JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7831–7837 7831
DOI:10.1021/jf101526c

Seasonal Variation in Nutrient Composition of *Mytilus coruscus* from China

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Seasonal variation in the nutrient composition of *Mytilus coruscus* (thick shell mussel), cultivated in Shengsi Islands, Zhejiang Province, China, was investigated in this study. Proximate composition, mineral and amino acid concentrations, as well as the compositions of the lipid and fatty acid of thick shell mussels were analyzed. Proximate composition and mineral contents were seasonally varied significantly except for calcium (Ca) and lead (Pb). Glycine was the predominant amino acid in mussels throughout all seasons, while lysine, threonine, phenylalanine, and arginine were the main essential amino acids. The predominant lipids were phospholipids, followed by triacylglycerols and sterol esters. Polyunsaturated fatty acids (PUFA) predominated over saturated and monounsaturated fatty acids throughout the year. Docosahexaenoic acid (12.44−18.34% of total fatty acids) and eicosapentaenoic acid (10.79−14.60%) were the most abundant PUFA. Significant seasonal variations were observed in the compositions of most amino acids, lipid classes, and fatty acids. Cultivated Chinese thick shell mussels represent a source of the health benefiting long chain *n*-3 PUFA, essential amino acids, and minerals for human consumption.

KEYWORDS: *Mytilus coruscus*; thick shell mussel; seasonal variation; proximate composition; minerals; amino acids; lipid class; fatty acids

INTRODUCTION

Mussels are one of the most widely distributed mollusc species in the world. According to the report, the annual world production of mussels is approximately 1,500,000 tonnes (*1*). They are easy to cultivate because of their fast-growing ability, high reproductive capacity, and strong adaptability. They are playing an ever-increasing role in the human diet since they are rich in nutrients and functional components, especially *n*-3 polyunsaturated fatty acids (PUFA), which are reported to have beneficial effects in patients with cardiovascular disease (*2*) or inflammatory disease (*3*). It is known that the consumption of mussels makes a large contribution to amino acid, mineral, and fatty acid intake (*1*, *4*, *5*).

China is the number one producer of mussels in the world, producing over 600,000 tonnes per year (*6*). Blue mussel (*Mytilus edulis*), thick shell mussel (*Mytilus coruscus*), and green mussel (*Perna viridis*) are the three main species cultivated in China. They are widely cultivated in coastal areas of Huanghai Sea, Bohai Sea, and East China Sea and have been the most represented mollusc in the Chinese bivalve market (*6*). Though the production of *Mytilus coruscus* (50,000 tonnes, accounting for 8% of total mussel production) in China is less than those of *Mytilus edulis* and *Perna viridis*, it is more appreciated by consumers for its high organoleptic quality. Zhejiang province is the major producer of *Mytilus coruscus* in China, with an annual production of over 25,000 tonnes (*7*). The samples for this study came from Shengsi Islands in Zhejiang province, one of the original and main culture areas of *Mytilus coruscus* in the Eastern China Sea.

The quality requisites of mussels are primarily dependent on their safety, nutrients, and functional components (*5*). From a nutritional standpoint, several characteristics are involved in product quality. Protein, lipid, and mineral contents, together with their compositions contribute to the nutritional value of mussels (*5*, *8*). However, many factors may influence their nutrient compositions. It has been reported that different mussel species, such as zebra mussel, blue mussel, and green mussel, have different nutrient compositions (*9*, *10*). For the same species, a large number of studies have proved that water temperature, culture areas, environmental conditions, life stage, and harvest time influence the nutrient compositions of mussels (*1*, *5*, *11*−*13*). Up to now, seasonal variations on the nutritional characteristics of some mussel species have also been reported (*5*, *14*−*16*). However, there is no data on the nutrient composition of the thick shell mussel, *Mytilus coruscus*, in the previous literature.

The present study was aimed at evaluating the seasonal variation in proximate composition, mineral, and amino acid contents, as well as the compositions of lipid and fatty acid of *Mytilus coruscus* cultivated in Shengsi Islands, one of the original and main culture areas in China. The results may provide useful information for both the harvest and consumption of thick shell mussels.

MATERIALS AND METHODS

Sample Collection and Preparation. Mussels (*Mytilus coruscus*) of commercial size were collected each season from Shengsi Islands during

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Published on Web 06/16/2010

2005−2006 (Autumn, in October, 2005; winter, in January, 2006; spring, in April, 2006; summer, in July, 2006).

Samples of each season were immediately transported under refrigeration (4 °C) to the Department of Food Science and Nutrition, Zhejiang University, China. Mussels were inspected and dead animal discarded as soon as they arrived. The live mussels were randomly divided into three pools ($n = 3$), each comprising 300 individuals, thus make a total of 900 individuals per mussel group. Mussel shells of each pool were removed, the fleshy portion of 50 individuals was used for proximate composition determination, and the remaining 250 individuals were cut into pieces, vacuum freeze-dried, homogenized to powder, and then stored at −80 °C for other nutrient composition determinations. All nutrient compositions were analyzed in triplicate (three pooled mussel samples for each season). In order to calculate the nutrient composition on a dry weight basis, the moisture contents of each freeze-dried mussel powder were measured before nutrient composition analysis. The mean moisture contents of mussel powder were 8.79%, 9.16%, 9.25%, and 8.98% for spring, summer, autumn, and winter, respectively.

Proximate Composition. Moisture, total lipid, protein, and ash contents of mussels were determined according to Association of Official Analytical Chemists (AOAC) methods 950.46, 991.36, 928.08, and 920.153, respectively (*17*). Glycogen was measured by the method of Carroll, Longley, and Roe (*18*).

