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Biochemical Composition and Nutritional Value of Different Shell Color Strains of Pacific Oyster *Crassostrea gigas*

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Abstract The shell color of Pacific oyster (*Crassostrea gigas*) is a desirable trait; but the nutritional studies on *C. gigas* with different shell colors have not been conducted. Through successive selective breeding, five shell color strains of black (B), purple (P), orange (O), golden (G) and white (W) *C. gigas* have been developed. The aim of this study was to evaluate the chemical composition and nutritional value of five shell color strains and one commercial population with a common color. The biochemical composition including moisture, total protein, glycogen, ash, total fat, fatty acids (FA), amino acids and minerals was detected. The results indicated that the protein (50.76%–56.57%) was the major component. The content of glycogen showed a significant difference between orange shell and golden shell strains, as well as between commercial population and golden shell strain. In addition, all shell color strains contained a large amount of essential amino acids (12.20–14.15 g $(100\,g)^{-1}$), of them leucine (2.81–3.29 g $(100\,g)^{-1}$) and lysine (2.79–3.28 g $(100\,g)^{-1}$) were predominant. The oysters were rich in polyunsaturated fatty acids (42.26%–45.24% of total fatty acid) with high levels of DHA (18.53%–21.16% of total fatty acid) and EPA (17.23%–18.68% of total fatty acid). Significant differences of mineral contents (Mg, Zn, Fe and Cu) were identified among the six populations. These results indicated that *C. gigas* with different shell colors presented rich nutritional value with high protein, glycogen, essential amino acids and polyunsaturated fatty acids. The biochemical composition obtained in this study is useful for selective breeding of *C. gigas* with different shell colors.

Key words *Crassostrea gigas*; biochemical composition; nutrition value; shell color; selective breeding

1 Introduction

Pacific oyster (*Crassostrea gigas*) is currently the most widely farmed oyster in the world. The production increased at an annual average of 7.8% over the last 30 years as was stimulated by market requirement (FAO, 2016). In view of its importance, selective breeding programs have been initiated in some countries (Dégremont *et al*., 2010; Langdon *et al*., 2003). Recently, with the improvement of selective breeding, the visual perception traits of Pacific oyster, such as shell and mantle pigmentations, have attracted more and more attentions (Brake *et al*., 2004). It has been shown that consumers are willing to pay more money for seafood with specific color, such as rich red salmon (Alfnes *et al*., 2006). Similarly, consumers' preference for Pacific oyster may also be influenced by shell color. Shell color is a high-valued trait which is known to appeal to consumer preference, and therefore, affecting product value (Kahn and Wansink, 2004). The oysters with different shell colors are rarely seen in the market and are sold at much higher prices than others (Nell, 2001). Thus, selective breeding was implemented for the shell color of *C. gigas*, and five strains characterized by black, purple, orange, golden and white shells were developed after successive five generations of selection.

The large variation in shell color of the cultured populations of *C. gigas* indicated that the shell color can be considered as a continuously distributed quantitative trait and can be stably inherited (Brake *et al*., 2004). The genetic analysis based on 133 single nucleotide polymorphism (SNP) markers demonstrated that there was significant genetic differentiations among different shell color strains, as well as between shell color strains and wild populations of *C. gigas* which shows a common color (Song *et al*., 2017). In previous studies, the relationship between shell color and phenotypic traits of *C. gigas* such as growth rate and survival has been reported (Cong *et al*., 2014; Ge *et al*., 2015). Cong *et al*. (2014) reported that the survival of the purple shell family was higher than that of the other shell color families, and the shell height and total weight were also significantly different between shell color strains and control family. So, there are concerns that whether there is any difference in biochemical composition and nutritional value among the five shell color strains of *C. gigas* after five generations of artificial selection. However, no information is currently available for the biochemical composition and nutritional value among different shell color strains of *C. gigas*.

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In present study, in order to provide useful information for selective breeding of shell color strains, biochemical composition and nutrition value of five shell color (black, purple, orange, golden and white) strains of *C. gigas* cultured in Rushan Bay, a predominant area for oyster culture, were determined and compared with a local commercial population.

