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Nutrient Components in the Siphon of the Surf Clam *Tresus keenae*

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We evaluated the nutritional composition of the siphon of the surf clam *Tresus keenae* in regard to the presence of nitrogenous [amino acids, nucleotides and their related compounds, total creatinine, betaine, trimethylamine oxide (TMAO), and trimethylamine (TMA)] and non-nitrogenous compounds (sugars and organic acids), lipid fatty-acid composition, and occurrence of minerals. The content of total free amino acids was 660.27 ± 7.94 mg/100 g, and the predominant amino acids were arginine, alanine, sarcosine, glycine, and glutamic acid. These amino acids accounted for 71% of the total free amino acids. Among the nucleotides and their related compounds, inosine was the major component and comprised 40.38 ± 0.02 mg/100 g. Free amino acids were the largest contributor to total extracted nitrogen, comprising 49.94%, followed by total creatinine, betaine, nucleotides, and ammonia; the contribution of TMAO and TMA was small. For the non-nitrogenous compounds, malic acid, propionic acid, and succinic acid comprised the major portion of the ten kinds of organic acids detected, and the sugars found were glucose, maltose, and arabinose, which were estimated to be 147.02±7.15, 34.45±1.09, and 1.21±0.02 mg/100 g, respectively. The predominant minerals were Na and K, which comprised 11.43±1.06 and 9.46±1.02 mg/100 g, respectively. The major fatty acids were C22:6, C20:5, C23:0, C18:3, and C16:0 in the lipid fractions. The 23:0 level of glycolipid (GL) was the highest of any other lipid fraction. The amount of total polyunsaturated fatty acids (PUFA) in the lipid fractions was higher, ranging from 58.22% in GL to 77.1% in phospholipid (PL), compared to the saturated and monounsaturated fatty acids. Of the n-3 fatty acids, C20:5 and C22:6 contributed 35.30-64.44% of PUFA in the lipid fractions. The ratios of n-3 to n-6 PUFA in total lipid (TL), neutral lipid (NL), PL, and GL were 4.35, 4.26, 6.69, and 2.04, respectively.

Key words: *Tresus keenae* , Surf clam, Siphon, Nitrogenous and non-nitrogenous compounds, Mineral, Fatty acid

Introduction

Shellfish include crustaceans, mollusks, and other invertebrates, and the major commercial shellfish are crabs, oyster, scallops, abalones, clams, and shrimp, which are usually consumed fresh or frozen. Historically, clams have been the most popular shellfish in Korea, Japan, Spain, Portugal, the People's Republic of China, and most Southeast Asian countries, and the species used in Korea and Japan are freshwater clams (*Corbicula leana*), hard clams (*Meretrix meretrix lusoria*), short-necked clams (*Venerupis philippinarum*), hen clams (*Mactra sachalinensis*), and surf clams (*Tresus keenae*; Tanikawa, 1985).

Of the commercially available clams, the surf clam, called 'wanguruk' in Korea, is a large bivalve with a shell that is normally about 140-180 mm in length, 90-100 mm in height, and 55-65 mm in width. The main locations of production for this shellfish in Korea are the coastal areas of Geojae, Sachun, Namhae, and Yeosu, and even though the harvest is not very large, a substantial portion is exported to Japan. The surf clam is marketed at a high price and is highly sought after for consumption because the clam tastes similar to umami (monosodium glutamate-like and meaty), is sweet, and has a good texture. Due to the excellent taste of the clam, the muscle is not only served raw but is also cooked or broiled with various seasonings.

Numerous studies have concentrated primarily on the taste-active components of shellfish (Ryu and Lee, 1978; Lee and Heo, 1980; Konosu, 1973; Fuke and Konosu, 1989; Konosu et al., 1988; Chung et al., 2001; Kim et al., 2000), but no analytical data on the

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composition of surf clams harvested in the costal area of Yeosu have been reported. In this paper, we report on the nitrogenous and non-nitrogenous compounds, fatty acid composition, and minerals found in the siphon, which is the major edible part, of the surf clam.

