

Monthly Variations in the Nutritional Composition of Antarctic Krill *Euphausia superba*

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Abstract

The proximate composition and various specific components of Antarctic krill *Euphausia superba*, in the catch season between March and August were investigated. Frozen krill were freeze-dried and milled. The proximate composition comprised water, proteins, fats, ash, fatty acids, and amino acids, while the specific components were vitamins, minerals, nucleotides, betaine, and astaxanthin. The moisture content of the krill ranged from 77 to 80%, with the highest value in June, and the ash content was between 12 and 13%. The protein content was lowest in May, and the fat content was 18–19%, with the highest value in March. The amino acid content varied according to the season: taurine and glycine were highest in August; β -alanine was higher in April and May; and arginine, ornithine, and lysine were highest in March. The unsaturated fat content was ~50% and omega-3 fatty acids were highest in June. Oil-soluble vitamins A and E were highest in March, and the water-soluble vitamin content was less than that of oil-soluble vitamins. The mineral content was highest in June, and the most abundant mineral was sodium at 235.60 mg/100 g krill. The content of other minerals was lowest (2.94 mg/100 g) in April, except for lead. The nucleotide content was highest in July, while the betaine content was highest in April and lowest in June. The astaxanthin content was highest in May and ranged from 6 to 10 ppm in other months.

Key words: *Euphausia superba*, Antarctic Krill, Krill, Food resource, Nutritional Composition

Introduction

Antarctic krill *Euphausia superba*, a shrimplike crustacean, is the most abundant oceanic food resource (Tou et al., 2007; Chen et al., 2009) and is usually caught in the months between March and August. The total amount of krill is estimated to be 379 million tonnes (Atkinson et al., 2009), and it is a key marine organism for sustaining the food chain in the ecosystem of the Antarctic ocean as a food for whales, seals, squids, and fish (Suh et al., 1991). On an annual basis, Antarctic organisms consume 152-313 million tonnes of krill: 63-130, 34-43, 15-20, 30-100, and 10-20 million tonnes are consumed by seals, whales, birds, squid, and fish, respectively (Miller and Hampton, 1989; Bonner, 1995). Since krill live in large masses, reaching densities of 10,000-30,000 individu-

als/m³ (Hamner et al., 1983), catching them is feasible using large-scale commercial fishing, and they offer much potential as a human food resource. Previous studies have shown that krill had a moisture content of 77.9-83.1%, lipid content of 0.4-3.6%, protein content of 11.9-15.4%, and chitin content of <2% (Grantham, 1977), although one study recorded a protein content of 72.9-75.8% (dry matter) and lipid content of 12-50% (dry matter; Saether et al., 1986; Jaczynski et al., 2011). Krill oil contains a high volume of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; Kolakowska et al., 1994; Jaczynski et al., 2011). Krill have a higher proportion of cholesterol than fish but less than shrimp (Tou et al., 2007), containing 123 μ g/g zinc (Zn; dry matter), 2.5 μ g/g cadmium



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(Cd; dry matter), and 3.4 µg/g lead (Pb; dry matter; Soszka et al., 1981; Kim et al., 1999), and the contents of Pb (2.76 ppm), chromium (Cr; 0.14 ppm), and arsenic (As; 1.29 ppm) were within safe levels for human consumption (Kim et al., 2004). The mineral contents of krill are known to be similar to those of shrimp (Tovar-Sanchez et al., 2007; Chen et al., 2009), and the crustacean has been shown to be rich in vitamins A and B (Kim et al., 1999), as well as astaxanthin (Suzuki and Shibata, 1990; Kim et al., 2004).

The potential of krill as a nutrient-rich human food source has been researched since it was first caught as a protein source in the 1960s (Kim et al., 2004). Krill protein is known to have sufficiently high nutritional value to compete with that of meat (Suzuki and Shibata, 1990; Tou et al., 2007), and its essential amino acid content meets the requirements of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Chen et al., 2007).

However, after harvest, krill rapidly degrades due to high enzyme activity, which produces unpleasant flavors and color changes (Anheller et al., 1989). Moreover, krill contains high levels of fluoride in its shell (Soevik and Breakkan, 1979; Christians and Leinemann, 1983; Nicol and Stolp, 1991; Virtue et al., 1995). Thus, human consumption of whole-body krill has been argued to present safety issues.

Captured krill is processed as fresh-frozen (34% of the catch), boiled-frozen (11%), peeled krill meat (23%), and meal (32% of the catch; Nicol and Endo, 1997). As such, krill is mostly used for feed and bait, and only a small proportion of the total catch is peeled and the meat used for human consumption (Budzinski et al., 1985; Kim et al., 2000). Therefore, despite being an abundant resource, the use of krill as a food for people has so far been limited, and to date, no systematic analysis has been performed of its food components according to the month of harvest.

This study investigated the monthly variation in the food components of Antarctic krill, including the proximate components, and the fatty acid and amino acid, cholesterol, astaxanthin, nucleotide, betaine, vitamin, mineral, heavy metal, and fluoride contents, with the objective of characterizing krill as a future human food resource.

Materials and Methods

Materials

Frozen Antarctic krill *Euphausia superba* was supplied by Dongwon Industry Co. (Busan, Korea) each month, and stored at 4°C after lyophilization until use. All reagents used in the analyses were analytical grade.

Determination of proximate components

Moisture, crude lipids, crude proteins, and crude ash were

analyzed according to the method of Horwitz (2005). Salinity was measured by the Mohr method (Kraemer and Stamm, 1924).

Analysis of amino acids

The total amino acids were analyzed using the method of Chen et al. (2009) with some modifications. The sample (0.5 g) was dissolved in 15 mL 6 N hydrogen chloride (HCl), sealed under reduced pressure, and hydrolyzed in a dry bath at 110°C for 24 h. The hydrolysate was filtered and concentrated to remove hydrochloric acid. The residue was dissolved with 50 mL sodium citrate buffer (pH 2.2) and examined with an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan).