2 Materials and Methods

2.1 Sample Collection

We surveyed one commercial population (C) and five shell color strains of *C. gigas*, named black (B), purple (P), orange (O), golden (G), and white (W) shell strains, respectively (Fig.1). Samples of five shell color strains of two-year-old *C. gigas* were collected in January 2016 from an oyster farm in Rushan Bay, Shandong Province, and the samples of two-year-old commercial *C. gigas* were collected from the same area synchronically. The five shell color strains (B, P, O, G, and W) were the fifth generation of successive selection (Cong *et al*., 2014) and exhibited steadily the hereditary shell color trait.

Thirty individuals each population were randomly selected for biometric measurements. The length, width and height of shells and the wet weight were measured individually (Table 1). The soft body of *C. gigas* was carefully separated from the shell, transferred into plastic storage bags, kept at −80℃ for 30 min, and then dried for 48 h using a freeze dryer (FD-1A-50, China). Dried samples were ground to fine powders using a mortar and pestle.

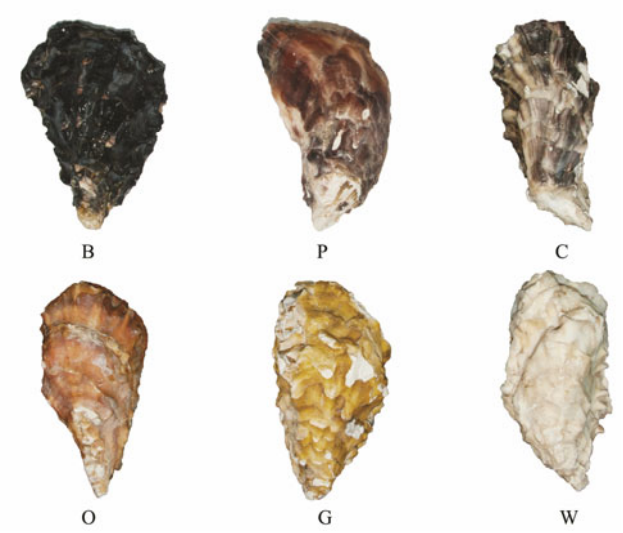

Fig.1 Six Pacific oyster populations with black (B), purple (P), orange (O), golden (G), white (W), and common (C) shell colors used in the study.

Table 1 Biometric characteristics of the different shell color and commercial population of *C. gigas*

Biometric characteristics	Black shell	Purple shell	Orange shell	Golden shell	White shell	Commercial population
Shell height (cm)	9.92±0.21	9.87±0.25	7.83±0.25	10.09±0.15	9.78±0.32	10.16±0.24
Shell length (cm)	5.77±0.71	5.79±0.51	4.90±0.34	4.98±0.86	5.04±0.09	4.99±0.34
Shell width (cm)	3.32±0.45	2.95±0.39	3.01±0.37	3.04±0.23	3.13±0.37	3.11±0.56
Wet weight (g)	77.21±12.40	80.59±10.71	60.09±1.30	76.39±10.05	82.93±2.90	76.05±10.54

Note: Mean±standard deviation (n=30).

2.2 Proximate Analysis

Moisture, protein and ash contents of the oysters were determined with the AOAC methods 950.46, 920.153 and 928.08, respectively (AOAC, 2000). The glycogen content was assayed with the method of Anthrone colorimetry (Horikoshi, 1958). Fat analysis was conducted according to the method of Folch *et al*. (1957) with some modifications. In brief, about 0.1 g (dry weight, w0) sample was added to 4 mL chloroform: methanol (C–M) (2:1, v/v) in tube A, and then tube A was settled at rest for over 24 h. Two microliters of C–M was then added to tube A, and tube A was again settled at rest for 4 h. After centrifugation at 3000×g and 4℃ for 10 min, the supernatant was transferred to tube B (constant weight, w1). After 2 μL C–M was added to the residue of tube A, tube A was settled for over 2 h and then centrifuged at 3000×g and 4℃ for 10 min. After the supernatant was transferred to tube B, 1.2 mL of 1.6% $CaCl_2$ was added to the supernatant and mixed. After tube B was settled at rest over 12 h, the upper phase was removed. The lower phase of tube B was dried under a pure nitrogen steam. Tube A was evaporated at 75℃ and reweighed (w2). Therefore, the fat content of the sample (%) is (w2−w1)/w0×100. Duplicate analyses were conducted for each sample.