Mineral Analysis. Minerals were measured by inductively coupled plasma mass spectrometry (ICP-MS) according to the method described by Airas (*19*) with some minor modifications. Mussel powder samples (0.2 g) were digested with 2.0 mL of nitric acid (65%, m/v) and 0.5 mL of hydrogen peroxide (30%, m/v) in sealed digestion vessels according to the digestion conditions. After complete digestion, the sample solutions were diluted with deionized water to the total volume of 25 mL. Blank samples were processed as follows: vessels were only filled with digestion acids and taken through the entire procedure to monitor the average and variation of the element blank value.

ICP-MS (Agilent 7500a, Agilent Corporation, USA) was used to determine the concentrations of minerals in the digested samples. Standard curves for all elements were calculated using five different concentrations. Data quality control was performed by a separate comparative study of a standard reference material (NRC, Canada TORT-1, lobster hepatopancreas) and procedural blanks, which were analyzed according to the same procedure every six samples. The agreement between the results for the reference biological material and the NRC certified values was satisfactory. Recovery (total certified concentrations of a metal versus total metal concentration in this study) and precision of the minerals were 95.4−108.6% and 1.3−6.8%, respectively.

Amino Acid Analysis. Amino acids were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) with precolumn derivation using phenylisothiocyanate (PITC) according to the method described by Dimova (*20*) with some minor modifications. Ten milligrams of freeze-dried mussel powder was hydrolyzed with 10 mL of 6.0 M hydrochloric acid in a sealed glass ampule at 110 °C for 24 h. The hydrolyzed sample solution was centrifuged at 3000*g* for 15 min. A 100 μL aliquot of the supernatant was mixed with 25 μL of 1 mM norleucine solution (internal standard) and then vacuum-dried. The vacuum-dried hydrolyzed sample was dissolved in 100 μL of ethanol−water−triethylamine (TEA) (2:2:1, v/v/v) and vacuum-dried again. Derivatization was carried out by the addition of 200 μL of ethanol−water−TEA−PITC (7:1:1:1, v/v/v/v) to the dried samples. After derivatization, the phenylthiocarbamyl (PTC) amino acids were vacuum-dried again. Then a 1000 μL aliquot of diluents (5 mM disodium hydrogen phosphate, adjusted to pH 7.4 with phosphate containing 5% acetonitrile) was added and filtered for RP-HPLC analysis.

The PTC-amino acids were analyzed by using a Waters 2695 HPLC system fitted with a variable wavelength UV detector (Waters Corporation, USA). The separation of amino acids was achieved on a reversed-phase HP ODS Hypersil column (200 mm × 4.6 mm I.D., 5 μm particle size), thermostatted at 40 °C. The solvent system consisted of two eluents: 0.1 M sodium acetate containing 7% of acetonitrile, adjusted to pH 6.4 with glacial acetic acid (mobile phase A); acetonitrile−water 80:20 (v/v) (mobile phase B). The gradient used is listed in **Table 1**. The injection volume was 10 μL. The chromatograms were monitored at a wavelength of 254 nm.

Identification was carried out by comparison of the retention times of unknowns with amino acid standards run under the same conditions.

Table 1. Elution Program for HPLC Analysis[a]

time (min)	flow (mL/min)	mobile phase A (%)	mobile phase B (%)
0	1.0	100	0
13	1.0	93	7
23	1.0	77	23
29	1.0	65	35
35	1.0	60	40
40	1.0	0	100
45	1.0	0	100
47	1.0	100	0
50	1.0	100	0

[a] Mobile phase A, 0.1 M sodium acetate containing 7% of acetonitrile, adjusted to pH 6.4 with glacial acetic acid; mobile phase B, acetonitrile−water 80:20 (v/v).

Quantification was done by using calibration curves of peak area ratios (compound/internal standard) versus concentration ratios (compound/internal standard), under identical chromatographic conditions.

Lipid Analysis. The total lipids of mussel powder were extracted with a chloroform−methanol (2:1, v/v) solvent system containing 10 mg/L of butylated-hydroxytoluene (BHT, Sigma Chemical Co. St Louis, USA), following the methods described by Folch, Less, and Stanley (*21*). Lipid composition was analyzed by using Iatroscan TLC-FID Analyzer (Iatron Laboratories Inc., Japan) (*22*). Briefly, the lipid extract of mussels, which has been dissolved in chloroform at the concentration of 10−20 μg/μL, was spotted on the starting point of the chromarods as 2 μL using autospotter. The rods, after spotting, were developed in developing tanks with a petroleum ether−ethylether−acetic acid (60:15:0.1, v/v/v) solvent system for 25 min and a petroleum ether−ethylether (56:4, v/v) solvent system for 30 min successively. Each time after development, the rods were placed in an oven (53 °C) for 3 min to evaporate the residual solvents. Then, the chromarods were scanned by Iatroscan MK-6s TLC-FID Analyzer (Iatron Laboratories Inc., Japan). The air and hydrogen flow rates for Iatroscan MK-6s Analyzer were 2000 mL/min and 160 mL/min, respectively, and the scan speed was set at 30 s/scan. The compositions of lipid classes were expressed in relative percentage of the total lipid according to their peak areas, which were recorded and processed by Chormstar software. Sterol palmitate, sterol, palmitic acid, tripalmitin, and lecithin (Sigma Corporation, USA) were employed as authentic standards for the quantitative analysis of the sterol esters, sterols, free fatty acids, triacylglycerols, and phospholipids.