Materials and Methods

Materials

Surf clams (700-1,000 g) were purchased from a retailer in Yeosu in 2002. The siphons from five surf clams were mixed in a homogenizer (KNIFETEC 1095 Sample Mill, FOSS TECATOR, Sweden) and kept frozen at -80°C until use.

Proximate composition

Total nitrogen (TN) was determined by the semi-micro-Kjeldahl method (AOAC, 1984). Crude protein was expressed as total nitrogen (TN)×6.25. Ash, fat, and moisture contents were measured according to standard methods (AOAC, 1984), and the amount of carbohydrate was calculated by the difference, subtracting contents (g) of moisture, fat, ash, and crude protein from 100 g.

Total and free amino acid analysis

The total amino acid composition of the sample was determined using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Uppsala, Sweden). The sample was hydrolyzed with 6 M HCl in evacuated sealed tubes at 110°C for 24 h. Free amino acids were extracted into 80% ethanol at room temperature for 12 h, reextracted with 4 mL of water for 4 h, and then deproteinized with 5-sulfosalicylic acid.

Analysis of ATP and related compounds

A 10-g sample was homogenized with 25 mL of chilled 10% $HClO_4$ for 15 min. The homogenate was centrifuged at 10,000×*g* (4°C) for 10 min, and the residue was reextracted twice more in the same manner. The combined supernatant was neutralized with 5.0 N KOH and centrifuged at 10,000×*g* for 10 min, and the final volume was increased to 10 mL with neutralized $HClO_4$. Aliquots were filtered through a Millipore filter (0.45 μm) for analysis. Nucleotide analysis was performed by a high-performance liquid chromatographic (HPLC) method similar to that reported by Ryder (1985). An HPLC system (Shimadzu LC-10A system, Shimadzu Co., Tokyo, Japan) with a Cosmosil C18 column (4.6 mm×250 mm, Nacalai Tesque, Inc.) was used at room temperature. The mobile phase was 0.04 M KH_2PO_4-0.06 M K_2HPO_4 (pH 7.0), and the flow rate was 1.0 mL/min. A signal was detected at 254 nm with an SPD-10A UV-VIS detector (Shimadzu Co.).

Betaine, TMA, TMAO, and total creatinine analysis

The amount of betaine was determined according to the method of Konosu and Kasai (1961) and TMAO and TMA according to the methods of Sasaki et al. (1953) and Hashimoto and Okaichi (1957). Total creatinine was determined according to the method described by Suyama et al. (1977).

Mineral analysis

The samples were prepared by the wet ashing method (AOAC, 1984). A 5-g sample was placed into an 800-mL Kjeldahl flask, and 20 mL of concentrated nitric acid and 10 mL of 70% perchloric acid were added. The flask was then heated until the solution was colorless and dense white fumes appeared. The addition of the acids was repeated as necessary to produce the colorless solution. The volume of the digest solution was increased to 25 mL with deionized water. The minerals were then evaluated with an atomic absorption spectrophotometer (AAS-6501, Shima-dzu, Japan) equipped with a graphite chamber and were quantified on the basis of peak areas by comparison to a calibration curve obtained with corresponding standards.

Organic acid analysis

A 5-g sample was homogenized using a Waring blender (Nihonseiki Co., Japan) in 100 mL of deionized water for 2 min and then extracted using a magnetic stirrer for 3 h at room temperature. The mixture was centrifuged at 3,000 rpm for 20 min, and the supernatant was recovered. The residue was extracted twice with 100 mL of deionized water after shaking for 1 h. The extracts were combined and filtered. The filtrate was concentrated to 100 mL using a rotary evaporator at 45°C. The filtrate was analyzed by a Dionex DX 500 (Sunnyvale, CA, USA) equipped with a conductometric detector and interfaced to a computer running PeakNet 6.1 software (Sunnyvale, CA, USA). The separation was carried out using an IonPac ICE AS6 column (4 mm, Dionex) and an Anion ICE MicroMembrane suppressor with external regeneration. The mobile phase was 0.4 mM heptafluorobutyric acid at a flow rate of 1.00 mL/min. The regenerating phase was 5.00 mM tetrabutylammonium hydroxide, and the flow rate was 4 mL/min.