Free amino acids were measured using the method of Horwitz (2005) with some modifications. The sample (1 g) was dissolved in 10 mL distilled water and stirred at 100°C for 24 h to precipitate the proteins. After filtration, the filtrate was concentrated and then dissolved with 50 mL 0.2 N lithium citrate buffer (pH 2.2). The solution was filtered through a 0.45-µm membrane and studied using an amino acid analyzer (Biochrom-30, Amersham, Little Chalfont, Buckinghamshire, UK).

Analysis of fatty acids

The fatty acids were analyzed using the methods of Folch et al. (1957) and Horwitz (2005) with some modifications. Chloroform/methanol (200 mL, 2:1, v/v) was added to 5 g of sample and stirred at 60°C for 12 h. The extracted lipids (100 mg) were dissolved with 5 mL toluene and 3 mL BF₃-methanol, and then methylated at 75°C for 30 min. The fatty acid methyl esters were extracted with 3 mL hexane and 1 mL 10% NaCl. The solution was filtered through a 0.45-µm membrane filter and analyzed using gas chromatography [GC; GCMS QP-5050A, Shimadzu, Otsu, Japan; Hp-INNO wax capillary column, 30 m × 0.32 mm i.d., 0.5 µm, Hewlett-Packard, Palo Alto, CA, USA; carrier gas, helium (He); detector, flame ionization detector (FID); column temperature, 170-225°C at a rate of 1°C/min]. Each fatty acid was calculated by comparing it to a standard fatty acid methyl ester mixture (Sigma-Aldrich, St. Louis, MO, USA) with the retention time under identical conditions, and the fatty acid content was expressed as a percentage of each peak area.

Determination of astaxanthin

The astaxanthin content was measured according to the method of Pavasant et al. (2008). The sample (1 g) was transferred to a Soxhlet apparatus to extract the lipids with diethyl ether at 60°C for 24 h. The extracted fat (1 mg) was dissolved with 50 mL dichloromethane/ethanol (1:4) and filtered through a 0.45-µm membrane filter. The filtrate was transferred to high-performance liquid chromatography

(HPLC) equipment (model 2000, Hitachi, Tokyo, Japan). The analytical conditions were the following: C18 column (250 × 4.6 mm, 5 μm, Waters, Milford, MA, USA), acetonitrile/dichloromethane/ethanol (5:10:85) as the mobile phase; 1.0 mL/min flow rate; and a detection wavelength of 470 nm. The astaxanthin content was calculated from a calibration curve using standard astaxanthin (Dr Ehrenstorfer GmbH Co., Augsburg, Germany).

Determination of vitamins A and E

Vitamins A and E were analyzed using the method of Horwitz (2005) with some modifications. The sample (10 g) was added to a brown mass flask and mixed with 30 mL ethanol, 3 mL KOH, and 1 mL 10% pyrogallol. The mixture was saponified for 30 min in a reflux condenser and cooled, after which the cooled solution was mixed with 30 mL water, poured into a separation funnel, and extracted with 30 mL diethyl ether. The diethyl ether layer was dehydrated with sodium sulfate, transferred into a brown flask, and then concentrated. The aliquot was dissolved with 5 mL isopropanol and analyzed using HPLC with the following conditions for vitamin A: ODS 2 column (250 × 4.6 mm, 5 μm, Waters); mobile phase, 1 N H₂SO₄ liquid at pH 3.7; flow rate, 0.5 mL/min; detection wavelength, 325 nm. For vitamin E, the HPLC conditions were the following: mobile phase, methanol/water (7:3); flow rate, 1.0 mL/min; detection wavelength, 298 nm.

Determination of vitamin B groups

Vitamins B1, B, and B6 were analyzed using the method of Horwitz (2005) with some modifications. The sample (5 g) was homogenized with 50 mL 50% acetonitrile, and the final volume was made up to 50 mL. The solution was centrifuged at 3,000 rpm for 20 min and the supernatant was filtered through a 0.45-μm membrane filter, after which the filtrate was used for analysis. For vitamin B5, 50 mL phosphoric acid buffer solution was added to 5 g of sample and extracted in an ultrasonic device for 30 min. The solution was centrifuged at 3,000 rpm for 10 min, and the supernatant was filtered through a 0.45-μm membrane filter and used for the analysis assay. The HPLC conditions were the following: column, C18 (250 × 4.6 mm, 5 μm, Water); mobile phase, A solvent, 20 mM potassium phosphate (pH 2.1); B solvent, 20 mM potassium phosphate (pH 2.1)/acetonitrile (80/20); A/B ratio (96/4) until 37 min for the gradient; flow rate, 1.0 mL/min; wavelength, 254 nm.

Determination of minerals and heavy metals

The mineral and heavy metal contents were analyzed using the methods of Horwitz (2005) and Chen et al. (2009) with some modifications. The sample (10 g) was homogenized with 20 mL 70% nitric acid. It was heated for 4 h at 100 ± 5°C in a

heating mantle until a clear solution was obtained. After cooling, the solution was made up to 100 mL with 0.2 N nitric acid. The aliquot was assayed using an inductively coupled plasma mass spectrometer (ICP-MS; Elan 6100, PerkinElmer, Waltham, MA, USA). A flow injection mercury system (FIMS 400, Perkin Elmer) was used to analyze mercury (Hg).

Determination of betaines

The betaines were analyzed using the method of Lee et al. (2004) with some modifications. The sample (10 g) was added to 50% methanol with a density of 10% (w/v), homogenized, and extracted for 12 h in a vibrating water bath at 40°C. The extracts were centrifuged at 3,000 rpm for 15 min, and the supernatant was adjusted to 10 mL by evaporating the solution. The solution was loaded on an Amberlite IRA 400 and Amberlite IR 120 column (Sigma-Aldrich) and eluted with distilled water. The eluate was added to 300 μL methanol-KOH (15 mg KOH in 10 mL methanol), 200 μL acetonitrile-crown ether (15 mg 18-crown-6-ether in 10 mL acetonitrile), and 1.8 mL acetonitrile-bromophenacyl bromide (20 mg 4-bromophenacyl bromide in 10 mL acetonitrile), and then reacted for 30 min at 40°C. The solution was filtered through a 0.45-μm membrane filter and analyzed using HPLC (Hitachi 2000). The HPLC conditions were the following: ODS2 column (250 × 4.6 mm, 5 μm, Waters); mobile phase, mixture of 13 mM sodium heptane sulfonic acid and 5 mM Na₂SO₄ adjusted to pH 3.7 with 1 N H₂SO₄; flow rate, 0.5 mL/min; wavelength, 200 nm.

