[14]., Kim[11] and Choi *et al*.[12] reported that lycine (3.5%), glutamic acid (2.8%) were contained high content in Carp and Israeli carp. Lee *et al*[14] reported that lycine (13.8%) and alanine (13.0%) were detected the predominant component in *Corbicula elatior*. It is probably caused by condition of growing environment; depth of inhabitation and kinds of feed[1-3].

국문요약

다슬기 조직의 지방은 chloroform-methanol로 추출하여 관 크로마토그래피로 극성 및 비극성 지방질로 분획하였으며, 이를 기체-액체 크로마토그래피를 이용하여 지방산 조성을 정량하였고, 구성 아미노산은 산가수분해하여 아미노산 자동분석기를 이용하여 정량하였다. 총 지방질 함량은 1.4%이였으며, 총 지방질중 중성지방질, 당 지방질 및 인 지방질의 함량은 각각 67.9%, 19.3% 및 12.8%이었다. 총 지방질중 주요 지방산 조성은 $C_{16:0}$가 20.5%, $C_{16:1}$이 16.4% 그리고 $C_{18:3}+C_{20:1}$이 13.3% 순이었다. 중성 지방질중 주요 지방산 조성은 $C_{16:0}$가 24.8%, $C_{18:1}+C_{20:1}$이 15.0% 그리고 $C_{18:2}$가 13.1% 순이었다. 당 지방질중 주요 지방산 조성은 $C_{16:0}$가 41.9%, $C_{16:1}$이 19.7% 그리고 $C_{18:1}$이 11.7% 순이었다. 인 지방질중 주요 지방산 조정은 $C_{18:3}+C_{20:1}$이 55.1%, $C_{18:1}$이 17.3% 그리고 $C_{16:0}$가 11.4% 순이었다. 주요 아미노산 함량은 glutamic acid가 16.0%, aspartic acid가 11.1% 순이었다.

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