Sugar analysis

A suspension containing 10 g of homogenized

sample in 250 mL of 80% ethanol was heated at 90°C for 3 h under a reflex condenser, cooled, and centrifuged at 3000 rpm for 10 min. The ethanol was removed with a rotary evaporator at 45°C. The samples were redissolved in double-distilled water and filtered through 0.22-μm filters. The amount of carbohydrates was determined by anion-exchange chromatography with a pulsed amperometry detector on a DX 500 chromatography system (Sunnyvale, CA, USA). A gold electrode was used as the working electrode and silver/silver chloride as the reference electrode. A CarboPac PA1 guard column (4×25 mm) and CarboPac PA1 analytical column (4×250 mm) were used. The eluent was 16 mM NaOH with a flow rate of 1 mL/min.

Lipid extraction and fractionation

Total lipid (TL) extraction was conducted according to Bligh and Dyer (1959). The TL was separated into three classes by silicic acid column chromatography according to the method of Rouser et al. (1967). Thirty grams of silicic acid (100 mesh, Sigma, St. Louis, MO, USA) were poured onto the column (40×3 cm, o.d.) with chloroform, and then 350 mg of TL were applied. Nonpolar lipids (NPL) were eluted with chloroform (400 mL), glycolipids (GL) with acetone (600 mL), and phospholipids (PL) with chloroform-methanol (1:1, 300 mL) and methanol (300 mL), successively. The eluent was monitored by thinlayer chromatography (TLC). NPL, GL, and PL fractionated by silicic acid column chromatography were separated by TLC and identified by comparing their R_f values to authentic compounds. NPL and polar lipid were developed using hexane/diethyl ether /glacial acetic acid (90:10:1) and using chloroform/ methanol/water (65:25:4), respectively.

Fatty acid analysis

Fatty acid methyl esters from the lipid extraction were prepared according to the AOCS Official Method Ce 1-62 (AOCS, 1988). They were analyzed by gas-liquid chromatography (HP 5890, Hewlett-packard Co., USA) using an SP-2560 capillary column (100 m×0.25 mm i.d., Supelco Inc., Bellefonte, PA, USA). The injector and flame-ionization detector temperature were maintained at 250°C. The column was programmed to operate from 180 (initial holding time 10 min) to 240°C at 3°C/min with the final time set for 15 min. Helium was used as the carrier gas at a linear velocity of 0.8 mL/min with a split ratio of 1:50. Fatty acids were identified by comparison to authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Data were calculated as the peak area percent of the total area of fatty acids. Methyl tricosanoate (99%, Aldrich Chem. Co., Milwaukee, WI, USA) was used as an internal standard for the quantitative calculation of fatty acid. All data are presented as the mean values of four determinations (two groups×two determinations) for each sample.

Statistical analysis

All determinations were done in triplicate, and data were analyzed using a one-way analysis of variance (ANOVA) and Duncan's multiple range test (Steel and Torrie, 1980).

Results and Discussion

Proximate compositions

Table 1 shows the results of proximate analyses of the siphons of surf clams. Compared to fish, bivalves are generally higher in carbohydrates, because of glycogen storage, but lower in lipids. As shown in Table 1, the carbohydrate content was higher, comprising 3.28±0.42 g/100 g, and lipid was somewhat lower, comprising 1.19±0.05 g/100 g, than fish (NFRDA, 1995).

Table 1. Proximate composition in the siphon of the surf clam. Data are mean values of triplicate determinations ± standard deviation.

(g/100 g)

Moisture	Crude lipid	Crude protein	Carbohydrate	Ash
77.50±0.67	1.19±0.05	16.19±0.42	3.28±0.42	1.84±0.02

Free and total amino acids

The total free amino acid content of the siphon was 660.27±7.94 mg/100 g, and the major amino acids were arginine (186.89±5.82 mg/100 g), alanine (106.84 ±4.01 mg/100 g), sarcosine (76.27±3.09 mg/100 g), glycine (63.90±2.38 mg/100 g), and glutamic acid (37.99±2.97 mg/100 g). These five amino acids accounted for 71% of the total amino acids (Table 2).