Fatty Acid Analysis. Fatty acids were determined by gas−liquid chromatography (GLC) (*23*). The extraction of the total lipids was the same as described above. The methyl esters of the fatty acids from the lipid extract were transesterified with H_2SO_4 in methanol (5%, v/v), together with toluene, in sealed tubes at 70 °C for 2 h. The derived fatty acid methyl esters were analyzed by using Shimadzu GC-14C (Shimadzu Corporation, Japan) fitted with a flame ionization detector (FID) and a 60 m × 0.25 mm (I.D) × 0.25 μm (film thickness) fused silica bonded phase column (DB-23, Aglient Corporation, USA). Nitrogen was the carrier gas at the pressure of 300 kpa. The injector and detector temperatures were both 270 °C. The column temperature was programmed from 150 to 180 °C at a rate of 10 °C/min, with an initial hold time of 2 min; the temperature was then further increased to 215 at 2.5 °C/min and held for 6 min; finally, it was increased to 230 at 10 °C/min and held for another 5 min. Fatty acids were identified by the comparison of retention time with standard mixtures of fatty acid methyl ester. The compositions of fatty acids were expressed in relative percentages of the total fatty acids according to their peak areas.

Statistical Analysis. All data are presented as the means ± standard deviation (SD). Moisture contents are presented in g/100 g wet weight (WW) of mussel meat, while other proximate compositions are expressed as g/100 g dry weight (DW) of mussel meat. Mineral concentrations are presented in mg/kg DW of mussel powder. Amino acid contents are measured in g/100 g DW of mussel powder. Lipid and fatty acid compositions are reported as relative percentages of total lipids and fatty acids, respectively. Comparisons of nutrient composition in mussels of different seasons were done by one-way analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means at 5% significance level. Statistical analyses were performed by using PC professional software SPSS 16.0.

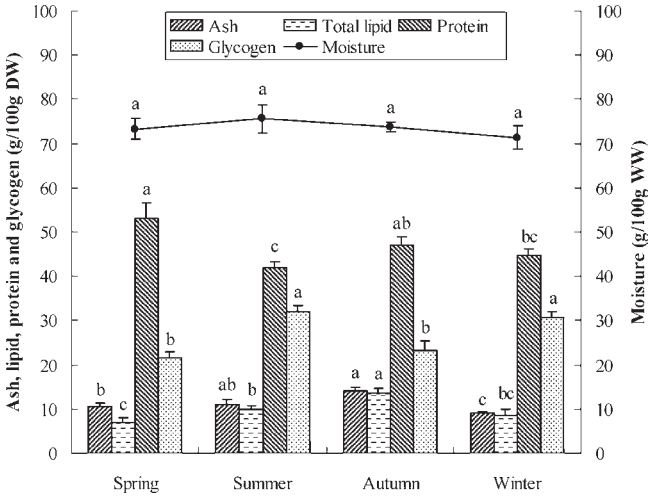

Figure 1. Seasonal variation of proximate composition in *Mytilus coruscus*. Results are presented as the mean ± SD (n = 3). Different letters in the bars of the same type indicate significant differences between different seasons in the composition of the same nutrient (P < 0.05).

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of *Mytilus coruscus* in different seasons is shown in **Figure 1**. Moisture contents ranged from 71.4 g/100 g WW in winter to 75.7 g/100 g WW in summer. Such contents were much lower than those of *Mytilus galloprovincialis*, whose moisture contents were reported above 80 g/100 g WW (*1*, *19*). Protein (42.0−53.2 g/100 g DW), followed by glycogen (21.6−32.2 g/100 g DW), was the predominant nutrient in *Mytilus coruscus*, while total lipid and ash were low in content, ranging from 7.0 to 13.6 g/100 g DW and 9.0 to 14.0 g/100 g DW, respectively. Many studies have demonstrated similar proximate composition patterns in mussels of *Mytilus galloprovincialis* (*1*, *5*), *Dreissena polymorpha*, and *Amblema plicata* (*9*). But in relation to the contents of individual nutritional components, different results were found. Compared with *Mytilus coruscus* in this study, much higher protein levels, ranging from 61 to 79 g/100 g DW, were measured in juvenile *Mytilus galloprovincialis* (*12*). Mediterranean mussels, harvested in Adriatic Sea, were reported to have much wider lipid contents (5.09−17.38 g/100 g DW) and higher ash contents (11.39−22.90 g/100 g DW) (*14*).

All proximate compositions, except moisture contents, displayed significantly seasonal variations in this study (P < 0.05). With regard to protein and glycogen, they followed opposite seasonal change trends. In spring, mussels showed the highest protein but lowest glycogen contents, while in summer, the mussels had the lowest protein but highest glycogen contents. As for total lipid and ash contents, they followed an almost similar change pattern and reached the highest levels in autumn but lower levels in spring and winter. Such seasonal fluctuations can be explained to some extent by the spawning periods of mussels. Okumus and Stirling (*24*) found an accumulation of glycogen in mussels (*Mytilus edulis*) a few months before spawning and a drop during spawning. Surh et al (*25*) concluded that lipid levels in shellfish species were higher at the beginning of spawning and lower at the end of spawning. Thick shell mussels in Shengsi Islands spawn twice every year, from March to April and from October to November (*26*). In our study, spring mussels were collected in April, which was the end of the spawning period, and they displayed the lowest levels of total lipid and glycogen, while autumn mussels which were collected in October (the beginning of the spawning period) displayed the highest levels of total lipid and lower levels of glycogen. Summer (in July) and winter (in January) mussels were collected a few months before spawning periods, and they presented higher levels of glycogen. Similar seasonal changes were also found in *Mytilus galloprovincialis* from Adriatic Sea (*5*).