Determination of nucleotides

The nucleotides were analyzed according to the method of Ryu et al. (2009). The sample (0.5 g) was added to 10 mL 10% perchloric acid (PCA), homogenized, and centrifuged at 4,000 rpm for 10 min. The supernatant was filtered and adjusted to pH 6.5 with 5 N KOH; then 10% PCA was added to the final 100 mL. The solution was left for 30 min at 0°C and then filtered through a 0.45-μm membrane filter, after which the filtrate was added to the HPLC. The HPLC conditions were the following: C18 column (250 × 4.6 mm, 5 μm, Waters); mobile phase, 50 mM KH₂P₄ (pH 7.5); flow rate, 0.8 mL/min; wavelength, 254 nm (Hitachi 2000).

Determination of total cholesterol

The total cholesterol was analyzed using the method of Horwitz (2005) with modifications. The sample (5 g), 30 mL deionized water, and 1 mL 5α cholestane were mixed and homogenized. The solution was transferred to a separation funnel containing 200 mL chloroform, after which methanol (2:1) was added, and then extracted for 5 min. The chloroform layer was washed with 100 mL 0.5% NaOH, dehydrated with Na₂SO₄, and concentrated. The residue was added to 20 mL 2 N

NaOH–ethanol, fluxed for 1 h at 85°C, cooled, and transferred to a separation funnel. Then, 20 mL distilled water was added and extracted four times with 20 mL diethyl ether. The extract was washed with 20 mL distilled water, dehydrated with Na₂SO₄, and concentrated. It was dissolved in 2 mL hexane and analyzed using gas liquid chromatography (GLC). The GLC conditions were the following: column, 30 m × 0.32 mm ± 0.25 µm; column temperature, 290°C at 1°C/min; carrier gas, He; detector, FID (Clarus 500, PerkinElmer).

Determination of fluoride

The total fluoride was measured using the methods of the American Society for Testing and Materials (ASTM, 2002) and Horwitz (2005). The sample (0.5 g) and 10 mL 1 N NaOH were transferred into a combustion device (1108 oxygen combustion bomb, Parr Instrument Co., Moline, IL, USA) and the device was sealed. Oxygen was injected and the sample was burned completely for 5 min. After combustion, the vaporized fluoride was dissolved in 1 N NaOH by shaking. Subsequently, the combustion device was maintained under atmospheric pressure and left open. The NaOH solution containing the flu-

oride was washed with 100 mL deionized water and transferred to a 500-mL flask. A sulfuric acid:water solution (2:3, v/v, 150 mL) was added to the flask and the flask was connected to the distillation apparatus, then distilled for 3 h at 170°C. The distillate was made up to 500 mL with deionized water, and 5 mL 5% alfosone (Dojindo, Tokyo, Japan) and 10 mL acetone were added to 25 mL distillate to make up the solution to 50 mL. The solution was left for 1 h at ambient temperature and measured at 620 nm using an ultraviolet (UV) spectrophotometer (UV-1800, Shimadzu). Fluoride was measured using the calibration curve of fluoride standard solution (Kanto Chemical Co., Tokyo, Japan).

Statistical analysis

All results are indicated as the mean ± S.E.M (standard error of the mean; $n = 3$) and an analysis of variance (ANOVA) was used for multiple comparisons. SPSS v12.01 (SPSS Inc., Chicago, IL, USA) software was used to perform the ANOVA, and the difference in statistical significance was estimated using Duncan's multiple range tests ($P < 0.05$).

Table 1. Monthly changes in proximate compositions of Antarctic krill *Euphausia superba* (% dry weight)

Components	March	April	May	June	July	August
Moisture*	77.7 ± 0.0 ^c	78.4 ± 0.4 ^d	79.2 ± 0.2 ^a	80.7 ± 0.1 ^b	80.1 ± 0.0 ^b	79.31 ± 0.5 ^a
Crude protein	71.4 ± 1.8 ^a	65.1 ± 2.9 ^b	48.0 ± 1.0 ^e	74.1 ± 4.0 ^a	70.7 ± 4.1 ^a	73.8 ± 1.0 ^b
Crude lipid	22.1 ± 0.1 ^a	23.7 ± 0.1 ^b	20.2 ± 0.2 ^c	17.8 ± 0.3 ^d	22.7 ± 1.0 ^e	21.2 ± 0.3 ^f
Crude ash	13.5 ± 0.1 ^b	13.1 ± 0.0 ^a	12.7 ± 0.1 ^c	13.3 ± 0.1 ^a	12.4 ± 0.1 ^d	12.9 ± 0.0 ^e
Salinity	5.0 ± 0.0 ^a	5.1 ± 0.1 ^a	5.0 ± 0.1 ^a	5.6 ± 0.1 ^b	5.0 ± 0.3 ^a	3.8 ± 0.2 ^e

*Moisture values from fresh-frozen krill before freeze drying. Different letters indicate significant differences at $P < 0.05$.

