

## Seasonal Variation in the Nutritional Content of Mideodeok *Styela clava*

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We evaluated changes in the nutritional composition of Mideodeok *Styela clava* harvested during the months of January, March, and May 2005. Glutamic acid, aspartic acid, arginine, leucine, and lysine were the most dominant amino acids present. Ratios of essential amino acids to nonessential amino acids were comparable to those of many fish species, with values ranging from 0.55 to 0.61 and 0.66 to 0.67 for muscle of Mideodeok from Geoje and Tongyeong, respectively. Mideodeok seems to be a suitable source of important fatty acids as it contains high levels of polyunsaturated fatty acids. Eicosapentanoic acid EPA; 20:5n-3 and docosahexanoic acid DHA; 22:6n-3 were the most dominant fatty acids, ranging from 20.0 to 22.3% and 16.5 to 17.9% in muscle, and 20.3 to 23.2% and 15.2 to 18.8% in tunic, respectively. The total mineral fraction of Mideodeok was 22.2-27.3% of dry matter. Sodium, calcium, magnesium, and potassium were the most dominant minerals in both muscle and tunic.

Key words: Essential amino acids, Fatty acids, Minerals, *Styela clava*

### Introduction

*Styela clava* is highly esteemed seafood on the south coast of Korea and is particularly liked for its distinctive pine flavor. Locally known as Mideodeok, this leathery sea squirt is harvested in large volumes from the middle of winter to the end of summer. The annual production levels of Mideodeok in Korea for 2001, 2002, and 2003 were relatively high, at up to 15,133 tons, 5325 tons and 3041 tons, respectively (MOMAF, 2005).

Despite its remarkable abundance, very little investigation has been undertaken regarding the possible uses of this species. Past studies of *S. clava* mainly focused on its embryological development (Ermak, 1975; Kusakabe, 1995), antimicrobial properties (Lehrer et al., 2002; Menzel et al., 2002), and immune defense systems (Kelly et al., 1992; Wright and Cooper, 1984). There are limited data on the nutritional composition of ascidians. Kim et al. (1985) reported the presence of high levels of eicosapentanoic acid (EPA; 20:5n-3), docosahexanoic acid (DHA; 22:6n-3), hexadecanoic acid (16:0), and cis-9-octadecanoic acid (18:1n-9) in the total lipids of *S. clava*, with average values of 18.3, 14.2, 16.3, and

7.0%, respectively. A more recent study by Jiang et al. (2005) also reported high concentrations of polyunsaturated fatty acids PUFAs in *S. clava*, especially EPA and DHA. Likewise, Popov et al. (2002) observed high levels of the following fatty acids in the triacylglycerol and phospholipid fractions of *Styela* sp., respectively: 16:0 (25.4%, 22.4%), 18:1 (18.4%, 11.8%), 20:5n-3 (10.7%, 10.3%), 18:0 (8.3%, 9.7%), and 18:2 (8.2%, 4.8%). Popov et al. 2002 also noted the presence of the PUFAs EPA 1.2% and DHA 7.0% concentrated in the phospholipids fraction.

The tunics of four species of ascidian contained 37-67% protein (dry weight basis) and exhibited a striking similarity in relative amino acid composition, with all four displaying high levels of acidic amino acids (Smith and Dehnel, 1971). The free amino acid composition in *S. clava* was dominated by taurine, proline, glutamic acid, and glycine (Lee et al., 1975; Lee et al., 1995). Little other data are available on the biochemical composition of this species, although Ermak (1975) reported the presence of a large glycogen deposit in its pyloric gland. Although Mideodeok possesses a distinct, delicious flavor, its use for human consumption (i.e., in soup, steamed, fermented) is limited to Koreans. This may partly be because of a lack of public knowledge regarding its nutritional

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quality.

The biochemical contents of marine organisms are directly affected by seasonal changes (Ackman, 1995; Orban et al., 2002) and geographical location (Leu et al., 1981; Constantinides et al., 1995). Both season and geographical location have profound effects on temperature, salinity levels, and food availability in the marine environment Socal et al., 2004; Pedersen et al., 2005. A study of the relative sensitivity of different developmental stages of another *Styela* species, *S. pilicata* (Lesueur), to various temperatures and salinity showed that both temperature and salinity have significant effects on embryonic and post larval development (Qian and Thiyagarajan, 2003). In addition, *Halocynthia aurantium* has a very limited capacity to survive an acute temperature elevation, (e.g. through the appearance of thermal currents), because they lack effective homeoviscous mechanisms (Sanina and Kostetsky, 2002).

Our objectives were to evaluate and compare the effects of seasonal changes on the nutritional composition of Mideodeok muscle and tunic, harvested from two different areas off the southern coast of Korea. Our aim was to provide baseline information on the nutrient value of Mideodeok for both consumers and researchers of ascidians, thus paving the way for the wider use of this marine organism, especially as a source of food for humans.

## Materials and Methods

### Materials

*Styela clava* samples were collected from two different culture sites, i.e., Geoje and Tongyeong, off the south coast of Korea in the first week of January, March, and May 2005. Upon arrival at the laboratory, the tunic and muscle were separated and frozen at -40°C until further analysis. Environmental conditions such as the salinity, temperature, and chlorophyll levels of the culture ground were obtained from the National Fisheries Research and Development Institute (NFRDI, 2005).

### Proximate composition

Moisture and ash contents were determined according to standard AOAC methods (AOAC, 1990). Moisture content was measured by drying the tissues to a constant weight in an oven at 110°C. Crude protein levels were quantified using a micro-Kjeldahl method; crude lipid contents were determined using the method of Bligh and Dyer (1959). Ash content was determined by burning to constant weight in a muffle furnace at 550°C.