2.3 Amino Acid Analysis

The amino acid analysis was conducted according to the method of GB/T 5009.124-2003 (ICS 67.040 C53). Frozen-dried oyster portions were hydrolyzed with 10 volumes of 6 mol L^{-1} HCl under reduced pressure at 110℃ for 22 h. The hydrolysates were pipetted into 10 mL volumetric flask and diluted to 10 mL using 0.02 mol L^{-1} HCl. Triethylamine solution and pheny isothiocyanate were added and then mixed. Thereafter, normal hexane was added to the mixture. An aliquot of 50 μL was detected with an amino acid analyzer (SYKAM, S-433D). Amino acid contents were expressed as g per 100 g dry sample.

2.4 Determination of Fatty Acids

For fatty acid analysis, the lipids in the samples were extracted with a 2:1 (v/v) chloroform/methanol mixture according to the method of Folch *et al*. (1957). Fatty acid methyl esters (FAMEs) were prepared by esterification using 2% sulfuric acid methanol (Gao *et al*., 2006). FAMEs were separated and quantified by means of a gas chromatography (Shimadzu GC-2010) equipped with a RTX-wax plus capillary column and flame ionization detector (GC-FID). Hydrogen was used as carrier gas (4 mL min^{-1}),

while injector and detector with temperatures of 250 and 260℃ respectively were used. The temperature program was: initial temperature 50℃, increasing up to 190℃ at 40℃ per minute and then at 2℃ per minute to 240℃ and maintained at this temperature for 2 min. Methyl nonadecanoate (19:0) was used as internal standard. Each of the specific FAME peaks was identified by the retention time with reference to the known standard (Supelco, Inc., Bellefonte, Pennsylvania, USA). The relative amount of each fatty acid in each shell color oyster was expressed as the percentage of the specific fatty acid in the sum of total fatty acids.

2.5 Determination of Minerals

Calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu) and selenium (Se) contents of frozen-dried portions were determined by the inductively coupled plasma atomic emission spectrometer (ICP-AES, Model VISTA-MPX, VARIAN, USA) according to the method of AOAC (2000). The contents were expressed as $g\,kg^{-1}$ dry sample.

2.6 Statistical Analysis

All data were subjected to a one-way ANOVA and differences between the means were tested by Tukey's multiple range test. The level of significance was set at $P<0.05$. The results are presented as mean values with their standard errors ($n=3$), and all statistical analyses were performed using SPSS 21.0 (SPSS Inc., USA).

3 Results

3.1 Proximate Composition

Proximate composition of five shell color strains and control population of *C. gigas* are presented in Table 2. Protein was accounted as the major component in all the samples (50.76%–56.57% dry weight), followed by glycogen (16.65%–22.09% dry weight) and fat (3.58%–5.15% dry weight). The protein content of golden shell strain was slightly higher than that of others. The analysis of variance revealed a significant effect of shell color on the content of glycogen. Orange shell strain showed significantly higher glycogen ($P<0.05$) but lower fat contents than golden shell strain. Between the shell color strains and commercial population, no obvious difference in the contents of moisture, protein, fat and ash was observed ($P>0.05$).