Table 2. Monthly changes in total amino acid compositions of Antarctic krill *Euphausia superba* (mg/100 g dry weight)

Amino acids	March	April	May	June	July	August
Aspartic acid	1187.95	1111.86	1097.75	993.49	1027.93	1141.65
Threonine	458.39	415.14	418.26	379.45	387.50	450.31
Serine	463.31	421.65	415.63	388.85	404.74	440.88
Glutamic acid	1510.13	1335.24	1386.74	1299.29	1315.47	1467.23
Proline	716.07	530.11	561.85	494.63	500.82	509.85
Glycine	562.97	507.17	480.42	478.54	507.26	568.20
Alanine	645.42	572.46	582.91	533.77	546.80	603.77
Cystine	305.07	300.98	290.76	278.60	289.94	298.49
Valine	579.54	543.81	540.91	493.87	508.36	554.40
Methionine	309.85	292.24	292.34	271.41	276.90	300.87
Isoleucine	560.94	538.98	533.83	485.55	504.08	546.09
Leucine	857.52	810.23	802.59	743.46	761.50	831.46
Tyrosine	388.37	372.65	359.06	333.31	356.32	376.01
Phenylalanine	469.27	441.42	437.98	403.89	410.70	463.31
Histidine	256.46	235.35	239.15	228.85	226.29	249.17
Lysine	971.82	860.39	856.43	759.30	671.47	860.82
Arginine	715.72	656.81	658.60	646.35	624.01	722.18
Tryptophan	97.10	77.75	74.75	80.26	84.62	83.67
Total	11,055.91	10,024.25	10,029.96	9,292.86	9,404.71	10,468.36

*Different letters indicate significant differences at $P < 0.05$.

Results and Discussion

Proximate components

Table 1 shows the monthly variations in the proximate components of krill. The moisture content ranged from 77.7 to 80.7% (moisture values from fresh-frozen krill before freeze-drying). The moisture content was slightly, but not significantly, higher in June and July. The protein content ranged from 48.0 to 74.1% on a dry weight (DW) basis and was lowest in May. No large differences in protein content were observed among March, April, June, July, and August. The crude lipid content varied from 17.8 to 23.7% (DW basis), and in contrast to that of crude protein showed the highest level in April and the lowest in June. The crude ash content ranged from 12.4 to 13.5% (DW basis), and the salinity content was 3.8-5.6% (DW basis). The crude ash and salinity content showed no significant monthly variability.

Jaczynski et al. (2011) reported a protein content for krill of 72.9-75.8% (DW basis), which is similar to the crude protein content in our study for each month except April and May, while Saether et al. (1986) described lipid contents in the range of 12-50% (DW basis). These values are, however, significantly different from the crude lipid contents measured in our study. Shon et al. (1994) reported that krill contained 61.1% crude protein, 15.8% crude lipids, and 12.5% crude ash, values similar to those for April in our study. The small differences in the proximate composition among different studies is believed to be due to variations in the size, age, and season of the krill catch.

Amino acids

Monthly total amino acid compositions in krill are shown in Table 2. Glutamic acid showed the highest levels at 1,510.13-1,299.29 mg/100 g, followed by aspartic acid (1,187.95-993.49 mg/100 g), leucine (857.52-743.46 mg/100 g), and arginine (715.72-624.01 mg/100 g). Glutamic acid, aspartic acid, and leucine exhibited their highest levels in March and lowest levels in June, while arginine was highest in May and lowest in July. Chen et al. (2009) reported that glutamic acid, aspartic acid, lysine, and leucine all had high levels, while Mohr and Saether (1987) reported that glutamic acid, aspartic acid, glycine, alanine, lysine, and leucine exhibited high levels, similar to the results of our study.

The total amino acids showed the highest level (11,055.91 mg/100 g) in March and lowest level (9,292.86 mg/100 g) in June. In other studies, Sriket et al. (2007) reported that arginine, proline, leucine, isoleucine, phenylalanine, and glutamic acid showed high levels, and that the total amino acid content was 29,808 mg/100 g in black tiger shrimp meat and 29,121 mg/100 g in white shrimp meat, while Heu et al. (2003) reported that aspartic acid, glutamic acid, leucine, arginine, lysine, and glycine were present at high levels and that the total amino acid content was 12,588 mg/100 g in northern pink shrimp muscle and 14,479 mg/100 g in spotted shrimp muscle.

The free amino acid compositions of krill for each month are shown in Table 3. Proline had the highest levels at 330.22-108.37 mg/100 g, followed by lysine (233.26-108.39 mg/100 g), taurine (171.48-152.19 mg/100 g), arginine (145.70-135.79 mg/100 g), and glycine (131.87-73.93 mg/100 g) in

Table 3. Monthly changes in free amino acid compositions of Antarctic krill *Euphausia superba* (mg/100 g, dry weight)

Amino acids	March	April	May	June	July	August
Taurine	154.41	154.84	152.19	155.63	154.29	171.48
Aspartic acid	18.68	8.53	10.44	10.78	8.68	11.88
Threonine	38.82	12.76	10.47	13.67	14.92	16.85
Serine	11.72	12.55	12.61	13.11	14.63	15.78
Glutamic acid	15.94	2.49	3.33	2.36	3.32	3.68
Proline	330.22	190.51	157.24	135.54	108.37	112.74
Glycine	119.80	86.32	73.93	88.59	112.80	131.87
Alanine	72.75	38.38	38.34	35.93	42.71	41.77
Cystine	5.37	3.10	3.36	2.94	2.24	3.45
Valine	34.11	10.81	10.81	10.41	11.13	11.49
Methionine	19.38	7.02	9.26	7.28	8.46	9.01
Isoleucine	21.41	5.50	6.24	6.21	7.97	8.05
Leucine	55.12	12.28	14.81	12.41	14.15	15.57
Tyrosine	28.78	6.93	9.84	10.82	11.57	13.56
Phenylalanine	31.37	6.36	5.41	6.13	7.65	8.43
Histidine	10.81	3.31	3.95	3.62	4.99	5.24
Lysine	233.26	151.88	141.87	108.39	134.57	128.64
Arginine	145.70	102.27	110.01	132.02	104.96	135.79
Tryptophan	15.48	6.18	6.20	5.38	6.21	6.90
Total	1,363.14	822.01	780.29	761.25	773.63	852.19

*Different letters indicate significant differences at $P < 0.05$.

order of concentration from highest to lowest. Proline showed the highest level in March and lowest level in July, while taurine displayed the highest level in August and lowest level in May. Generally, the free amino acid levels were highest in March and lowest in June: 1,363.14 mg/100 g in March and 761.25 mg/100 g in June. Both the total and free amino acids were at their highest levels in March. In other studies, Chen et al. (2009) reported that krill protein included essential amino acids, while Mohr and Saether (1987) reported that krill contained high levels of taurine, glycine, arginine, and alanine. The amino acid content of krill meets the requirements of the FAO/WHO/UNU (United Nations University) for essential amino acid intake in adults (Chen et al., 2009). Tou et al. (2007) reported that the nutritional components of the proteins in krill are almost equal to those of meat. Furthermore, krill is expected to play an important role as a dietary source of taurine because of its high levels of this key amino acid.