### Glycogen analysis

Glycogen contents were assayed according to the method of Lo et al. (1970). The samples were boiled with 3 mL of 30% potassium hydroxide for 20 min. After cooling, 50 µL of saturated sodium sulfate solution and 5 mL of 95% ethanol were added. The samples were placed in an ice bath for 30 min to facilitate precipitation. After centrifugation, the precipitate was dissolved in 2 mL of distilled water, re-precipitated with 2.5 mL of ethanol, and re-dissolved in distilled water to a total volume of 25 mL. The glycogen content was measured using the phenol-sulfuric acid method, and the absorbance was measured at 490 nm (Shimadzu UV-1200, Kyoto, Japan). Oyster glycogen type II (Sigma Chemical, St. Louis, MO, USA) was used to prepare a calibration curve.

### Total amino acid analysis

Homogenized tissue samples were hydrolyzed with 6 N HCl in a sealed vacuum ampoule at 110°C for 24 h. The HCl was removed from the reaction mixture on a rotary evaporator and the hydrolyzed sample was diluted to a total volume of 10 mL with 0.2 M sodium citrate buffer (pH 2.2). A Biochrom 20 amino acid analyzer (Pharmacia Biotech., Cambridge, UK) was used to determine the amino acid content.

### Lipid extraction and fatty acid composition analysis

Individual samples were homogenized in a mixture of chloroform and methanol (1:2, v/v) and extracted using the Bligh and Dyer (1959) method. For saponification and methylation, 0.5 M NaOH 1.5 mL was added to 25 mg of the lipid extract and boiled for 7 min at 100°C; 2 mL of 8% boron trifluoride in methanol solution was then added and boiled for another 5 min. After cooling, 1 mL of iso-octane was added to the mixture, blanketed with nitrogen, and vortexed for 30 s. Saturated NaCl solution 5 mL was added and mixed thoroughly by agitation. The iso-octane layer was separated, and the methanol/water phase was re-extracted by adding 1 mL of iso-octane. The combined iso-octane extracts were dried to 1 mL under nitrogen. Fatty acid methyl esters were analyzed using gas chromatography (Shimadzu GC-17A, Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) equipped with an Omegawax-320 fused-silica capillary column (30 m×0.32 mm i.d., Supelco Co., Bellefonte, PA, USA). The oven was programmed at an initial temperature of 185°C for 8 min, after which it was increased to 230°C at a rate of 3°C/min and was held at 230°C for 15 min. The injector and detector temperatures were 250°C and 260°C, respec-

tively. Helium was used as the carrier gas at a constant column inlet pressure of 1.0 kg/cm<sup>2</sup> and a split ratio of 1:50. Fatty acid methyl esters in the samples were identified by comparison to equivalent chain length (ECL) standards (Sigma Chemical Co., St. Louis, MO, USA). Methyl tricosanate (99%: Aldrich Chemical Co., Milwaukee, WI, USA) was used as an internal standard.

### Mineral analysis

Mineral components were identified using inductively coupled plasma (ICP) emission-spectrophotometry (Sequential ICP-AES, Varian, Palo Alto, CA, USA), the quantity of each inorganic component was determined from the peak area using a standard calibration curve. Specifically, 1 g of each sample was incinerated in a furnace 550°C for 12 h. ICP samples were prepared by dissolving the ash in 1 N HCl to a total volume of 200 mL. Identification and quantitative analyses of individual mineral components were conducted at specific wavelengths.

### Statistical analysis

All data are expressed as the mean  $\pm$  SD of triplicate samples. Data were subjected to -Tukey-Kramer honestly significant difference tests using JMP Statistical Discovery Software™, version 5 (SAS Institute Inc., Cary, NC, USA) at 5% level of significance.

## Results and Discussion

### Culture ground environmental conditions

Environmental data for the culture sites were obtained from NFRDI (2005). All data represented mean values at a depth of 0-10 m. The temperature did not differ significantly in Geoje and Tongyeong. The lowest temperatures occurred in February (5.3°C in Geoje, 5.0°C in Tongyeong) and the highest in November (18.8°C, 18.6°C). Salinity levels did not exhibit significant change, and ranged from 31.04 to 33.7 g/L. Chlorophyll a was highest in November, with concentrations of 4.35 and 6.53  $\mu$ g/L in Geoje and Tongyeong, respectively.

### Proximate composition

There was seasonal variation in the proximate composition of Mideodeok (Table 1). The moisture content of both muscle and tunic was slightly higher in Tongyeong (87.0-87.4% and 86.1-86.7%, respectively) than in Geoje (85.7-86.7% and 84.3-84.5%, respectively). The highest moisture content occurred in May, although differences between months were not significant ( $P > 0.05$ ).

The protein content of Mideodeok muscle from Geoje (GM) was significantly lower in May than in January by 12%. No significant changes were found in the protein content of Mideodeok muscle from Tongyeong (TM), although it was somewhat lower in

Table 1. Proximate composition of Mideodeok muscle and tunic from Geoje and Tongyeong, Korea