Table 2 Proximate compositions of soft tissues of five shell color and commercial population of *C. gigas* (% dry weight)

Proximate compositions	Black shell	Purple shell	Orange shell	Golden shell	White shell	Commercial population
Moisture	80.79 ± 0.87^{a}	80.80 ± 0.26^{a}	79.73 ± 0.82^{a}	80.28 ± 0.59^{a}	80.80 ± 0.23^{a}	79.92 ± 0.53^{a}
Protein	50.76 ± 2.04^{a}	52.33 ± 1.68^{a}	51.98 ± 2.96^{a}	56.57 ± 6.10^{a}	52.42 ± 1.91^{a}	52.76 ± 0.89^{a}
Fat	4.96 ± 0.52^{a}	4.80 ± 0.44^{a}	3.58 ± 1.57^{a}	5.04 ± 1.85^{a}	5.15 ± 0.68^{a}	4.95 ± 0.62^{a}
Glycogen	20.32 ± 3.66^{ab}	19.28 ± 3.27^{ab}	22.09 ± 0.59^{b}	16.65 ± 0.32^{a}	17.91 ± 2.01^{ab}	21.96 ± 1.16^{b}
Ash	13.40 ± 2.59^{a}	11.73 ± 0.30^{a}	9.71 ± 2.06^{a}	10.67 ± 4.03^{a}	12.07 ± 0.20^{a}	10.71 ± 2.39^{a}

Notes: Data are mean ± standard deviation ($n=3$). Different letters in the same row indicate significant difference ($P<0.05$).

3.2 Amino Acid Composition

Different amino acid compositions were found in different populations (Table 3). The major non-essential amino acids were glutamic acid, aspartic acid, taurine, glycine, arginine and alanine, which accounted for more than 50% of the total. Leucine (2.81–3.29 g (100 g dry weight)$^{-1}$) and lysine (2.79–3.28 g (100 g dry weight)$^{-1}$) were the predominant essential amino acids, followed by valine (1.89–2.18 g (100 g dry weight)$^{-1}$), isoleucine (1.72–2.02 g (100 g dry weight)$^{-1}$), threonine (1.65–1.85 g (100 g dry weight)$^{-1}$), phenylalanine (1.22–1.44 g (100 g dry weight)$^{-1}$) and methionine (0.04–0.10 g (100 g dry weight)$^{-1}$). The concentration of phenylalanine in the golden shell strain was significantly higher than that in the commercial population ($P<0.05$). Between the commercial population and cultured five shell color strains, there was no significant difference ($P>0.05$) in the contents of delicious amino acids, such as glycine, aspartic acid, glutamic acid and alanine. The content of taurine indicated high statistical differences ($P<0.05$) among the studied populations. The golden shell color strain was observed to have the highest quantities of total amino acids and total essential amino acids. In addition, the values of EAA/total amino acids and EAA/total non-essential amino acids were slightly higher in golden shell strain than those in the other color strains and commercial population ($P>0.05$).

3.3 Fatty Acid Profile

Palmitic acid 16:0 (24.43%–27.10%) was the most dominant saturated fatty acid (SFA) in *C. gigas* for all the studied populations, and the content of this SFA showed no obvious difference ($P>0.05$) (Table 4). Among the monounsaturated fatty acids (MUFAs), oleic acid 18:1n-9 (6.51%–7.19%) was the most dominant species, while the concentration of 18:1n-9 did not show a significant difference between the commercial population and cultured five shell color strains. The content of 16:1n-7 in commercial population (3.92%) was significantly higher than that in black shell color strain (2.64%) ($P<0.05$). The total MUFAs of *C. gigas* showed the highest value for golden shell strain and lowest value for purple shell strain ($P<0.05$). 22:6n-3 (DHA) (18.53%–21.16%) and 20:5n-3 (EPA) (17.23%–18.68%) were the most dominant polyunsaturated fatty acids (PUFAs) for all shell color strains and commercial population. The content of 18:2n-6c in golden shell strain (2.24%) was significantly higher than that in orange shell strain (1.63%) ($P<0.05$), whereas 18:3 n-3 contents in purple shell strain (0.51%) and commercial population (0.41%) was significantly higher than that in golden shell color strain (0.31%) ($P<0.05$). In addition,

concentration of 20:4 n-6 in the orange shell strain (2.89%) and commercial population (2.83%) was significantly higher than that in purple shell color strain (2.21%). As a result, the ratio of total n-3 PUFA to total n-6 PUFA in golden shell strain was significantly lower than that in black, purple and white shell color strains ($P<0.05$).