Fatty acids

Monthly fatty acid compositions in krill are shown in Table 4. The saturated fatty acid contents (51.10-54.91%) were at similar levels to those of unsaturated fatty acids at 48.00-51.21%. The saturated fatty acid contents showed the highest level in August and the lowest level in April, while the unsatu-

rated fatty acid contents exhibited the highest level in April and the lowest in August.

Among the saturated fatty acids, C16:0 (25.07-27.64%) and C14:0 (16.44-18.19%) were the most abundant: C16:0 had the highest level in August and the lowest in April, while C14:0 had the highest level in June and the lowest in April. Among the unsaturated acids, C16:1 (5.70-9.21%), C18:1 (14.02-17.50%), C20:5 (9.64-14.13%), and C22:6 (5.95-10.42%) showed the highest contents, with C16:1 having the highest level in August and the lowest in March and C18:1 having the highest level in July and the lowest in March. In contrast, the n-3 polyunsaturated fatty acids (PUFAs), C20:5 (EPA), and C22:6 (DHA) exhibited high levels in March and low levels in August. Overall, the fatty acid content showed high levels in August and low levels in March, while unsaturated fatty acid was highest in March and lowest in August. Yoshitomi and Nagano (2012) reported 15.6% palmitic acid and 16.5% oleic acid, similar to values observed in our study, although their EPA content (7.8%) was lower than that observed our study. Kolakowska et al. (1994) also showed a similar result for EPA and DHA, while Cho et al. (1999) reported that palmitic acid, EPA, and DHA were found at levels of 19.95, 16.14, and 14.09%, respectively, in the lipids from whole krill powder. Sriket et al. (2007) reported DHA and EPA at levels of 14.9 and 8.58%, respectively, in the lipids from black tiger shrimp

Table 4. Monthly changes in fatty acid compositions of Antarctic krill *Euphausia superba* (% dry weight)

Fatty acids	March	April	May	June	July	August
C12:0	0.40	0.39	0.38	0.43	0.35	0.35
C13:0	0.11	0.12	0.17	0.15	0.11	0.12
C14:0	16.46	16.44	16.85	18.19	16.97	17.35
C15:0	0.49	0.54	0.76	0.63	0.54	0.57
C16:0	25.10	25.07	25.26	25.34	27.03	27.64
C17:0	7.08	6.72	7.04	5.99	6.30	6.77
C18:0	1.44	1.54	1.41	1.61	1.82	1.80
C22:0	0.03	0.04	0.04	0.04	0.04	0.04
C23:0	0.23	0.25	0.30	0.27	0.27	0.28
C24:0	0.00	0.00	0.00	0.00	0.02	0.00
	51.33	51.10	52.22	52.65	53.45	54.91
C14:1	0.33	0.31	0.33	0.35	0.26	0.28
C16:1	5.70	7.26	7.24	8.21	8.82	9.21
C17:1	0.20	0.18	0.28	0.21	0.17	0.17
C20:1	0.77	0.97	0.70	0.93	1.09	1.04
C24:1	0.00	0.00	0.00	0.00	0.10	0.09
	7.00	8.72	8.55	9.70	10.45	10.79
C18:1n9c	14.02	15.50	15.09	16.97	17.50	17.33
C18:2n6c	2.04	2.11	2.55	2.26	2.07	2.15
C18:3n3	2.58	2.09	2.02	1.81	1.26	1.32
C18:3n6	0.16	0.20	0.18	0.20	0.21	0.21
C20:3n3	0.16	0.12	0.00	0.10	0.00	0.00
C20:5n3	14.13	13.01	11.96	10.54	10.18	9.64
C22:1n9	0.46	0.00	0.40	0.44	0.71	0.61
C22:6n3	10.42	9.46	9.64	7.30	6.38	5.95
	43.96	42.49	41.85	39.61	38.32	37.21

*Different letters indicate significant differences at $P < 0.05$.

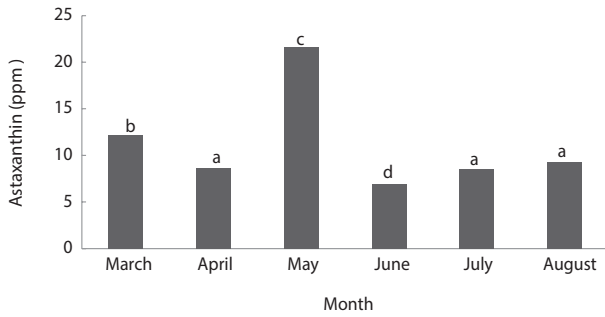


Fig. 1. Monthly changes in astaxanthin contents of Antarctic krill *Euphausia superba* (ppm, dry weight). Different letters indicate significant differences at $P < 0.05$.

meat, while Heu et al. (2003) reported DHA and EPA at levels of 14.5 and 11.6%, respectively, in the lipids from northern pink shrimp muscle and 14.2 and 13.2% in the lipids from spotted shrimp muscle. Krill is assumed to be a rich source of PUFAs, which are reported to be associated with preventing arteriosclerosis and heart disease by reducing cholesterol and neutral fat in blood (Venugopal, 2009).