Sampling date	Proximate composition (%) <sup>1</sup>				
	Moisture	Crude protein	Crude lipid	Ash	Glycogen
Muscle					
Geoje					
January	86.1 $\pm$ 1.0 <sup>ab</sup>	5.0(36.0) $\pm$ 0.2 <sup>a</sup>	1.0(7.2) $\pm$ 0.1 <sup>b</sup>	3.8(27.3) $\pm$ 0.3 <sup>a</sup>	3.0(21.6) $\pm$ 0.2 <sup>b</sup>
March	85.7 $\pm$ 1.1 <sup>a</sup>	4.9(34.3) $\pm$ 0.1 <sup>a</sup>	1.3(9.1) $\pm$ 0.1 <sup>a</sup>	2.8(19.6) $\pm$ 0.2 <sup>b</sup>	3.7(25.9) $\pm$ 0.3 <sup>a</sup>
May	86.7 $\pm$ 0.7 <sup>a</sup>	4.4(33.1) $\pm$ 0.1 <sup>b</sup>	1.3(9.8) $\pm$ 0.1 <sup>a</sup>	3.0(22.6) $\pm$ 0.4 <sup>b</sup>	3.1(23.3) $\pm$ 0.3 <sup>b</sup>
Tongyeong					
January	87.0 $\pm$ 0.7 <sup>a</sup>	4.9(37.7) $\pm$ 0.3 <sup>a</sup>	1.1(8.5) $\pm$ 0.1 <sup>a</sup>	3.1(23.8) $\pm$ 0.2 <sup>a</sup>	2.7(20.8) $\pm$ 0.4 <sup>b</sup>
March	87.0 $\pm$ 0.5 <sup>a</sup>	4.6(35.4) $\pm$ 0.2 <sup>a</sup>	0.9(6.9) $\pm$ 0.1 <sup>ab</sup>	2.9(22.3) $\pm$ 0.1 <sup>a</sup>	3.6(27.7) $\pm$ 0.3 <sup>a</sup>
May	87.4 $\pm$ 1.2 <sup>a</sup>	4.5(35.7) $\pm$ 0.3 <sup>a</sup>	0.8(6.3) $\pm$ 0.1 <sup>b</sup>	2.8(22.2) $\pm$ 0.2 <sup>a</sup>	3.0(23.8) $\pm$ 0.3 <sup>ab</sup>
Tunic					
Geoje					
January	84.4 $\pm$ 0.8 <sup>a</sup>	2.8(17.9) $\pm$ 0.1 <sup>b</sup>	0.3(1.9) $\pm$ 0.0 <sup>a</sup>	3.9(25.0) $\pm$ 0.3 <sup>a</sup>	n.d.
March	84.5 $\pm$ 0.6 <sup>a</sup>	3.6(23.2) $\pm$ 0.2 <sup>a</sup>	0.2(1.3) $\pm$ 0.0 <sup>b</sup>	3.5(22.6) $\pm$ 0.2 <sup>a</sup>	n.d.
May	84.3 $\pm$ 0.5 <sup>a</sup>	3.0(19.1) $\pm$ 0.1 <sup>b</sup>	0.2(1.3) $\pm$ 0.0 <sup>b</sup>	3.9(24.8) $\pm$ 0.2 <sup>a</sup>	n.d.
Tongyeong					
January	86.1 $\pm$ 0.5 <sup>a</sup>	1.3 (9.4) $\pm$ 0.2 <sup>b</sup>	0.2(1.4) $\pm$ 0.0 <sup>a</sup>	3.8(27.3) $\pm$ 0.2 <sup>a</sup>	n.d.
March	86.1 $\pm$ 0.4 <sup>a</sup>	1.8(12.9) $\pm$ 0.2 <sup>a</sup>	0.1(0.7) $\pm$ 0.0 <sup>a</sup>	3.6(25.9) $\pm$ 0.1 <sup>a</sup>	n.d.
May	86.7 $\pm$ 0.8 <sup>a</sup>	1.5(11.3) $\pm$ 0.1 <sup>ab</sup>	0.1(0.8) $\pm$ 0.0 <sup>a</sup>	3.3(24.8) $\pm$ 0.1 <sup>b</sup>	n.d.

<sup>1</sup>Mean  $\pm$  SD of three replicates; data in parentheses are expressed on a dry weight basis.

<sup>2</sup>Different letters within a row denote significant differences ( $P < 0.05$ ).

n.d.. not detected.

May than in January. The protein content ranged from 33.1 to 37.7% (dry weight basis); this was lower than the 50.2-55.6% levels reported in other ascidians (Oh et al., 1997), but was similar to levels found in high-protein vegetables such as soybean (Kumar et al., 2006). The protein content of mideodeok tunic from Geoje (GT) and Tongyeong (TT) ranged from 17.9 to 23.2% and 9.4 to 12.9%, respectively, with the highest levels in March.

The crude lipid content in muscle was high, ranging from 6.3 to 9.8% (dry weight basis). All samples had lower crude lipid concentrations in May, except for GM, which increased relative to that in January. The ash content remained fairly constant, except for that of GM in March and May, and that of TT in May, which decreased significantly. The ash content of GM and TM ranged from 19.6 to 27.3% and 22.2 to 23.8%, respectively. There was very little difference in the ash content between muscle and tunic tissues. In most cases, the skin or body wall of an organism contains a higher mineral residue than the muscle. Ascidian tunic contains high concentrations of fibrous carbohydrate, which may explain its low ash content relative to that of muscle. Also, because Mideodeok is a filter feeder, it may have accumulated minerals in its digestive tract, resulting in a high ash content.

The glycogen content ranged from 21.6 to 25.9% in GM and 20.8 to 27.7% in TM samples. The highest glycogen content was found in March, for both sampling grounds. No glycogen was detected in the tunic of Mideodeok.

### Total amino acid composition

There was seasonal variation in the amino acid profiles of GM (Table 2) and TM (Table 3) samples. Generally, the highest amino acid contents occurred in January and March. The most abundant amino acids were glutamic acid, aspartic acid, arginine, leucine, and lysine. In GM, these amino acids varied from 50 to 59, 42 to 44, 27 to 35, 24 to 25, and 29 to 35%, respectively; in TM, they ranged from 52 to 54, 40 to 41, 27 to 31, 26 to 29, and 31 to 34%, respectively. Among the essential amino acids (EAAs), leucine and lysine were found at the highest levels.