Table 2. Seasonal Variation of Mineral Contents (mg/kg DW) in *Mytilus coruscus*[a]

minerals	spring	summer	autumn	winter	P-value
Mg	1655 ± 377 a	1860 ± 261 a	644 ± 128 b	1929 ± 324 a	0.000
Al	37.4 ± 7.1 b	59.2 ± 15.0 ab	35.7 ± 8.7 b	76.0 ± 3.9 a	0.001
Ca	1408 ± 275 a	1222 ± 191 a	1430 ± 356 a	2194 ± 882 a	0.167
Cr	0.54 ± 0.03 b	0.79 ± 0.09 a	0.48 ± 0.03 b	0.69 ± 0.05 a	0.003
Mn	3.6 ± 0.2 b	3.4 ± 0.5 b	3.1 ± 0.5 b	4.6 ± 0.3 a	0.012
Fe	66.5 ± 3.0 b	98.0 ± 11.6 a	58.6 ± 10.5 b	116.3 ± 7.7 a	0.002
Ni	0.24 ± 0.02 b	0.42 ± 0.11 a	0.23 ± 0.05 b	0.34 ± 0.06 a	0.022
Cu	4.9 ± 0.2 d	8.2 ± 1.1 b	14.6 ± 0.6 a	6.4 ± 0.4 c	0.000
Zn	45.7 ± 2.0 b	48.9 ± 7.0 b	63.8 ± 3.7 a	46.3 ± 2.9 b	0.002
Se	2.2 ± 0.2 a	2.0 ± 0.3 ab	1.5 ± 0.1 b	1.7 ± 0.1 b	0.014
Pb	0.61 ± 0.12 a	0.63 ± 0.06 a	0.61 ± 0.16 a	0.64 ± 0.04 a	0.920
Cd	1.5 ± 0.4 ab	1.8 ± 0.3 a	1.5 ± 0.0 b	0.9 ± 0.1 c	0.000

[a] Data are presented as the mean ± SD (n = 3). Different letters within the same row indicate significant differences between different seasons in the content of the same mineral (P < 0.05). Mg = magnesium, Al = aluminum, Ca = calcium, Cr = chromium, Mn = manganese, Fe = ferrum, Ni = nickel, Cu = copper, Zn = zinc, Se = selenium, Pb = lead, and Cd = cadmium.

Mineral Contents. Twelve minerals, including calcium (Ca), magnesium (Mg), aluminum (Al), ferrum (Fe), lead (Pb), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr), and selenium (Se), were analyzed in all mussel samples during the period of this study as reported in **Table 2**. Ca, Mg, Al, Fe, and Zn were the predominant elements in mussels of all seasons. Significant seasonal variations were found in most of the mineral contents except for Ca and Pb. The Mg level was significantly lower in autumn mussels, while Al and Mn levels were significantly higher in winter mussels (P < 0.05). Levels of Cr, Fe, and Ni had a similar change tendency. They increased from spring to summer, then decreased in autumn months, and finally increased in winter months. Mussels in autumn and spring had significantly higher contents of Zn and Se (P < 0.05). Concentrations of Cu and Cd were varied significantly in different seasons (P < 0.05). The significant differences found in the mineral contents may be partly due to the seasonal variation in the metabolic activity of mussels and chemical properties of seawater (*16*, *27*). During the whole study period, the contents of Pb (0.14−0.18 mg/kg WW) and Cd (0.25−0.48 mg/kg WW) in thick shell mussels were well below the maximum limits (1.5 mg/kg WW for Pb and 1 mg/kg WW for Cd) set by European Union (*28*). Other heavy metals including Cr (0.13−0.19 mg/kg WW) and Cu (1.3−3.8 mg/kg WW) were also below the upper limits (2.0 mg/kg WW for Cr and 50 mg/kg WW for Cu) set by General Administration of Quality Supervision, Inspection and Quarantine of China (AQSIQ) (*29*).

The mineral concentrations of mussels have been reported in a number of studies (*1*, *13*, *27*, *30*). However, no data on the minerals studied in *Mytilus coruscus* were found in the previously literature. Comparing the results obtained in this article on *Mytilus coruscus* with previously published data on other mussel species, some discrepancies in concentrations of the minerals can be observed. In the case of major elements (Ca and Mg), the concentrations reported here were higher than those observed in *Mytilus chilensis* from Strait of Magellan (600 and 750 mg/kg DW for Ca and Mg, respectively) (*4*) and *Mytilus galloprovincialis* from Ebro Delta (370 and 410 mg/kg DW for Ca and Mg, respectively) (*1*). With regard to trace elements, the Cu, Zn, Pb, and Cd concentrations analyzed in present study were lower than those found in *Mytilus edulis* from Southwest Iceland (9.6, 162, and 0.82 mg/kg DW for Cu, Zn, and Pb, respectively) (*31*) and