Table 3 Amino acid profiles of soft tissues of five shell color and commercial population of *C. gigas*

Amino acid	Black shell	Purple shell	Orange shell	Golden shell	White shell	Commercial population
Aspartic**	4.63±0.22	4.53±0.23	4.41±0.46	5.00±0.13	4.30±0.34	4.42±0.23
Threonine*	1.71±0.04	1.71±0.07	1.69±0.14	1.85±0.06	1.65±0.15	1.68±0.08
Serine	1.87±0.03	1.87±0.07	1.93±0.12	2.02±0.06	1.84±0.14	1.87±0.12
Glutamic acid**	6.57±0.17	6.75±0.34	6.60±0.67	7.41±0.10	6.47±0.53	6.99±0.39
Glycine**	3.03±0.12	3.21±0.22	3.36±0.29	2.97±0.19	3.04±0.30	3.01±0.09
Alanine**	2.39±0.01	2.47±0.10	2.49±0.19	2.71±0.09	2.36±0.20	2.48±0.16
Cysteine	0.17±0.05	0.17±0.05	0.12±0.05	0.09±0.01	0.17±0.02	0.12±0.04
Valine*	1.99±0.04	1.96±0.09	1.99±0.18	2.18±0.10	1.89±0.18	1.92±0.08
Methionine*	0.04±0.01	0.08±0.01	0.10±0.02	0.09±0.05	0.08±0.01	0.06±0.03
Isoleucine*	1.82±0.06	1.79±0.09	1.84±0.19	2.02±0.10	1.72±0.17	1.79±0.08
Leucine*	2.94±0.09	2.97±0.18	2.95±0.27	3.29±0.15	2.81±0.28	2.97±0.16
Tyrosine	1.19±0.03	1.18±0.05	1.18±0.08	1.30±0.05	1.13±0.12	1.14±0.04
Phenylalanine*	1.33 ± 0.04^{ab}	1.34 ± 0.06^{ab}	1.35 ± 0.11^{ab}	1.44 ± 0.06^{b}	1.27 ± 0.12^{ab}	1.22 ± 0.03^{a}
Histidine	1.34±0.02	1.31±0.05	1.34±0.05	1.42±0.02	1.25±0.13	1.27±0.09
Lysine*	2.94±0.09	2.93±0.16	2.97±0.27	3.28±0.12	2.79±0.27	3.01±0.19
Arginine	2.80±0.06	2.81±0.21	2.87±0.25	3.06±0.12	2.59±0.25	2.77±0.20
Proline	1.83±0.06	1.72±0.12	1.83±0.08	1.95±0.07	1.78±0.16	1.76±0.09
Taurine	4.28 ± 0.03^{b}	4.08 ± 1.10^{ab}	4.47 ± 0.09^{bc}	4.87 ± 0.20^{c}	3.74 ± 0.27^{a}	4.45 ± 0.30^{bc}
TAA	42.85±0.98	42.88±1.65	43.67±3.29	46.95±1.23	40.85±3.55	42.97±1.95
TEAA	12.77±0.34	12.78±0.64	12.90±1.17	14.15±0.53	12.20±1.16	12.64±0.56
E/T (%)	29.80±0.15	29.79±0.42	29.51±0.54	30.13±0.34	29.86±0.25	29.43±0.05
E/N (%)	42.43±0.70	42.44±0.85	42.10±0.82	43.13±0.32	42.54±0.51	41.75±0.18

Notes: Data are mean ± standard deviation ($n=3$, unit: (g (100 g dry weight)$^{-1}$)). Different letters in the same row indicate significant difference ($P<0.05$). * Essential amino acids. ** Delicious amino acids. TAA, total amino acids; EAA, total essential amino acids. E/T means the ratio of EAA and TAA; E/N means the ratio of EAA and nonessential amino acid.