The ratios of essential amino acids to nonessential amino acids (NEAA) ranged from 0.55 to 0.61 and 0.66 to 0.67 in GM and TM, respectively. These values are similar to the average ratio of 0.70 found in many fish species, and higher than the 0.59 reported for crabs and squids (Constantinides et al., 1995). Thus, Mideodeok has a well-balanced amino acid composition-because it contains all the essential

Table 2. Amino acid composition (g/100 g) of Mideodeok muscle from Geoje, Korea

Amino acid	January	March	May
Aspartic acid	0.42 ± 0.03 <sup>ab2</sup>	0.44 ± 0.03 <sup>a</sup>	0.44 ± 0.02 <sup>a</sup>
Threonine <sup>3</sup>	0.24 ± 0.01 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	0.20 ± 0.00 <sup>b</sup>
Serine	0.22 ± 0.02 <sup>a</sup>	0.23 ± 0.00 <sup>a</sup>	0.19 ± 0.00 <sup>b</sup>
Glutamic acid	0.59 ± 0.01 <sup>a</sup>	0.59 ± 0.02 <sup>a</sup>	0.50 ± 0.03 <sup>b</sup>
Proline	0.24 ± 0.01 <sup>b</sup>	0.28 ± 0.00 <sup>a</sup>	0.21 ± 0.00 <sup>c</sup>
Glycine	0.26 ± 0.02 <sup>a</sup>	0.26 ± 0.00 <sup>a</sup>	0.21 ± 0.00 <sup>b</sup>
Alanine	0.26 ± 0.01 <sup>a</sup>	0.24 ± 0.00 <sup>b</sup>	0.19 ± 0.00 <sup>c</sup>
Cystine	0.07 ± 0.00 <sup>b</sup>	0.08 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>c</sup>
Valine <sup>3</sup>	0.24 ± 0.00 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>	0.18 ± 0.01 <sup>c</sup>
Methionine <sup>3</sup>	0.18 ± 0.00 <sup>a</sup>	0.15 ± 0.00 <sup>a</sup>	0.12 ± 0.00 <sup>a</sup>
Isoleucine <sup>3</sup>	0.27 ± 0.01 <sup>a</sup>	0.24 ± 0.02 <sup>a</sup>	0.18 ± 0.00 <sup>b</sup>
Leucine <sup>3</sup>	0.35 ± 0.02 <sup>a</sup>	0.32 ± 0.01 <sup>a</sup>	0.24 ± 0.01 <sup>b</sup>
Tyrosine	0.27 ± 0.01 <sup>a</sup>	0.25 ± 0.00 <sup>b</sup>	0.20 ± 0.00 <sup>c</sup>
Phenylalanine <sup>3</sup>	0.26 ± 0.01 <sup>b</sup>	0.28 ± 0.00 <sup>a</sup>	0.21 ± 0.01 <sup>c</sup>
Histidine	0.14 ± 0.00 <sup>b</sup>	0.16 ± 0.00 <sup>a</sup>	0.11 ± 0.00 <sup>c</sup>
Lysine <sup>3</sup>	0.34 ± 0.01 <sup>a</sup>	0.35 ± 0.00 <sup>a</sup>	0.29 ± 0.02 <sup>b</sup>
Ammonia	0.26 ± 0.00 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>b</sup>
Arginine	0.35 ± 0.01 <sup>a</sup>	0.34 ± 0.02 <sup>a</sup>	0.27 ± 0.01 <sup>b</sup>
Total	4.96	4.94	4.03
EAA <sup>3</sup> /NEAA <sup>4</sup>	0.61	0.58	0.55

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences (P < 0.05).

<sup>3</sup>Essential amino acids.

<sup>4</sup>Nonessential amino acids.

Table 3. Amino acid composition (g/100 g) of Mideodeok muscle from Tongyeong, Korea

Amino acid	January	March	May
Aspartic acid	0.40 ± 0.03 <sup>ab2</sup>	0.40 ± 0.02 <sup>a</sup>	0.41 ± 0.05 <sup>a</sup>
Threonine <sup>3</sup>	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.00 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>
Serine	0.21 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.19 ± 0.00 <sup>a</sup>
Glutamic acid	0.54 ± 0.04 <sup>a</sup>	0.54 ± 0.04 <sup>a</sup>	0.52 ± 0.03 <sup>a</sup>
Proline	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>
Glycine	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>
Alanine	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>	0.21 ± 0.00 <sup>a</sup>
Cystine	0.07 ± 0.00 <sup>a</sup>	0.06 ± 0.00 <sup>b</sup>	0.05 ± 0.00 <sup>c</sup>
Valine <sup>3</sup>	0.20 ± 0.01 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>
Methionine <sup>3</sup>	0.13 ± 0.00 <sup>a</sup>	0.13 ± 0.00 <sup>a</sup>	0.12 ± 0.00 <sup>a</sup>
Isoleucine <sup>3</sup>	0.22 ± 0.02 <sup>a</sup>	0.22 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>
Leucine <sup>3</sup>	0.29 ± 0.02 <sup>a</sup>	0.29 ± 0.02 <sup>a</sup>	0.26 ± 0.01 <sup>a</sup>
Tyrosine	0.24 ± 0.01 <sup>a</sup>	0.24 ± 0.01 <sup>a</sup>	0.21 ± 0.02 <sup>b</sup>
Phenylalanine <sup>3</sup>	0.25 ± 0.01 <sup>b</sup>	0.25 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>b</sup>
Histidine	0.15 ± 0.00 <sup>a</sup>	0.15 ± 0.00 <sup>a</sup>	0.16 ± 0.00 <sup>a</sup>
Lysine <sup>3</sup>	0.34 ± 0.01 <sup>a</sup>	0.33 ± 0.03 <sup>a</sup>	0.31 ± 0.02 <sup>a</sup>
Ammonia	0.24 ± 0.02 <sup>a</sup>	0.24 ± 0.00 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>
Arginine	0.31 ± 0.03 <sup>a</sup>	0.30 ± 0.02 <sup>a</sup>	0.27 ± 0.03 <sup>a</sup>
Total	4.54	4.50	4.27
EAA <sup>3</sup> /NEAA <sup>4</sup>	0.67	0.67	0.66

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences (P < 0.05).