Astaxanthin

Monthly astaxanthin contents in krill are shown in Fig. 1. In general, the astaxanthin contents were in the range of 6.94–21.59 ppm. Astaxanthin showed the highest level (21.59 ppm) in May and the lowest level (6.94 ppm) in June, while Kim et al. (2004) reported an astaxanthin content in krill of 10 ppm, similar to the contents for each month in our study except May. Yamaguchi et al. (1983) reported that the carotenoid contents in krill were 3–4 mg/100 g, and that astaxanthin diester, astaxanthin monoester, and astaxanthin comprised 40–50, 30–40, and 15–25%, respectively. Astaxanthin, unlike carotenes, is devoid of vitamin A activity, but has strong antioxidant effects, with its antioxidant capacity being 10 times stronger than that of β -carotene and 100 times stronger than vitamin E. In addition, the immune stimulation effect of astaxanthin is reported to be superior to that of β -carotene (Mortensen and Skibsted, 1997; Kim et al., 2004). Astaxanthin helps to improve cardiovascular, immune, and inflammatory status, and also protects organs from oxidative damage (Guerin et al., 2003; Fassett and Coombes, 2009).

Vitamins

Monthly vitamin contents in krill are shown in Table 5. Vitamins were present in the order of increasing to decreasing concentration of $B_6 > B_1 > E > A > B_5$. Water-soluble vitamins were present at higher concentrations than fat-soluble vitamins, among which vitamin A showed the highest level (7.1 mg/100 g) in May and lowest level (5.2 mg/100 g DW) in June, and vitamin E had the highest level (13.4 mg/100 g) in March and the lowest one (9.8 mg/100 g) in July. Tou et al. (2007) reported a higher vitamin A content of 380 I.U./100 g (11.4 mg/100 g) than our study, while their value of the vitamin E content of 15 mg/100 g was similar to our finding. Among the water-soluble vitamins, B_1 had the highest level (17.78 mg/100 g) in March and lowest level (11.40 mg/100 g) in July, B_6 had the highest level (109.50 mg/100 g) in March and lowest (63.77 mg/100 g) in May (no significant difference was detected from April to July), and B_5 showed the highest level (0.75 mg/100 g) in July and the lowest level (0.47 mg/100 g) in March. Tou et al. (2007) reported that vitamin B_1 was not detected and vitamin B_6 was present at 0.001 mg/100 g, which was lower than in our study.

Minerals

Minerals are essential substances that at low concentrations in conjunction with vitamins sustain life and maintain health by modulating body functions. Monthly mineral contents in krill are shown in Table 6. Sodium (Na) showed the highest level (233.6 mg/100 g) in April and the lowest level (203.6 mg/100 g) in July. Earlier, Kim et al. (2004) had reported a Na content in krill of 2.85%. The magnesium (Mg) content was at its highest level (38.86 mg/100 g) in March and lowest level (30.74 mg/100 g) in July, and significant differences were noted for each month in calcium (Ca), Mg, and Na. Iron (Fe) was present at its highest level (19.05 mg/100 g) in March and lowest level (3.69 mg/100 g) in June, while Ca (6.28–10.16 mg/100 g) and Zn (3.95–4.97 mg/100 g) exhibited lower levels than other minerals with their highest level in June and lowest in April. Copper (Cu) had the highest level at 6.32–7.456 mg/100 g among the trace minerals, reaching its highest level in May and lowest level in March. The high Cu levels and their monthly variations are believed to be caused by the effect of hemocyanin, a blood pigment containing Cu in crustaceans.

Table 5. Monthly changes in vitamin contents of Antarctic krill *Euphausia superba* (mg/100 g, dry weight)

Vitamins	March	April	May	June	July	August
A	6.10 ± 0.74 ^a	6.60 ± 0.12 ^a	7.10 ± 0.40 ^a	5.20 ± 0.21 ^a	5.30 ± 1.41 ^a	5.80 ± 1.91 ^a
B_1	17.78 ± 4.59 ^a	15.88 ± 2.38 ^a	15.50 ± 3.42 ^a	11.95 ± 1.11 ^a	11.40 ± 0.66 ^a	14.12 ± 2.86 ^a
B_5	0.47 ± 0.04 ^b	0.56 ± 0.06 ^a	0.56 ± 0.01 ^a	0.54 ± 0.08 ^a	0.75 ± 0.05 ^c	0.69 ± 0.06 ^d
B_6	109.50 ± 4.72 ^b	67.44 ± 2.38 ^a	63.77 ± 6.94 ^a	64.66 ± 6.96 ^a	66.95 ± 1.40 ^a	82.54 ± 5.22 ^c
E	13.40 ± 0.00 ^a	12.40 ± 0.00 ^b	11.13 ± 0.00 ^c	11.21 ± 0.00 ^d	9.83 ± 0.00 ^e	10.78 ± 0.00 ^f

* Different letters indicate significant differences at $P < 0.05$.

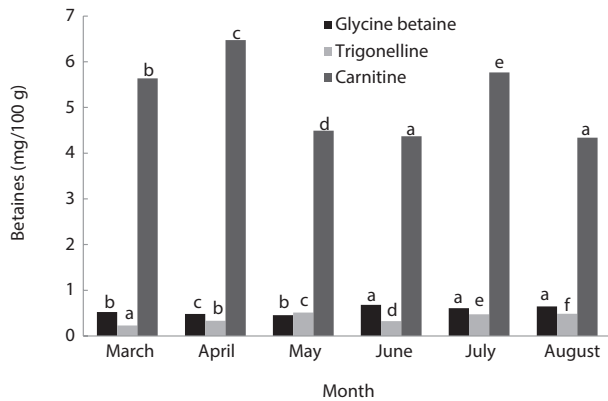


Fig. 2. Monthly changes in betaine contents of Antarctic krill *Euphausia superba* (dry weight). Different letters indicate significant differences at $P < 0.05$.