Table 4 Fatty acid profiles of soft tissues of five shell color and commercial population of *C. gigas* (% dry matter basis)

Fatty acid	Black shell	Purple shell	Orange shell	Golden shell	White shell	Commercial population
C14:0	3.02±0.31	3.22±0.14	2.73±0.89	2.79±0.48	3.26±0.45	2.87±0.93
C15:0	0.90±0.11	0.77±0.21	0.96±0.04	0.83±0.24	0.85±0.12	0.77±0.12
C16:0	24.61±2.14	25.51±1.62	24.43±4.36	25.42±3.24	25.40±1.55	27.10±2.01
C17:0	2.21±0.09	2.24±0.16	2.23±0.13	2.24±0.41	2.06±0.36	1.99±0.04
C18:0	5.30±1.61	5.49±0.02	6.23±1.70	5.59±0.84	4.96±1.26	5.75±1.10
C20:0	2.80 ± 0.46^{ab}	2.22 ± 0.21^{a}	3.85 ± 1.14^{b}	2.48 ± 0.40^{ab}	2.25 ± 0.30^{a}	2.93 ± 0.45^{ab}
C23:0	0.92 ± 0.10^{ab}	0.77 ± 0.11^{a}	0.67 ± 0.06^{a}	1.14 ± 0.16^{b}	0.88 ± 0.05^{ab}	0.75 ± 0.08^{a}
Total saturated	39.76±2.02	40.23±1.93	41.10±3.80	40.50±4.50	39.68±1.54	42.17±2.08
C15:1	1.42±0.20	1.02±0.04	1.26±0.33	1.33±0.21	1.07±0.09	0.97±0.16
C16:1 n-7	2.64 ± 0.30^{a}	3.33 ± 0.17^{ab}	3.60 ± 0.44^{ab}	3.55 ± 0.14^{ab}	3.71 ± 0.32^{ab}	3.92 ± 0.89^{b}
C17:1 n-7	0.36±0.01	0.38±0.00	0.38±0.03	0.34±0.02	0.35±0.02	0.33±0.09
C18:1 n-9t&C18:1 n-9c	6.86±0.41	6.57±0.07	7.19±0.85	7.12±0.37	6.51±0.18	6.75±0.23
C20:1 n-9	4.36±0.83	3.23±0.19	3.39±0.33	4.30±0.53	3.79±0.57	3.59±0.44
Total monounsaturated	15.64 ± 0.86^{ab}	14.53 ± 0.45^{a}	15.82 ± 0.59^{ab}	16.64 ± 0.74^{b}	15.43 ± 0.32^{ab}	15.57 ± 0.84^{ab}
C18:2 n-6c	1.83 ± 0.03^{ab}	1.89 ± 0.20^{ab}	1.63 ± 0.06^{a}	2.24 ± 0.38^{b}	2.00 ± 0.18^{ab}	1.81 ± 0.07^{ab}
C18:2 n-6t	0.44±0.05	0.56±0.19	0.47±0.10	0.44±0.25	0.48±0.09	0.55±0.07
C18:3 n-3	0.37 ± 0.02^{ab}	0.51 ± 0.01^{c}	0.40 ± 0.05^{ab}	0.31 ± 0.02^{a}	0.40 ± 0.06^{ab}	0.41 ± 0.01^{b}
C20:2 n-6	0.33±0.09	0.31±0.01	0.34±0.10	0.37±0.01	0.33±0.01	0.35±0.09
C20:4 n-6	2.77 ± 0.12^{ab}	2.21 ± 0.13^{a}	2.89 ± 0.25^{b}	2.77 ± 0.26^{ab}	2.55 ± 0.16^{ab}	2.83 ± 0.26^{b}
C20:5n-3 (EPA)	17.77±0.44	18.68±0.68	17.23±1.29	17.90±1.65	17.97±0.69	17.76±1.26
C22:6n-3 (DHA)	21.08±0.59	21.08±0.64	20.13±1.68	18.81±1.52	21.16±0.72	18.53±1.50
Total polyunsaturated	44.60±1.17	45.24±2.02	43.08±3.55	42.85±4.13	44.90±1.82	42.26±3.09
n-3	39.22±1.01	40.27±1.31	37.75±2.96	37.02±3.17	39.54±1.42	36.71±2.73
n-6	5.04±0.17	4.96±0.72	5.17±0.63	5.83±0.96	5.13±0.41	5.55±0.36
Ratio n-3/n-6	7.78 ± 0.22^{bc}	8.11 ± 0.24^{c}	7.30 ± 0.63^{abc}	6.35 ± 0.50^{a}	7.71 ± 0.30^{bc}	6.61 ± 0.50^{ab}

Notes: Data are mean ± standard deviation ($n=3$). Different letters in the same row indicate significant difference ($P<0.05$).