Table 3. Seasonal Variation of Amino Acid Contents (g/100g DW) in *Mytilus coruscus*[a]

amino acids	spring	summer	autumn	winter	*P*-value
asparticacid	1.7 ± 0.2 a	0.5 ± 0.1 b	0.6 ± 0.1 b	0.7 ± 0.1 b	0.000
glutamate	0.8 ± 0.1 a	0.5 ± 0.0 b	0.7 ± 0.1 a	0.5 ± 0.1 b	0.002
serine	4.4 ± 0.3 a	3.1 ± 0.2 b	3.2 ± 0.2 b	3.3 ± 0.2 b	0.000
glycine	13.4 ± 0.9 a	10.7 ± 1.5 ab	9.7 ± 0.5 b	10.6 ± 1.1 b	0.013
histidine[b]	0.3 ± 0.0 c	0.5 ± 0.0 b	0.6 ± 0.1 a	0.5 ± 0.1 ab	0.002
arginine[b]	2.1 ± 0.4 b	1.6 ± 0.1 b	2.9 ± 0.2 a	2.0 ± 0.2 b	0.002
threonine[b]	3.0 ± 0.7 a	2.3 ± 0.2 a	2.6 ± 0.1 a	2.3 ± 0.6 a	0.307
alanine	3.7 ± 0.4 a	3.2 ± 0.4 a	2.8 ± 0.7 a	3.2 ± 0.5 a	0.289
proline	4.1 ± 0.5 a	3.2 ± 0.3 ab	3.8 ± 0.2 ab	3.1 ± 0.1 b	0.023
tyrosine	2.5 ± 0.1 a	2.5 ± 0.1 a	2.8 ± 0.6 a	2.2 ± 0.4 a	0.278
valine[b]	3.5 ± 0.4 a	1.2 ± 0.4 b	1.6 ± 0.4 b	1.7 ± 0.3 b	0.000
methionine[b]	0.9 ± 0.1 c	0.8 ± 0.0 c	1.9 ± 0.2 b	2.9 ± 0.5 a	0.000
cysteine	0.8 ± 0.2 a	0.6 ± 0.2 a	0.6 ± 0.1 a	0.3 ± 0.1 b	0.006
isoleucine[b]	1.1 ± 0.2 b	2.7 ± 0.6 a	1.1 ± 0.1 b	0.9 ± 0.1 b	0.001
leucine[b]	1.1 ± 0.2 a	1.0 ± 0.1 a	1.0 ± 0.1 a	0.8 ± 0.1 a	0.098
phenylalanine[b]	2.2 ± 0.1 b	1.8 ± 0.3 b	3.2 ± 0.5 a	2.2 ± 0.4 b	0.006
lysine[b]	2.3 ± 0.3 bc	1.7 ± 0.1 c	3.3 ± 0.4 a	2.5 ± 0.3 b	0.001
total EAA	16.4 ± 1.1 ab	13.5 ± 1.3 b	18.3 ± 1.2 a	15.8 ± 1.4 ab	0.011
total NEAA	31.1 ± 3.5 a	24.4 ± 2.1 b	24.3 ± 1.0 b	23.9 ± 2.3 b	0.017
TAA	47.6 ± 3.2 a	37.9 ± 3.4 b	42.6 ± 2.8 ab	39.7 ± 4.1 ab	0.038

[a] Data are presented as the mean ± SD ($n = 3$). Different letters within the same row indicate significant differences between different seasons in the content of the same amino acid ($P < 0.05$). NEAA = nonessential amino acids and TAA = total amino acids. [b] EAA = essential amino acids.

Mytilus galloprovincialis from Camburnu in Eastern Black Sea (190, 630, 21.0, and 4.0 mg/kg DW for Cu, Zn, Pb, and Cd, respectively) (*13*), but higher than those in *Mytilus chilensis* from Strait of Magellan (0.67, 15.7, and 0.168 mg/kg DW for Cu, Zn, and Cd, respectively) (*27*). The concentrations of Fe, Mn, Ni, and Cr obtained in this article were much lower than those observed in *Mytilus galloprovincialis* from Camburnu (3340, 59, 6.0, and 3.0 mg/kg DW for Fe, Mn, Ni, and Cr, respectively) (*13*) and *Mytilus trossulus* from the gulf of Gdansk, Baltic Sea (219−1027, 20.3−55.2, and 2.0−5.0 mg/kg DW for Fe, Mn, and Ni, respectively) (*30*), but higher than those in *Mytilus chilensis* from Strait of Magellan (63.1 and 2.8 mg/kg DW for Fe and Mn, respectively) (*27*).

Amino Acid Contents. The amino acid contents of *Mytilus coruscus* in different seasons are presented in **Table 3**. A total of 17 common amino acids, including 9 essential (EAA) and 8 nonessential amino acids (NEAA), were measured. All mussels demonstrated high contents of total amino acids (TAA), ranging from 37.9 g/100 g DW in summer to 47.6 g/100 g in spring. The highest concentration of total EAA was found in autumn (18.3 g/100 g), while the lowest concentration was observed in summer (13.5 g/100 g). Mussels in spring showed a significantly higher concentration of NEAA than those in other seasons ($P < 0.05$). Most of the amino acid contents analyzed in *Mytilus coruscus* demonstrated significant seasonal variations except threonine, alanine, tyrosine, and leucine. In the case of EAA, lysine, threonine, phenylalanine, and arginine were present in high concentrations (> 2 g/100 g), while histidine was present in low concentrations (< 1 g/100 g). Maximum contents of histidine, arginine, lysine, and phenylalanine were all observed in autumn mussels, while threonine, valine, and leucine reached their highest concentrations in spring samples. With regard to NEAA, the contents of glycine, ranging from 9.7 g/100 g in autumn to 13.4 g/100 g in spring mussels, were much higher than any other amino acids. Mussels in spring and autumn had the highest or higher levels of aspartic acid, glutamate, serine, glycine, alanine, and cysteine, while lower levels of these amino acids were all observed in summer and winter samples.