The free amino acid composition of animal muscle is generally quite different from species to species, and a few amino acids dominate the total amount of amino acids. Komata et al. (1962) and Lee (1968) reported that amino acids such as glycine, alanine, and proline comprised a large portion of the total free amino acids in many invertebrate animals. Fujita et al. (1968) reported that taurine, glycine, and arginine were abundant in the free amino acids of the adductor muscle, and Konosu et al. (1965) also reported that taurine, glycine, alanine, glutamic acid, and arginine comprised the dominant portion of free amino acids

Table 2. Free amino acids in the siphon of the surf clam. Data are means of triplicate measurements.

Amino acid	Content (mg/100 g)	Content (N-mg/100 g)	% to total free amino acid
Phosphoserine	2.07±0.07	0.16	0.31
Taurine	16.02±1.24	1.79	2.43
Urea	50.69±3.21	23.65	7.68
Aspartic acid	2.24±0.05	0.24	0.33
Hydroxyproline	5.56±0.52	0.59	0.84
Threonine	8.95±1.32	1.05	1.36
Serine	13.45±1.13	1.79	2.03
Asparagine	20.10±1.86	4.26	3.04
Glutamic acid	37.99±2.97	3.62	5.75
Sarcosine	76.27±3.09	11.99	11.55
Proline	1.02±0.02	0.12	0.15
Glycine	63.90±2.38	11.92	9.68
Alanine	106.84±4.01	16.80	16.18
-Aminobutyric acid	3.79±0.05	0.33	0.57
Valine	4.99±0.11	0.60	0.76
Cystine	5.81±0.29	0.68	0.88
Methionine	4.38±0.31	0.41	0.66
DL-Allocysthathionine	3.58±0.58	0.45	0.54
Isoleucine	3.89±0.39	0.42	0.59
Leucine	4.04±0.23	0.43	0.61
Tyrosine	2.36±0.17	0.18	0.36
Phenylalanine	2.85±0.08	0.24	0.43
Homocystine	0.14±0.01	0.01	0.02
-Aminobutyric acid	3.29±0.89	0.45	0.50
Ethanolamine	3.97±0.24	0.91	0.60
Ammonia	12.28±1.09	10.11	1.86
DL-Allohydroxylysine	0.76±0.02	0.13	0.12
Ornithine	4.93±0.23	1.05	0.75
Lysine	1.53±0.19	0.29	0.23
Histidine	5.69±0.95	1.54	0.86
Arginine	186.89±5.82	60.10	28.31
Total	660.27±7.94	156.31	99.98

of the short-necked clam. On the other hand, Konosu and Maeda (1961) found that the content of taurine, arginine, and glycine were the dominant amino acids in abalone extracts. Konosu et al. (1978) indicated that glycine and alanine occupied approximately 50% of the total free amino acid in the extracts of boiled crabs.

Fuke (1994) summarized the contribution of various components to the flavor of five kinds of seafood (sea urchin, snow crab, scallop, short-necked clam, and dried skipjack) by an omission test. Glutamine (Glu) and glycine (Gly) were the taste-active amino acids that were common to the five kinds of seafood irrespective of their amounts. Glu in the synthetic extract not only elicits umami but also improves the overall preference as a result of imparting continuity, thickness, complexity, and mildness. Elevation of sweetness by Glu was recognized in all synthetic extracts except sea urchin, in which the extract without Glu was sweeter. Gly imparted sweetness to the synthetic extracts. Alanine was taste-active only in samples of sea urchin, snow crab, and scallop, in which the contents were relatively high. Arginine was found to be taste-active in snow crab, scallop, and short-necked clam. Taking into account the contribution of glutamic acid, glycine, and arginine to seafood as mentioned above, these amino acids, which have relatively high concentrations, may play an important role in eliciting the flavor of the siphons of surf clams. Table 3 indicates the profiles of total amino acids. The predominant amino acid was glutamic acid (3,223.26±12.28 mg/100 g), which comprised 20.43% of the total amino acid content. Arginine (2,783.89±10.56 mg/100 g), lysine (2,192.56 ±9.07 mg/100 g), glycine (1,692.08±6.35 mg/100 g), and leucine (1,111.13±7.39 mg/100 g) were also prominent components. Proline (1.15±10.56 mg/100 g, 0.01%) was the limiting amino acid in the siphons of surf clams.