Yamamoto et al. (1987) reported that Cu, Zn, and Fe in krill were 12.7, 9.6, and 3.6 $\mu\text{g/wet g}$, respectively. The selenium (Se) content was in the range of 0.02-0.18 mg/100 g. Se is an essential trace element nutrient for humans and plays an important role as a component in the antioxidant enzyme glutathione peroxidase to eradicate reactive oxygen species in the body. Fish and crustaceans contain high levels of Se (Barclay et al., 1995), and krill is also thought to be an important source of Se. Moreover, Tovar-Sanchez et al. (2007) and Chen et al. (2009) reported that the mineral contents in krill were similar to those of shrimp.

Heavy metals

Monthly heavy metal contents in krill are shown in Table 6. Hg exhibited no significant monthly variation (0.03–0.05 mg/100 g). Cd showed the highest level (0.05 mg/100 g) in May and lowest level (0.03 mg/100 g) in April, July, and August, while Pb had a high level (2.94 mg/100 g) in March and low level (0.01 mg/100 g) in May and July. As (arsenic) was

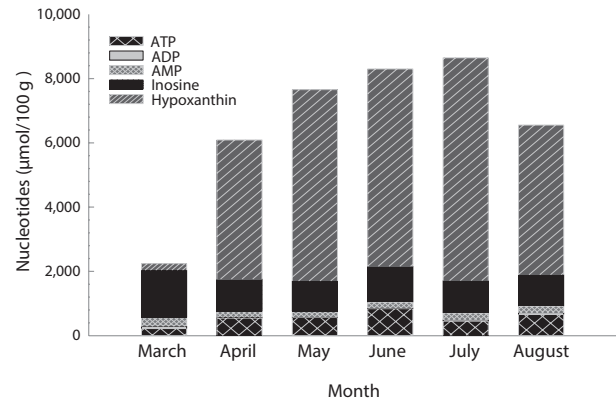


Fig. 3. Monthly changes in nucleotide contents of Antarctic krill *Euphausia superba* (dry weight). Different letters indicate significant differences at $P < 0.05$.

present at its highest level (1.01 mg/100 g) in June and lowest level (0.52 mg/100 g) in April. Yamamoto et al. (1987) reported heavy metal contents of Cd, Pb, and Hg in krill of 0.43, 0.04, and 0.008 $\mu\text{g/wet g}$, respectively, while Kim et al. (2004) reported that krill contained 2.76 ppm Pb, 0.14 ppm Cd, and 1.29 ppm As, which are within safety levels. Codex regulations for heavy metals such as Hg, As, and Pb are 0.1, 0.1, and 0.3 ppm, respectively, and the heavy metal contents of krill have been found to comply with Codex and European Union regulations. The standard regulations for foods are Hg < 0.5 mg/kg, Pb < 2.0 mg/kg, and Cd < 2.0 mg/kg.

Betaines

Monthly variations in the betaine contents in krill are shown in Fig. 2. The glycine betaine content showed the highest levels (0.49-0.69 mg/100 g) in June. Trigonelline levels were high (0.52 mg/100 g) in May and low (0.23 mg/100 g) in March, but carnitine showed the highest levels and was present at its highest level (6.63 mg/100 g) in April and lowest

Table 6. Monthly changes in mineral and heavy metal contents of Antarctic krill *Euphausia superba* (mg/100 g, dry weight)

Minerals	March	April	May	June	July	August
Ca	6.47 ± 0.01 ^a	6.28 ± 0.01 ^b	7.50 ± 0.01 ^c	10.16 ± 0.01 ^d	9.51 ± 0.00 ^e	8.53 ± 0.02 ^f
Mg	38.86 ± 0.04 ^a	35.12 ± 0.10 ^b	33.48 ± 0.05 ^c	36.64 ± 0.07 ^d	30.74 ± 0.01 ^e	31.4 ± 0.03 ^f
Na	216.40 ± 2.08 ^a	233.60 ± 3.27 ^c	219.60 ± 1.54 ^b	235.60 ± 1.27 ^c	203.60 ± 2.10 ^a	206.40 ± 0.55 ^a
Fe	19.05 ± 0.04 ^d	6.67 ± 0.03 ^b	7.55 ± 0.00 ^c	3.44 ± 0.00 ^a	3.83 ± 0.01 ^a	3.69 ± 0.00 ^a
Zn	4.66 ± 0.01 ^b	4.30 ± 0.02 ^a	3.95 ± 0.01 ^c	4.97 ± 0.01 ^d	4.27 ± 0.02 ^a	4.60 ± 0.01 ^b
Cu	6.32 ± 0.01 ^a	7.39 ± 0.0 ^a	7.45 ± 0.01 ^a	7.26 ± 0.00 ^a	6.77 ± 0.03 ^b	6.67 ± 0.00 ^b
Se	0.07 ± 0.01 ^c	0.18 ± 0.00 ^c	0.05 ± 0.00 ^f	0.09 ± 0.00 ^d	0.02 ± 0.00 ^a	0.03 ± 0.00 ^b
Hg	0.05 ± 0.00 ^a	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.04 ± 0.00 ^a
As	0.63 ± 0.00 ^a	0.52 ± 0.00 ^b	0.79 ± 0.01 ^c	1.01 ± 0.00 ^d	0.56 ± 0.00 ^c	0.63 ± 0.00 ^a
Cd	0.04 ± 0.00 ^b	0.03 ± 0.00 ^a	0.05 ± 0.00 ^c	0.04 ± 0.00 ^d	0.03 ± 0.00 ^e	0.03 ± 0.00 ^a
Pb	2.94 ± 0.00 ^c	0.02 ± 0.01 ^b	0.01 ± 0.00 ^a	0.02 ± 0.00 ^a	0.11 ± 0.00 ^a	0.02 ± 0.00 ^b

*Different letters indicate significant differences at $P < 0.05$.