<sup>3</sup>Essential amino acids.

<sup>4</sup>Nonessential amino acids.

amino acids, except tryptophan. However, the amino acid contents are low compared to FAO guidelines for amino acid requirements (FAO/WHO/UNU, 1985).

### Fatty acid composition

The major fatty acid compositions of GM and TM (Table 4), and GT and TT (Table 5), are shown. Both muscle and tunic contained very high amounts of EPA, similar to earlier findings (Kim et al., 1985; Popov et al., 2002), with values ranging from 20.0 to 22.3% and 20.3 to 23.2%, respectively. Saturated and monounsaturated fatty acids, including tetradecanoic acid (14:0), hexadecanoic acid (16:0), 9-hexadecenoic acid (16:1n-7), octadecanoic acid (18:0), 9-octadecenoic acid (18:1n-9), and 11-octadecenoic acid (18:1n-7), were also present, with respective overall ranges as follows: 2.3-6.6%, 10.3-13.6%, 6.0-13.9%, 3.6-5.3%, 2.2-9.5%, and 2.2-9.1%. Most of the fatty acids showed statistically significant seasonal changes.

High levels of polyenes 47.8-54.9% and low levels

of saturates 22.8-26.6% and monoenes 19.7-27.4% characterized both muscle and tunic from both sampling sites. The seasonal fluctuations in EPA ( $P \leq 0.05$ ) and DHA ( $P \leq 0.05$ ) may be attributed to the type and abundance of food (i.e., phytoplankton and zooplankton) consumed by Mideodeok during the year. These important fatty acids, which are generally widespread in the marine environment, are synthesized by bacteria, zooplankton, and phytoplankton (Saito et al., 2002; Valentine and Valentine, 2004). The high levels of EPA and DHA in Mideodeok make it a very suitable dietary source of these important fatty acids.

### Mineral contents

Because Mideodeok is a filter feeder, it can draw an incomparable wealth of mineral elements, macroelements, and trace elements from the sea. The total mineral fraction of Mideodeok ranged from 22.2 to 27.3% of dry matter. The mineral content of muscle (Table 6) and tunic (Table 7) are shown. Four macro-

Table 4. Major fatty acid composition of Mideodeok muscle and tunic from Geoje, Korea

Fatty acid	Muscle (%) <sup>1</sup>			Tunic (%)		
	January	March	May	January	March	May
14:0	4.7 ± 0.1 <sup>az</sup>	4.5 ± 0.5 <sup>ab</sup>	3.7 ± 0.4 <sup>b</sup>	3.6 ± 0.3 <sup>b</sup>	4.9 ± 0.1 <sup>a</sup>	3.0 ± 0.2 <sup>c</sup>
16:0	10.3 ± 0.1 <sup>a</sup>	11.6 ± 0.9 <sup>a</sup>	11.0 ± 0.3 <sup>a</sup>	12.9 ± 0.3 <sup>a</sup>	12.1 ± 0.1 <sup>b</sup>	11.9 ± 0.4 <sup>b</sup>
16:1n-7	11.3 ± 0.0 <sup>c</sup>	12.7 ± 0.6 <sup>b</sup>	13.9 ± 0.4 <sup>a</sup>	10.1 ± 0.2 <sup>b</sup>	13.6 ± 0.6 <sup>a</sup>	13.2 ± 0.6 <sup>a</sup>
18:0	4.0 ± 0.1 <sup>ab</sup>	3.8 ± 0.1 <sup>b</sup>	4.1 ± 0.1 <sup>a</sup>	3.9 ± 0.1 <sup>b</sup>	3.9 ± 0.1 <sup>b</sup>	5.2 ± 0.1 <sup>a</sup>
18:1n-9	7.6 ± 0.1 <sup>a</sup>	5.4 ± 0.2 <sup>b</sup>	5.2 ± 0.3 <sup>b</sup>	3.0 ± 0.2 <sup>a</sup>	2.5 ± 0.0 <sup>b</sup>	2.2 ± 0.0 <sup>c</sup>
18:1n-7	6.0 ± 0.1 <sup>a</sup>	6.2 ± 0.2 <sup>a</sup>	6.0 ± 0.2 <sup>b</sup>	9.1 ± 0.2 <sup>a</sup>	7.1 ± 0.2 <sup>b</sup>	7.2 ± 0.3 <sup>b</sup>
20:5n-3	20.0 ± 0.0 <sup>c</sup>	21.3 ± 0.5 <sup>b</sup>	21.6 ± 0.4 <sup>a</sup>	22.2 ± 0.1 <sup>b</sup>	22.6 ± 0.6 <sup>ab</sup>	23.2 ± 0.2 <sup>a</sup>
22:6n-3	17.9 ± 0.8 <sup>a</sup>	16.8 ± 0.2 <sup>ab</sup>	16.5 ± 0.5 <sup>b</sup>	15.2 ± 0.8 <sup>b</sup>	17.5 ± 0.5 <sup>a</sup>	15.2 ± 0.7 <sup>b</sup>
Saturates	22.8	24.8	23.4	24.2	24.9	24.2
Monoenes	27.4	26.9	27.1	24.8	24.4	24.8
Polyenes	49.0	47.8	49.3	50.7	50.3	51.0

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences ( $P < 0.05$ ).