3.4 Mineral Content

Based on dry weight, Ca (3.94–5.16 g kg^{-1}) was the most abundant macro-minerals in *C. gigas*, followed by Mg (2.92–3.71 g kg^{-1}) (Table 5). In general, the black shell and golden shell strains showed higher content of

Mg than the orange shell strain and control population ($P<0.05$), whereas the content of Ca showed no significant difference between the shell color strains and commercial population ($P>0.05$). When considering the micro-minerals, Fe content in the black shell strain (1.31 g kg^{-1}) was significantly higher than those in the orange shell strain (0.56 g kg^{-1}) and control population (0.58 g kg^{-1}) ($P<0.05$). The observed Zn concentration ranged from 0.82 to 1.64 g kg^{-1}, and the highest Zn content was found in orange shell color strain ($P<0.05$). In addition, the concentration of Cu were significantly higher in the orange shell (0.34 g kg^{-1}), purple shell (0.27 g kg^{-1}) and black shell color (0.26 g kg^{-1}) strains than in the commercial population (0.22 g kg^{-1}) ($P<0.05$). Between the cultured five shell color strains and the commercial population, no obvious difference in the content of Se was observed ($P>0.05$).

Table 5 Mineral contents of soft tissues of five shell color and commercial population of *C. gigas* (dry weight)

Minerals	Black shell	Purple shell	Orange shell	Golden shell	White shell	Commercial population
Ca (g kg^{-1})	4.45±0.59^{a}	4.11±1.35^{a}	3.94±0.63^{a}	5.15±0.58^{a}	4.36±1.36^{a}	5.16±2.63^{a}
Mg (g kg^{-1})	3.71±0.12^{b}	3.44±0.39^{ab}	2.93±0.02^{a}	3.57±0.20^{b}	3.39±0.13^{ab}	2.92±0.03^{a}
Zn (g kg^{-1})	1.24±0.06^{ab}	1.36±0.03^{bc}	1.64±0.11^{c}	1.20±0.06^{ab}	1.33±0.37^{bc}	0.82±0.11^{a}
Fe (g kg^{-1})	1.31±0.30^{b}	0.88±0.14^{ab}	0.56±0.04^{a}	0.97±0.58^{ab}	0.88±0.20^{ab}	0.58±0.05^{a}
Cu (g kg^{-1})	0.26±0.01^{bc}	0.27±0.01^{c}	0.34±0.00^{d}	0.23±0.02^{ab}	0.24±0.03^{abc}	0.22±0.02^{a}
Se (mg kg^{-1})	11.05±1.6^{1a}	11.18±2.72^{a}	9.16±0.94^{a}	9.74±0.93^{a}	10.97±5.03^{a}	8.97±2.81^{a}

Notes: Data are mean±standard deviation ($n=3$). Different letters in the same row indicate significant difference ($P<0.05$).

4 Discussion

In general, high protein content and low fat content have become symbolic of the ideal food (Hao *et al*., 2015). In this study, the results showed that *C. gigas* was rich in protein and low in fat content, and with a high content of glycogen, which may be reflected in the quality and taste of oysters (Oliveira *et al*., 2006). In similar studies, Karnjanapratum *et al*. (2013) reported that Asian hard clam *Meretrix lusoria* contained about 53.82% protein and 14.96% carbohydrate. From the results of this study, the content of glycogen in *C. gigas* was higher than that in Asian hard clam; however, the protein content was similar to that of Asian hard clam. The contents in proximate compositions of oysters agreed with previous findings reported for glycogen (Wang *et al*., 2015; Li *et al*., 2006), protein (Wang *et al*., 2015) and fat (Li *et al*., 2006). In addition, the data analyses revealed distinct differences in lipid and glycogen contents between the orange shell strain and golden shell strain. The orange shell strain exhibited a significantly higher glycogen content as compared to the golden shell strain, while fat dropped drastically. This may be explained by the conversion of energy (Pogoda *et al*., 2013), as the glycogen reserve may be used in the synthesis of lipids (De la Parra *et al*., 2005). The simultaneous glycogen decrease and fat increase may indicate the conversion of carbohydrates into lipids during ontogenesis ((De la Parra *et al*., 2005; Robinson, 1992). The variations in proximate compositions of marine seafood are closely related to biological factors, including species, diet, harvest area, catching season, seasonal and sexual variations (Karnjanapratum *et al*., 2013). Moreover, the differences in compositions between the studied populations may relate closely to the growth speed of oysters (Chi *et al*., 2007). The results revealed that *C. gigas* harvested from Rushan Bay can be a rich source of nutrients, including protein, fat and glycogen.