Table 3. Total amino acids in the siphon of the surf clam. Data are means of triplicate measurements.

Amino acid	Content (mg/100 g)	% to total amino acid
Aspartic acid	899.89± 7.28	5.70
Threonine	191.04± 2.43	1.21
Serine	427.65± 4.98	2.71
Glutamic acid	3,223.26±12.28	20.43
Proline	1.15± 0.04	0.01
Glycine	1,692.08± 6.35	10.73
Alanine	654.28± 5.34	4.15
Valine	140.41± 3.49	0.89
Methionine	322.87± 4.25	2.05
Ileucine	300.84± 5.02	1.91
Leucine	1,111.13± 7.39	7.04
Tyrosine	429.08± 5.01	2.72
Phenylalanine	450.17± 4.33	2.85
Histidine	714.08± 6.83	4.53
Lysine	2,192.56± 9.07	13.89
Ammonia	240.71± 2.57	1.53
Arginine	2,783.89±10.56	17.65
Total	15,775.09±78.49	100.00

ATP and related compounds

Adenosine 5'-triphosphate (ATP) and related compounds are very important for improving the characteristic taste of each seafood as a result of a synergistic effect with some amino acids, especially monosodium glutamate (Yamaguchi et al., 1971), and are used as indices of freshness in a wide variety of fish (Surette et al., 1988; Greene and Bubbitt, 1990; Hattula et al., 1993).

ATP and ATP-degradation products, such as ADP (adenosine 5'-diphosphate), AMP (adenosine 5'-monophosphate), IMP (inosine 5'-monophosphate), HxR

(inosine), and Hx (hypoxanthine), were detected (Table 4). Among these, inosine (40.38±0.02 mg/100 g) was present in the highest amount, followed by AMP (32.84±0.42 mg/100 g), ADP (12.02±0.05 mg/100 g), hypoxanthine (5.83±0.02 mg/100 g), ATP (3.11±0.67 mg/100 g), and IMP (1.45± 0.03 mg/100 g) (Table 4).

Table 4. ATP and its related compounds in the siphon of the surf clam. Data are means of triplicate measurements.

Compound	Content (mg/100 g)
ATP	3.11±0.67
ADP	12.02±0.05
AMP	32.84±0.42
IMP	1.45±0.03
Inosine	40.38±0.02
Hypoxanthine	5.83±0.02

The post-mortem patterns of ATP have not been thoroughly investigated in invertebrates. Arai (1966) found no IMP in certain marine invertebrates and demonstrated that in many species, AMP is dephosphorylated to adenosine, which is then deaminated to inosine. Stone (1970) also reported that the enzymatic deamination of AMP did not significantly contribute to the IMP content of crab or scallop muscles. On the other hand, the accumulation of IMP in post-mortem storage has been observed in the muscles of several crustaceans, including king crab (Porter, 1968), snow crab (Hayashi et al., 1978), lobster (Dingle et al., 1968), and Antarctic krill (Shibata and Nakamura, 1981). Furthermore, Konosu et al. (1965) detected a large amount of IMP in the soft parts of the short-neck clam, and Konosu et al. (1966) detected IMP in the whole body of a marine worm. Suwetja et al. (1989) reported that IMP was detected in all mollusk and crustacean species examined.