Table 5. Major fatty acid composition of Mideodeok muscle and tunic from Tongyeong, Korea.

Fatty acid	Muscle (%) <sup>1</sup>			Tunic (%)		
	January	March	May	January	March	May
14:0	6.6 ± 0.7 <sup>az</sup>	5.3 ± 0.4 <sup>b</sup>	4.3 ± 0.2 <sup>b</sup>	4.0 ± 0.2 <sup>a</sup>	4.5 ± 0.3 <sup>a</sup>	2.3 ± 0.0 <sup>b</sup>
16:0	12.2 ± 0.3 <sup>b</sup>	13.2 ± 0.2 <sup>a</sup>	11.4 ± 0.4 <sup>c</sup>	13.4 ± 0.3 <sup>a</sup>	11.6 ± 0.3 <sup>b</sup>	13.6 ± 0.3 <sup>a</sup>
16:1n-7	7.4 ± 0.1 <sup>c</sup>	10.2 ± 0.0 <sup>b</sup>	10.6 ± 0.2 <sup>a</sup>	6.0 ± 0.2 <sup>b</sup>	10.7 ± 0.5 <sup>a</sup>	8.2 ± 0.3 <sup>c</sup>
18:0	3.6 ± 0.1 <sup>b</sup>	3.7 ± 0.1 <sup>b</sup>	5.3 ± 0.1 <sup>a</sup>	4.0 ± 0.2 <sup>ab</sup>	3.8 ± 0.2 <sup>b</sup>	4.7 ± 0.4 <sup>a</sup>
18:1n-9	5.9 ± 0.3 <sup>a</sup>	5.7 ± 0.2 <sup>a</sup>	4.2 ± 0.1 <sup>b</sup>	9.5 ± 1.0 <sup>a</sup>	5.4 ± 0.2 <sup>b</sup>	5.3 ± 0.4 <sup>b</sup>
18:1n-7	7.9 ± 0.2 <sup>a</sup>	6.7 ± 0.1 <sup>b</sup>	6.9 ± 0.4 <sup>b</sup>	2.2 ± 0.2 <sup>c</sup>	6.2 ± 0.2 <sup>a</sup>	5.4 ± 0.1 <sup>b</sup>
20:5n-3	22.3 ± 0.2 <sup>a</sup>	22.3 ± 0.7 <sup>a</sup>	20.3 ± 0.3 <sup>b</sup>	20.3 ± 0.4 <sup>b</sup>	21.3 ± 0.6 <sup>ab</sup>	21.6 ± 0.3 <sup>a</sup>
22:6n-3	16.5 ± 0.2 <sup>a</sup>	17.2 ± 0.4 <sup>a</sup>	16.9 ± 0.4 <sup>a</sup>	18.0 ± 0.3 <sup>b</sup>	18.8 ± 0.2 <sup>a</sup>	17.1 ± 0.2 <sup>c</sup>
Saturates	25.8	26.0	26.6	25.2	24.5	24.6
Monoenes	23.3	24.8	23.9	19.7	25.6	23.5
Polyenes	51.6	49.2	49.6	54.9	49.4	52.4

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences ( $P < 0.05$ ).

Table 6. Mineral content (mg/100 g) of Mideodeok muscle and tunic from Geoje, Korea

Mineral	Muscle <sup>1</sup>			Tunic		
	January	March	May	January	March	May
Potassium	56.3 ± 5.0 <sup>az</sup>	43.0 ± 6.7 <sup>b</sup>	39.0 ± 4.5 <sup>b</sup>	98.3 ± 5.3 <sup>az</sup>	84.5 ± 5.4 <sup>b</sup>	57.3 ± 4.5 <sup>c</sup>
Calcium	129.1 ± 7.2 <sup>b</sup>	141.6 ± 2.3 <sup>b</sup>	192.3 ± 11.9 <sup>a</sup>	132.5 ± 6.0 <sup>c</sup>	168.6 ± 12.1 <sup>b</sup>	273.3 ± 8.3 <sup>a</sup>
Magnesium	68.3 ± 7.0 <sup>a</sup>	44.6 ± 4.2 <sup>b</sup>	42.8 ± 5.6 <sup>b</sup>	78.0 ± 6.1 <sup>a</sup>	67.1 ± 4.2 <sup>a</sup>	42.8 ± 2.7 <sup>b</sup>
Sodium	1,471.1 ± 21.8 <sup>a</sup>	1,257.9 ± 12.4 <sup>b</sup>	1,286.0 ± 34.3 <sup>b</sup>	1,335.1 ± 20.4 <sup>b</sup>	1,391.0 ± 12.4 <sup>a</sup>	1,286.0 ± 9.7 <sup>c</sup>
Manganese	5.2 ± 0.5 <sup>a</sup>	0.9 ± 0.0 <sup>b</sup>	1.0 ± 0.2 <sup>b</sup>	0.3 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>b</sup>	1.0 ± 0.2 <sup>a</sup>
Iron	2.5 ± 0.3 <sup>a</sup>	2.5 ± 0.3 <sup>a</sup>	2.3 ± 0.5 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	0.7 ± 0.2 <sup>b</sup>	2.3 ± 0.4 <sup>a</sup>
Copper	0.2 ± 0.0 <sup>a</sup>	trace	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	trace	0.1 ± 0.0 <sup>a</sup>
Zinc	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.1 <sup>a</sup>	0.4 ± 0.0 <sup>a</sup>	0.2 ± 0.0 <sup>b</sup>	0.3 ± 0.1 <sup>a</sup>
Chromium	trace	trace	trace	trace	trace	trace
Lead	trace	trace	trace	trace	trace	trace
Silver	trace	trace	trace	trace	trace	trace
Arsenic	trace	trace	trace	trace	trace	trace

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences (P < 0.05).