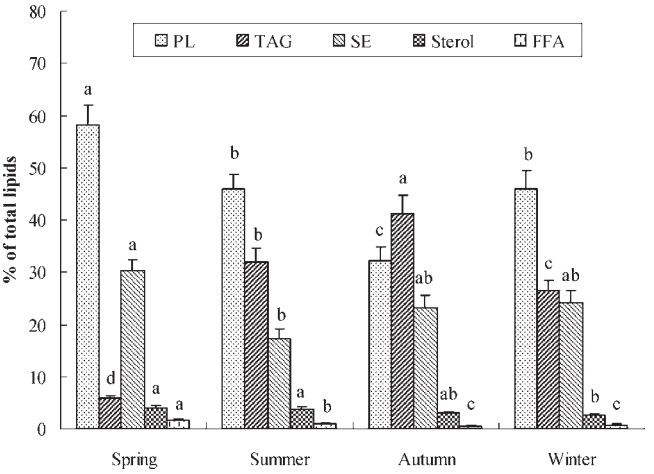

Figure 2. Seasonal variation of lipid composition in *Mytilus coruscus*. Results are presented as the mean ± SD ($n = 3$). Different letters in the bars of the same type indicate significant differences between different seasons in the composition of the same lipid class ($P < 0.05$). PL = phospholipids, TAG = triacylglycerols, SE = sterol ester, and FFA = free fatty acids.

The presence of 9 individual EAA throughout the four seasons in *Mytilus coruscus* demonstrated the good quality of mussel protein. This observation was in agreement with previous studies on other mussel species (*32*−*34*). In the present study, high contents of total EAA were found in *Mytilus coruscus*, accounting for 29.4−34.6% of the TAA. This was similar to *Perna canaliculus*, in which 33.2% of the TAA were reported as EAA (*34*). However, *Mytilus galloprovincialis* cultivated at Eceabat in Canakkale Strait was measured at a much higher relative percentage (44.44%) (*32*). Thirteen of the 17 amino acids analyzed in this study exhibited statistically significant seasonal variations ($P < 0.05$). Glycine, which was reported as the most abundant amino acid (10.4% of total amino acids) in *Perna canaliculus* from Guangdong province, China (*34*), predominated over any other amino acids in *Mytilus coruscus* in the present study. However, Konosu et al. (*33*) found arginine to be the predominant amino acid in *Mytilus* species, followed by alanine, glycin, glutamic acid, and aspartic acid. In the present investigation, a clear relationship between seasonal changes in amino acids and spawning was observed. A rise in the concentrations of some amino acids (glutamate, arginine, threonine, praline, cysteine, phenylalanine, and lysine) during spawning (spring and autumn) and a fall after spawning (summer and winter) were noticed. Such a relationship was also found by Hujita (*35*) in the shrimp *Penaeus japonicus*.

Lipid Profile. Seasonal variation of lipid profiles in *Mytilus coruscus* is reported in **Figure 2**. Phospholipids (PL, 32.1−58.1% of total lipid) were the predominant lipid class in all mussels, followed by triacylglycerols (TAG, 5.8−41.1%) and sterol esters (SE, 17.4−30.3%). Small portions of sterols (2.7−4.1%) and free fatty acids (FFA, 0.7−1.6%) were also observed. Similar lipid composition modes were observed in other mussel species, including *Mytilus galloprovincialis* (*36*), *Bathymodiolus* sp. (*37*), *Perna canaliculus*, and *Mytilus edulis* (*10*). As for the contents of individual lipid classes, they varied greatly in different mussel species due to the different food supplies, which have been considered as an important factor on the lipid composition of mussels (*38*). Compared with *Mytilus coruscus* in the present study, higher percentages of PL (73.6%) and FFA (7.3%) but lower level of TAG (13.5%) were found in Tasmanian blue mussels (*10*). *Mytilus galloprovincialis* from Northwest Spain displayed much wider ranges of individual lipid classes: PL from 40.46 to 77.67%, TAG from trace to 52.76%, and FFA from 0.14 to 4.77% (*36*).

Table 4. Seasonal Variation in Fatty Acid Composition (% of Total Fatty Acids) of *Mytilus coruscus*[a]