Distribution of nitrogen

The distribution of non-protein nitrogenous constituents in the siphon extract is presented in Table 5; the nitrogen content was calculated for each group of compounds and is shown as a percentage of the total extract nitrogen (EN). The ratio of the total EN (292.77 mg/100 g) to total nitrogen (2,590.4 mg/100 g) was 11.3%, which was lower than other bivalves, such as the pen shell, turban shell, abalone, and oriental hard clam, which exhibited ratios of 21.3%, 16.8%, 22.2%, and 22.4%, respectively (Suyama and Konosu, 1987). The contribution of free amino acids to the total EN is the most substantial, comprising 49.94% of the total EN, followed by total creatinine, betaine, nucleotides, and ammonia; the contribution of TMAO and TMA was small. The recovery of total EN by those compounds was 86.2%.

Table 5. Distribution of nitrogen in the extract of the siphon of surf clam.

Component	Content (mg/100 g)	% to Extract-N
Total extract-N	292.77	
Nucleotide-N	19.82	6.77
Free amino acid-N	146.20	49.94
Ammonia-N	10.11	3.45
TMA-N	2.07	0.71
TMAO-N	3.93	1.34
Betaine-N	25.48	8.70
Total creatinine-N	44.76	15.29
Recovery (%)		86.20

Organic acids, sugars, and minerals

Table 6 shows the contents of organic acids, sugars, and minerals in the siphons of surf clams. Ten organic acids were positively identified, among which malic acid (100.38±7.43 mg/100 g) predominated, followed by propionic acid (86.15±2.97 mg/100 g) and succinic acid (66.17±4.30 mg/100 g). Malic, propionic, and succinic acids contributed 60% to the total organic acid. Small quantities of fumaric, oxalic, and formic acid were also found in the surf clam siphons.

Table 6. Non-nitrogenous compounds in the siphon of the surf clam. Data are means of triplicate measurements.

Compound	Content (mg/100 g)
Organic acids	
Oxalic acid	2.60±0.08
Tartaric acid	22.50±0.74
Citric acid	57.36±0.01
Malic acid	100.38±7.43
Formic acid	2.72±0.06
Lactic acid	25.39±0.86
Acetic acid	54.62±3.07
Succinic acid	66.17±4.30
Fumaric acid	1.66±0.09
Propionic acid	86.15±2.97
Sugars	
Arabinose	1.21±0.02
Maltose	34.45±1.09
Glucose	147.02±7.15
Minerals	
Mn	0.03±0.02
Cu	0.02±0.01
Zn	1.19±0.04
Na	11.43±1.06
Fe	0.51±0.09
K	9.46±1.02
Ca	1.77±0.57

Organic acids are generally responsible for the sour, tart, acidic, and characteristic fruity tastes of many foods (Johnstone and Hammill, 1970; Ulrich, 1970).

Succinic acid is odorless and produces an almost astringent taste and feeling in the mouth when found in aqueous solutions at concentrations higher than 2,000 ppm (Arctander, 1969). Fuke (1994) explained that an aqueous solution of succinic acid can retain a strong flavor similar to the extracts from shellfish, such as short-necked clams, hard clams, and corbicula, and confirmed by the omission test using the synthetic short-necked clam extract that succinic acid was necessary for producing the characteristic taste. Therefore, succinic acid, which is found in a comparatively high amount, may also be an important component in the characteristic flavor of the clam siphon.

For sugars, only glucose, maltose, and arabinose, which were estimated to be 147.02±7.15, 34.45±1.09, and 1.21± 0.02 mg/100 g, respectively, were detected. As in many marine products (Lauer et al., 1974; RCJ, 1982), sodium (11.43±1.06 mg/100 g) and potassium (9.46±1.02 mg/100 g) comprised the major part of the minerals in the siphon. The sodium ion is extremely important in producing the characteristic tastes of crustaceans and shellfish (Konosu, 1973; Hayashi et al., 1979; Hayashi et al., 1981a; Hayashi et al., 1981b).