Table 7. Mineral content (mg/100 g) of Mideodeok muscle and tunic from Tongyeong, Korea

Mineral	Muscle <sup>1</sup>			Tunic		
	January	March	May	January	March	May
Potassium	77.9 ± 3.2 <sup>2</sup>	65.9 ± 3.5 <sup>b</sup>	47.0 ± 3.9 <sup>c</sup>	110.6 ± 2.1 <sup>az</sup>	81.5 ± 3.9 <sup>b</sup>	49.5 ± 4.5 <sup>c</sup>
Calcium	152.8 ± 7.3 <sup>b</sup>	162.4 ± 4.4 <sup>b</sup>	193.4 ± 8.1 <sup>a</sup>	112.5 ± 8.4 <sup>c</sup>	192.2 ± 2.3 <sup>b</sup>	211.3 ± 1.6 <sup>a</sup>
Magnesium	53.4 ± 3.3 <sup>a</sup>	46.1 ± 2.2 <sup>ab</sup>	42.0 ± 4.6 <sup>b</sup>	83.3 ± 5.2 <sup>a</sup>	64.5 ± 4.2 <sup>b</sup>	55.2 ± 5.6 <sup>b</sup>
Sodium	1,326.9 ± 16.0 <sup>a</sup>	1,286.2 ± 6.3 <sup>b</sup>	1,244.0 ± 19.0 <sup>c</sup>	1,128.7 ± 15.5 <sup>b</sup>	1,161.0 ± 10.4 <sup>b</sup>	1,368.5 ± 22.2 <sup>a</sup>
Manganese	1.9 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>c</sup>	0.5 ± 0.1 <sup>b</sup>	0.3 ± 0.0 <sup>b</sup>	trace	1.3 ± 0.2 <sup>a</sup>
Iron	1.7 ± 0.2 <sup>a</sup>	0.8 ± 0.0 <sup>b</sup>	0.5 ± 0.0 <sup>c</sup>	1.3 ± 0.3 <sup>b</sup>	1.5 ± 0.2 <sup>b</sup>	2.2 ± 0.0 <sup>a</sup>
Copper	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>
Zinc	3.1 ± 0.1 <sup>a</sup>	0.4 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>c</sup>	0.5 ± 0.1 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>
Chromium	trace	trace	trace	trace	trace	trace
Lead	trace	trace	trace	trace	trace	trace
Silver	trace	trace	trace	trace	trace	trace
Arsenic	trace	trace	trace	trace	trace	trace

<sup>1</sup>Mean ± SD of three replicates.

<sup>2</sup>Different letters within a row denote significant differences (P < 0.05).

elements (K, Ca, Mg, and Na) and eight microelements (Cr, Mn, Fe, Cu, Zn, Pb, Ag, and As) were detected using inductively coupled plasma emission spectrophotometry. Seasonal changes were observed in most of the minerals detected. Sodium levels, which ranged from 1,128.7 to 1471.1 mg/100 g, and comprised the largest fraction of the total mineral content, were significantly higher in January than they were in other months in both GM and TM, whereas they fluctuated in both GT and TT. Potassium and magnesium levels were highest in January in all samples. The average potassium content of GM, TM, GT, and TT was 46.1, 63.6, 80.0, and 80.5 mg/100 g, respectively. The corresponding average values for magnesium were 51.9, 42.7, 62.6, and 67.7 mg/100 g. The calcium contents of GM, TM, GT, and TT were higher in May than in January by 32.9, 26.6, 106.3, and 87.8%, respectively. The average calcium

content was 154.3, 169.6, 191.5, and 172.0 mg/100 g, respectively. Manganese, iron, and zinc, all of which are essential minerals for humans, were present in amounts ranging from 0.2 to 5.2, 0.5 to 2.5, and 0.2 to 3.1 mg/100 g, respectively. Copper was present in the muscle and tunic at an average of 0.1 mg/100 g. Heavy metals, such as chromium, lead, silver, and arsenic, were present at negligible levels. Thus, Mideodeok is a rich source of minerals and can provide a significant portion of the recommended dietary allowance (RDA) if consumed regularly (FNB, 2004).