fatty acid	spring	summer	autumn	winter	*P*-value
12:0	2.1 ± 0.6 a	1.3 ± 0.1 ab	0.6 ± 0.2 b	0.9 ± 0.1 b	0.002
14:0	1.3 ± 0.2 b	2.7 ± 0.2 a	2.5 ± 0.2 a	2.1 ± 0.1 ab	0.004
15:0	0.7 ± 0.2 b	0.8 ± 0.0 ab	0.8 ± 0.1 ab	1.2 ± 0.2 a	0.000
16:0	14.1 ± 1.4 b	20.9 ± 1.2 a	21.5 ± 1.2 a	22.5 ± 0.5 a	0.028
17:0	2.6 ± 0.1 a	2.5 ± 0.3 a	2.3 ± 0.1 a	2.7 ± 0.2 a	0.140
18:0	6.8 ± 1.7 a	7.4 ± 0.1 a	6.5 ± 0.6 a	7.6 ± 0.6 a	0.214
23:0	1.4 ± 0.1 b	1.3 ± 0.3 b	1.0 ± 0.2 b	2.6 ± 0.7 a	0.005
total SFA	29.0 ± 3.9 b	37.0 ± 0.9 a	35.2 ± 1.2 a	39.6 ± 0.6 a	0.002
15:1	0.5 ± 0.1 a	0.4 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.2 a	0.662
16:1*n*-7	11.3 ± 2.7 a	10.6 ± 0.9 a	7.5 ± 0.9 b	6.4 ± 0.6 b	0.013
17:1	4.1 ± 0.5 a	3.1 ± 0.0 b	3.8 ± 0.5 ab	3.0 ± 0.3 b	0.015
18:1*n*-9	1.7 ± 0.5 a	0.8 ± 0.1 b	1.0 ± 0.1 b	1.3 ± 0.0 ab	0.014
18:1*n*-7	1.9 ± 0.4 b	2.8 ± 0.4 a	3.1 ± 0.1 a	2.8 ± 0.4 a	0.010
20:1	2.8 ± 0.8 b	6.2 ± 0.8 a	6.2 ± 0.9 a	5.2 ± 0.9 a	0.004
total MUFA	22.3 ± 2.6 a	23.9 ± 0.7 a	22.1 ± 1.1 a	19.1 ± 1.4 a	0.057
18:3*n*-3	1.8 ± 0.3 a	1.5 ± 0.2 ab	1.6 ± 0.2 ab	1.1 ± 0.0 b	0.035
18:4*n*-3	6.5 ± 1.2 a	3.9 ± 0.6 ab	3.3 ± 0.3 b	5.1 ± 1.4 ab	0.017
20:5*n*-3	14.6 ± 2.5 a	11.5 ± 0.2 ab	12.2 ± 1.3 ab	10.8 ± 0.6 b	0.054
22:5*n*-3	0.4 ± 0.1 b	0.6 ± 0.1 b	1.1 ± 0.2 a	0.6 ± 0.1 b	0.002
22:6*n*-3	12.4 ± 2.6 b	16.7 ± 0.2 a	18.3 ± 1.1 a	16.7 ± 0.5 a	0.006
total *n*-3 PUFA	35.8 ± 2.3 a	34.2 ± 0.8 a	36.6 ± 0.5 a	34.3 ± 1.7 a	0.216
18:2*n*-6	2.2 ± 0.1 a	1.6 ± 0.1 a	1.9 ± 0.2 a	2.2 ± 1.1 a	0.525
20:2*n*-6	1.3 ± 0.1 a	0.2 ± 0.0 c	0.2 ± 0.0 c	0.7 ± 0.2 b	0.000
20:3*n*-6	1.5 ± 0.1 a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.2 ± 0.0 b	0.001
20:4*n*-6	3.0 ± 0.8 a	1.4 ± 0.0 b	2.2 ± 0.6 ab	1.6 ± 0.2 b	0.019
22:3*n*-6	3.9 ± 0.8 a	1.2 ± 0.1 b	1.2 ± 0.1 b	1.5 ± 0.3 b	0.000
22:4*n*-6	0.8 ± 0.1 a	0.1 ± 0.0 c	0.2 ± 0.1 bc	0.4 ± 0.1 b	0.000
22:5*n*-6	0.2 ± 0.0 a	0.4 ± 0.1 a	0.4 ± 0.2 a	0.4 ± 0.1 a	0.226
total *n*-6 PUFA	13.0 ± 1.9 a	4.9 ± 0.1 c	6.1 ± 1.0 bc	7.0 ± 0.6 b	0.000
total PUFA	48.8 ± 3.5 a	39.1 ± 0.9 b	42.8 ± 0.9 b	41.3 ± 0.9 b	0.002
n-6/*n*-3	0.36 ± 0.05 a	0.14 ± 0.02 c	0.17 ± 0.03 bc	0.21 ± 0.03 b	0.000

[a] Data are presented as the mean ± SD ($n = 3$). Different letters within the same row indicate significant differences between different seasons in the composition of the same fatty acid ($P < 0.05$). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.

All lipid classes underwent statistically significant seasonal changes. Mussels in spring had the highest levels of PL, SE, FFA, and sterols and the lowest level of TAG. Minimum FFA and PL and maximum TAG levels were found in mussels in autumn. However, SE reached the lowest level in summer samples. This seasonal variation may be caused by the changes of the reproduction activities and seawater temperature in different seasons, which could influence physiological activities and metabolism of mussels (*15*, *36*, *39*). The greatest seasonal difference was observed in TAG, where the autumn mussels had a value 7.06-fold greater than that of the spring mussels. In contrast, PL in the spring mussels was only 1.81-fold greater than that in autumn mussels. This is due to the fact that TAG, presenting an energy reserve function, is easily influenced by different seasons, while PL, mainly serving a structural-type function, is maintained virtually constant over the year (*40*). During the whole study period, the seasonal changes of total lipid contents were consistent with those of TAG percentage levels. This trend can be explained by the fact that seasonal variation in lipid contents is mainly caused by fluctuations in TAG (*40*).

Fatty Acid Composition. The fatty acid composition of *Mytilus coruscus* in different seasons is presented in **Table 4**. The PUFA, ranging from 39.1% of total fatty acids in summer mussels to 48.8% in spring samples, predominated over the saturated (SFA, 29.0−39.6%) and monounsaturated (MUFA, 19.1−23.9%) fatty acids. This composition pattern agreed with the results found in other mussel species (*5*, *10*, *11*), with wider ranges of PUFA (37−66%), SFA (19−38%), and MUFA (10−32%). However, some studies concluded that the SFA were the most abundant fatty acids in mussels (*1*, *8*). Significant seasonal changes of the levels of PUFA and SFA were observed in the present study ($P < 0.05$). Mussels in spring exhibited the highest level of PUFA and the lowest level of SFA, while mussels in summer displayed the lowest level of PUFA and the highest level of SFA. Such variations can be partly attributed to the seasonal changes of phytoplankton available in seawater. Mussels are filter feeders with diets consisting largely of phytoplankton that contain a high proportion of PUFA, especially long-chain *n*-3 PUFA (*10*, *41*). In Shengsi Island coastal areas, there is a boom of phytoplankton which provides abundant PUFA resources for mussels in spring (*42*). Consequently, the highest PUFA and the lowest SFA were observed. Besides, an inverse relationship between the percentage of PUFA in marine organisms and water temperature was observed (*43*), due to membrane fluidity. Thus, mussels in spring (the water temperature is relatively low) displayed higher PUFA levels than those in summer (the water temperature is relatively high).