Fatty acid composition

The fatty acid compositions of total lipid (TL), neutral lipid (NL), phospholipid (PL), and glycolipid (GL) of the surf clam siphons are shown in Table 7. The carbon number of fatty acids in the samples ranged from 14 to 23. The major fatty acids were C22:6, C20:5, C23:0, C18:3, and C16:0 in all lipid fractions. The C16:1 was also the prominent fatty acid in TL and GL, but not in PL and GL. The 23:0 level of GL was the highest of any fatty acid in the saturated fatty acids. The amount of total polyunsaturated fatty acids (PUFA) in all the lipid fractions was higher, ranging from 58.22% in GL to 77.1% in PL, compared to the saturated and monounsaturated fatty acids. Of the n-3 fatty acids, C20:5 (EPA, eicosapentaenoic acid) and C22:6 (DHA, docosahexaenoic acid) contributed 35.30-64.44% of PUFA in all the lipid fractions and were therefore primarily responsible for the highest level of PUFA. The ratios of n-3 to n-6 PUFA in TL, NL, PL, and GL were 4.35, 4.26, 6.69, and 2.04, respectively, showing the highest ratio in PL and the lowest ratio in GL due to the high level (41.93%) of C22:6 in PL and low level (13.14%) of C20:5 in GL.

Table 7. Fatty acid composition of total lipid, neutral lipid, phospholipid, and glycolipid separated from the siphon of the surf clam (weight %). Data presented as means value of 4 determinations. Different characters in the same row are significantly different ($p<0.05$).

Fatty acid	Total lipid	Neutral lipid	Phospholipid	Glycolipid
C14:0	1.31±0.03[a2)]	1.19±0.02[a]	1.16±0.02[a]	0.91±0.01[b]
C15:0	0.27±0.01[b]	0.30±0.01[b]	0.32±0.01[b]	2.27±0.43[a]
C16:0	6.05±0.91[a]	6.38±1.04[a]	4.19±0.71[b]	6.42±1.01[a]
C17:0	0.73±0.50[b]	0.58±0.03[b]	0.31±0.02[c]	1.81±0.04[a]
C18:0	4.01±0.03[b]	5.17±0.82[a]	3.08±0.33[c]	3.17±0.84[c]
C22:0	0.01±0.01[c]	0.05±0.01[c]	0.11±0.02[b]	0.19±0.03[a]
C23:0	10.42±0.98[b]	9.22±1.21[bc]	8.66±1.42[c]	19.90±2.06[a]
Saturated	22.80±0.69[b]	22.89±1.96[b]	17.83±0.97[c]	34.67±2.11[a]
C16:1	7.91±0.05[a]	7.52±1.04[a]	0.74±0.06[b]	0.19±0.03[c]
C18:1n-9(cis)	2.86±0.02[b]	3.64±0.75[a]	2.80±0.19[b]	1.66±0.29[c]
C20:1	1.91±0.07[c]	2.33±0.51[b]	1.54±0.04[d]	5.29±1.02[a]
Monounsaturated	12.68±0.54[a]	13.49±1.27[a]	5.08±0.98[c]	7.14±1.03[b]
C18:3n-3	0.91±0.08[b]	0.75±0.03[bc]	0.87±0.06[b]	2.98±0.03[a]
C20:3n-3	0.09±0.01[a]	0.05±0.01[b]	0.11±0.01[a]	0.04±0.01[b]
C20:5n-3	23.74±1.26[b]	28.13±2.06[a]	22.51±1.39[b]	13.14±0.92[c]
C22:6n-3	25.84±2.39[b]	20.90±0.98[c]	41.93±2.85[a]	22.16±1.53[c]
n-3	50.58±3.78[b]	49.83±2.93[c]	65.42±3.25[a]	38.32±2.71[d]
C18:2n-6(cis)	4.40±0.22[a]	3.27±0.08[c]	3.45±0.19[c]	3.81±0.65[b]
C18:3n-6	7.23±0.11[bc]	8.43±1.01[b]	6.33±0.12[c]	14.99±1.15[a]
n-6	11.63±0.23[b]	11.70±0.26[b]	9.78±0.62[c]	18.80±0.27[a]
C20:2	2.23±0.03[a]	2.10±0.05[ab]	1.90±0.02[b]	1.10±0.02[c]
Polyunsaturated	64.44±3.55[b]	63.63±2.95[b]	77.1±3.41[a]	58.22±2.28[c]
n-3/n-6 ratio	4.35	4.26	6.69	2.04

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