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## References

- Ackman, R.G. 1995. Composition and Nutritive Value of Fish and Shellfish Lipids. In: Fish and Fishery Products. Ruither, A. ed. CAB International, UK, 117-156.
- AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, D.C., USA.
- Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37, 911-917.
- Constantinides, S.M., P.A. Karakoltsidis and A. Zotos. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and molluscs. *J. Food Comp. Anal.* 8, 258-273.
- Ermak, T.H. 1975. Cell proliferation in the digestive tract of *Styela clava* Urochordata: Ascidiacea as revealed by autoradiography with tritiated thymidine. *J. Exp. Zool.*, 194, 449-465.
- FAO/WHO/UNU (Food and Agriculture Organization/World Health Organization/United Nations Union). 1985. Energy and protein requirements. Technical Report Series 724. -World Health Organization, Geneva, Switzerland.
- FNB Food and Nutrition Board. 2004. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. The National Academies Press, Washington, D.C., USA. (Online) URL: <http://www.nap.edu>.
- Jiang, A.L., X.M. Liu and C.H. Wang. 2005. Growth status and nutrition analysis of *Styela clava* in Yantai maritime space. *Mar. Sci. Bull.*, 24, 13-16.
- Kelly, K.L., E.L. Cooper and D.A. Raftos. 1992. Purification and characterization of a humoral opsonin from the solitary urochordate *Styela clava*. *Comp. Biochem. Physiol.*, Part B, 103, 749-753.
- Kim, K.S., E.H. Lee, K.S. Oh, T.H. Lee, C.H. Ahn and Y.H. Chung. 1985. Lipid components of sea squirt, *Halocynthia roretzi*, and Mideuduck, *Styela clava*. *Korean J. Food Sci. Technol.*, 17, 289-295.
- Kumar, V., A. Rani, S. Solanki and S.M. Hussain. 2006. Influence of growing environment on the biochemical composition and physical characteristics of soybean seed. *J. Food Comp. Anal.*, 19, 188-195.
- Kusakabe, T. 1995. Expression of larval-type muscle actin encoding genes in the ascidian *Halocynthia roretzi*. *Gene*, 153, 215-218.
- Lee, E.H., S.Y. Chung, J.H. Ha, N.J. Sung and K.O. Cho. 1975. Free amino acid content in the extract of mideudiuck, *Styela clava*. *J. Kor. Fish. Soc.*, 8, 177-180.
- Lee, K.H., M.G. Kim, B.I. Hong, B.C. Jung, D.H. Lee and C.H. Park. 1995. Seasonal variations in the taste components of warty sea squirt *Styela clava*. *J. Kor. Soc. Food Nutr.*, 24, 274-279.
- Lehrer, R.I., P.M. Lorenzo, I.H. Lee and B. Sjostrand. 2002. Immunolocalization of clavanins in *Styela clava* hemocytes. *Develop. Comp. Immunol.*, 26, 505-515.
- Leu, S.S., S.N. Jhaver, P.A. Karakoltsidis and S.M. Constantinides. 1981. Atlantic mackerel *Scomber scombrus*, L.: seasonal variation in proximate composition and distribution of chemical nutrients. *J. Food Sci.*, 46, 1635-1638.
- Lo, S., J.C. Russell and A.W. Taylor. 1970. Determination of glycogen in small tissue samples. *J. Appl. Physiol.* 28, 234-236.
- Menzel, L.P., I.H. Lee, B. Sjostrand and R.I. Lehrer. 2002. Immunolocalization of clavanins in *Styela clava* hemocytes. *Develop. Comp. Immunol.*, 26, 505-515.
- MOMAF (Ministry of Maritime Affairs and Fisheries). 2005. Fishery Production Survey. Ministry of Maritime Affairs and Fisheries, Korea. (Online) URL: <http://fs.fips.go.kr>.
- NFRDI (National Fisheries Research Development Institute). 2005. Korea Marine Environment Data. National Fisheries Research Development Institute, Korea. (Online) URL: <http://www.nfrda.re.kr>.
- Oh, K.S., J.S. Kim and M.S. Heu. 1997. Food constituents of edible ascidians *Halocynthia roretzi* and *Pyura michaelsoni*. *Kor. J. Food Sci. Technol.*, 29, 955-962.
- Orban, E., G.D. Lena, T. Nevigato, I. Casini, A. Marzetti and R. Caproni. 2002. Seasonal changes in meat content, condition index and chemical composition of mussels *Mytilus galloprovincialis* cultured in two different Italian sites. *Food Chem.*, 77, 57-65.
- Pedersen, S.A., M.H. Ribergaard and C.S. Simonsen. 2005. Micro and mesozooplankton in southwest Greenland waters in relation to environmental factors. *J. Mar. Syst.*, 56, 85-112.
- Popov, S., K. Slantchev, F. Yalcin, T. Ersoz, J. Nechev, I. Calis and K. Stefanov. 2002. Composition of lipophilic extracts from two tunicates, *Styela* sp. and *Phallusia* sp., from the eastern Mediterranean. *Z. Naturforsch.*, 57, 534-540.
- Saito, H., Y. Kotani, J.M. Keriko, C. Xue, K. Taki, K. Ishihara, T. Ueda and S. Miyata. 2002. High levels of n-3 polyunsaturated fatty acids in *Euphausia pacifica* and its role as a source of docosahexaenoic and icosapentaenoic acids for higher trophic levels. *Marine Chem.*, 78, 9-28.
- Sanina, N.M. and E.Y. Kostetsky. 2002. Thermotropic behavior of major phospholipids from marine invertebrates: changes with warm acclimation and seasonal acclimatization. *Comp. Biochem. Physiol.*, Part B, 133, 143-153.
- Smith, M.J. and P.A. Dehnel. 1971. The composition of tunic from four species of ascidians. *Comp. Biochem.*

- Physiol., Part B, 40, 615-622.
- Socal, G., F.B. Aubry, A. Berton, M. Bastianini and F. Acri. 2004. Phytoplankton succession in a coastal area of the NW Adriatic, over a 10-year sampling period 1990-1999. *Continental Shelf Res.*, 24, 97-115.
- Qian, P.Y. and V. Thiyagarajan. 2003. Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *Styela plicata* Lesueur. *J. Exp. Mar. Biol. Ecol.*, 290, 133-146.
- Valentine, R.C. and D.L. Valentine. 2004. Omega -3 fatty acids in cellular membranes: a unified concept. *Prog. Lipid Res.*, 43, 383-402.
- Wright, R.K. and E.L. Cooper. 1984. Protochordate immunity II. Diverse hemolymph lectins in the solitary tunicate *Styela clava*. *Comp. Biochem. Physiol., Part B*, 79, 269-277.

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