Palmitic acid (16:0) was the predominant SFA in *Mytilus coruscus* of all seasons, accounting for 14.1−22.5% of total fatty acids; this is agreed with the previous studies from other species (*5*, *8*, *10*, *11*, *13*). Stearic acid (18:0), another important SFA in *Mytilus coruscus*, ranged from 6.8 to 7.6%. Fuentes et al. (*1*) found a similar relative percentage of 18:0 (5−10%) in *Mytilus galloprovincialis* from Spanish origins. Other SFA, including 12:0, 14:0, 15:0, 17:0, and 23:0, were detected with relatively low levels (< 3% of total fatty acids).

In the case of MUFA, palmitoleic acid (16:1*n*-7), ranging from 6.4 to 11.3% of the total fatty acids, was the major one. Similar findings have been reported previously in other species (*1*, *5*, *8*, *11*, *13*, *36*). Though no remarkable variation was observed in total MUFA levels, most individual MUFA underwent statistically significant seasonal changes ($P < 0.05$). Maximum and minimum levels of 16:1*n*-7 and 17:1 were found in spring and

winter mussels, respectively. 20:1 and 18:1*n*-7 levels were significantly lower in spring than in the other three seasons ($P < 0.05$).

With regard to PUFA, eicosapentaenoic (EPA or 20:5*n*-3) and docosahexaenoic (DHA or 22:6*n*-3) acids were the most abundant fatty acids in *Mytilus coruscus*; this result is consistent with the results from other species such as *Mytilus galloprovincialis* (*1*, *36*), *Perna canaliculus*, and *Mytilus edulis* (*10*). Murphy et al. (*10*) and Taylor and Savage (*44*) attributed it to the diet (phytoplankton) of mussels in seawater which contains large amounts of EPA and DHA. During the whole study period, both EPA and DHA underwent significant seasonal variations. Mussels in spring demonstrated the highest content of EPA but the lowest content of DHA, while mussels in winter had the lowest level of EPA. In addition, the contents of DHA were always higher than those of EPA in mussels of all seasons except spring. This was contrary to numerous previous results from other mussel species which showed that levels of EPA were higher than those of DHA (*1*, *5*, *10*, *36*). Such results may be caused by the seasonal changes of phytoplankton organisms in the seawater. Generally, there are two kinds of phytoplankton prevailing in seawater, including diatoms and dinoflagellates, among which, diatoms contain considerable amounts of EPA, while dinoflagellates contain large quantities of DHA (*41*). In the Shengsi Island coastal areas, the predominant phytoplankton in seawater in spring are diatoms. However, in the other three seasons, the major phytoplankton are dinoflagellates (*42*). According to our study, all samples contained high levels of *n*-3 PUFA (34.2−36.6%) but low levels of *n*-6 PUFA (4.9−13.0%) and the *n*-6/*n*-3 ratio (0.14−0.36). It is reported that the intake of *n*-3 PUFA from a natural source may influence the onset and progression of several disease states, including cardiovascular disease and cancer (*45*). Ackman (*46*) concluded that the *n*-6/*n*-3 ratio was positively correlated to deaths caused by coronary illnesses.

In conclusion, *Mytilus coruscus* from Shengsi Islands displayed a high nutritional value. It is rich in minerals, amino acids, and PUFA, especially *n*-3 PUFA. However, the nutrient compositions of *Mytilus coruscus* were strongly affected by the harvest time (season). Significant seasonal variations in proximate composition, mineral, and amino acid contents, as well as lipid profile and fatty acid composition were observed in *Mytilus coruscus* in the present study. Mussels in spring and autumn had higher levels of TAA, total EAA, and total PUFA but lower levels of total SFA compared with those in other seasons. Thus, spring and autumn would be the most suitable seasons for the harvest of *Mytilus coruscus*. These results provide valuable information for both mussel producers and consumers. For mussel producers, the data obtained from the present study may be useful to indicate the periods of the year which are more suitable for harvesting and marketing. For mussel consumers, the present data can be used as guidance for the consumption of this product in the most appropriate seasons according to their own nutritional requirements. Further research is warranted to experimentally investigate the effects of some usual processing methods, such as freezing and cooking/heat processing, on the nutrient composition of *Mytilus coruscus*. This may be of interest to and useful for both mussel farmers and consumers.

ABBREVIATIONS USED

ICP-MS, inductively coupled plasma mass spectrometry; RP-HPLC, reversed-phase high-performance liquid chromatography; PITC, phenylisothiocyanate; TEA, triethylamine; PTC, phenylthiocarbamyl; GLC, gas−liquid chromatography; FID, flame ionization detector; SD, standard deviation; WW, wet weight; DW, dry weight; Mg, magnesium; Al, aluminum; Ca, calcium; Cr, chromium; Mn, manganese; Fe, ferrum; Ni, nickel; Cu, copper; Zn, zinc; Se, selenium; Pb, lead; Cd, cadmium; PL, phospholipids; TAG, triacylglycerols; SE, sterol ester; EAA, essential amino acids; NEAA, nonessential amino acids; TAA, total amino acids; FFA, free fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

ACKNOWLEDGMENT

We thank Bureau of Science and Technology of Shengsi Island for providing the mussel samples.

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Received for review April 21, 2010. Revised manuscript received June 9, 2010. Accepted June 9, 2010. This study was supported by Science and Technology Department of Zhejiang Province, China.