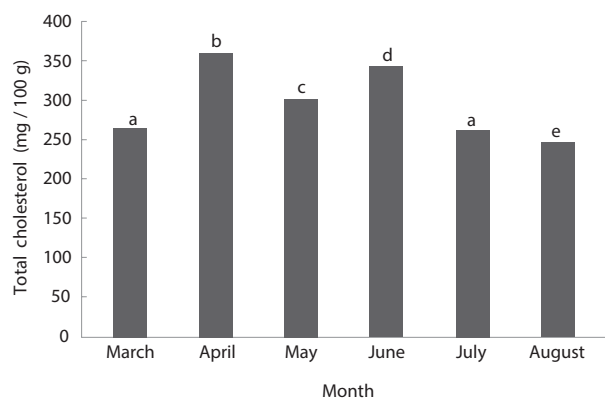


Fig. 4. Monthly changes in total cholesterol contents of Antarctic krill *Euphausia superba* (dry basis). Different letters indicate significant differences at $P < 0.05$.

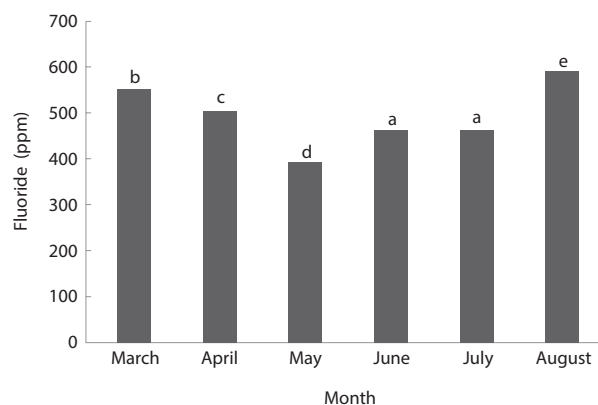


Fig. 5. Monthly changes in fluoride contents of Antarctic krill *Euphausia superba* (dry weight). Different letters indicate significant differences at $P < 0.05$.

level (4.46 mg/100 g) in June. Generally, carnitine is known to be an essential substance associated with fat metabolism, taking part in the transportation of fat to mitochondria. It is reported to have effects in preventing osteoporosis with enriched osteocalcin (Cavazza, 2002a) and in preventing peroxidation of phospholipid membranes and to act as an antioxidant against oxidative stress (Cavazza, 2002b). Betaines are widely distributed in animals and plants, and play a role as methyl group donors to create methionine from homocysteine in the liver (Garrow and Park, 1999). Betaine supplementation is able to prevent cardiovascular disease by reducing the density of homocysteine in blood (Garrow and Park, 1999).

Nucleotides

Adenosine triphosphate (ATP) is a major adenine nucleotide found in the muscle of animals. ATP generates adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine, and hypoxanthin through the action of phosphatases. Monthly nucleotide contents in krill are shown in Fig. 3 and range from 2,242.71 to 8,645.90 $\mu\text{mol}/100\text{ g}$. Overall, inosine had the highest level and ADP had the lowest level. ATP showed the highest level (840.52 $\mu\text{mol}/100\text{ g}$) in June and lowest level (221.55 $\mu\text{mol}/100\text{ g}$) in March, while ADP had the highest level (73.16 $\mu\text{mol}/100\text{ g}$) in March and the lowest level (16.86 $\mu\text{mol}/100\text{ g}$) in May; AMP also showed a high level (272.73 $\mu\text{mol}/100\text{ g}$) in March and low level (179.22 $\mu\text{mol}/100\text{ g}$) in May. Inosine reached its highest level (1,470.67 $\mu\text{mol}/100\text{ g}$) in March, similar to ADP and AMP, but hypoxanthin showed its highest level (6,930.58 $\mu\text{mol}/100\text{ g}$) in July and lowest level (204.60 $\mu\text{mol}/100\text{ g}$) in March. Hypoxanthin had the highest level among whole nucleotides, and IMP was not detected. Fish have been reported to accumulate large amounts of IMP through the degradation of ATP (Oba et al., 1991), and the ATP content is reportedly closely related to changes as the freshness of fish and shellfish

deteriorates (Koseki et al., 2006). The fact that IMP was not detected, while inosine and hypoxanthine were found at high levels, is believed to result from inosine and hypoxanthine formation via the adenosine pathway from AMP, or inosine and hypoxanthine formation by the degradation of IMP, which are dependent on the elapsed time after catch.

Cholesterol

Cholesterol is known to play an important role in the biosynthesis of steroid hormones and vitamin D, and is believed to be an essential substance for maintaining the life of crustaceans (Kanazawa et al., 1976). Major human dietary sources of cholesterol are cheese, egg yolk, beef, and shrimp (Jensen et al., 1978), and cholesterol coexists with large amounts of fatty substances (Kanazawa et al., 1976). Monthly total cholesterol contents in krill are shown in Fig. 4. The total cholesterol content ranged from 249.65 to 348.88 mg/100 g, showing the highest level (348.88 mg/100 g) in April and the lowest level (249.65 mg/100 g) in August. Shrimp meat has been reported to contain 150-160 mg/100 g (wet weight) of cholesterol (Krzynowek and Panunzio, 1989). Tou et al. (2007) reported cholesterol levels in krill of 66.1 mg/100 g and 152 mg/100 g in shrimp (Penaeidae and Pandalidae), and the cholesterol content in krill was higher than that of fish, but lower than that of shrimp. However, our study revealed a cholesterol content for krill that was slightly higher than that reported for shrimp.

Fluoride

Since Antarctic krill contains high levels of fluoride in its shell (Soevik and Breakkan, 1979; Christians and Leinemann, 1983; Nicol and Stolp, 1991; Virtue et al., 1995), potential safety issues arise if the whole body is consumed for food. Monthly contents of fluoride in krill are shown in Fig. 5. The total fluoride content was highest (599.63 ppm) in August and

lowest (397.51 ppm) in May. Soszka et al. (1981) had reported a fluoride content in krill of 1,330–2,400 mg F/kg, while Moren et al. (2007) reported a value of $1,160 \pm 230$ mg F/kg and Mohr and Saether (1987) described the fluoride content in North Antarctic krill as being 1,040–3,200 ppm. The difference between these values and those of our study is believed to be attributable to differences in the catch season, size, and sampling and analysis methods.

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