The most abundant amino acid in five shell color strains and commercial population was glutamic acid, and this observation is in line with the finding reported by Karnjanapratum *et al*. (2013) for Asian hard clam. In *Mytilus galloprovincialis*, Babarro *et al*. (2011) reported the soft tissue consisted of 3.09% glutamic acid and 1.01% aspartic. However, in this study, *C. gigas* showed much higher compositions of glutamic acid (6.47%–7.41%) and aspartic (4.3%–5%) than *M. galloprovincialis*. From these results, the taste of oysters could be stronger than the mussels cultured on a raft system. Several recent studies reported that some amino acids especially histidine, proline, valine, methionine, cysteine, tyrosine, and phenylalanine play significant roles in the activities of antioxidative peptides (Bougatef *et al*., 2010; Samaranayaka and Li-chan, 2011). These seven amino acids accounted for approximately 18% of the total amino acids detected in this study, which suggests that there is strong antioxidant activity in these oysters. In general, taurine is found in breast milk, suggesting that it is particularly important at this stage. However, the capacity of endogenous synthesis of taurine is limited in humans (particularly in infants), therefore, the majority of body taurine usually are from food sources especially seafood and meat (El Zahraa *et al*., 2012). Additionally, some previous studies have indicated that taurine can decrease blood lipids (Pandya *et al*., 2010). Li *et al*. (2015) demonstrated that the content of taurine was 1.51 g (100 g)$^{-1}$ and 1.21 g (100 g)$^{-1}$ in *Ruditapes philippinarum* and *Mactra veneriformis,* respectively, from the east coast of Jiangsu Province. The taurine concentration in soft tissue of the two species was less than half of that in *C. gigas* studied here. Therefore, *C. gigas* is a good source of taurine, especially the golden shell color strain.

Content and composition of total amino acids of *C. gigas* investigated in this study are somewhat in agreement with the values (42.8%–50.6%) provided for *M. lusoria* harvested from the coast of Andaman Sea (Karnjanapratum *et al*., 2013). Difference in the content of total amino acids indicated the different protein nutritional value between the shell color strains and commercial population. Based on the amino acid profiles, TEAA content was higher in the golden shell color strain, indicating that its protein fraction was of higher nutritional value than that

of the other strains, especially the white shell color strain. The content of amino acids satisfy the suggested profile of essential amino acid requirements for adult humans, however, the mechanism of different shell color effects on amino acid content need to be elucidated in future studies.

Similar to our FAMEs results, Pogoda *et al*. (2013) reported palmitic (23.5%), EPA (16.4%), DHA (21.3%) were major fatty acids found in *C. gigas* harvested in October from the test site of Nordergründe. Dridi *et al*. (2007) reported the fatty acid profile of *C. gigas* from the Bizert lagoon in winter, and found the three major fatty acids were DHA (20.4%), EPA (12.15%), and palmitic acid (19.77%). One of the most striking differences among the results of Pogoda (2013), Dridi (2007) and ours is in the level of EPA. This difference is likely a result of the environmental temperature in which the oysters resided prior to harvest. The PUFA levels are high when temperature is low, while the PUFA levels are low when temperature is high (Dridi *et al*., 2007). Moreover, the polyunsaturated fatty acid 20:5n-3 and 22:6n-3 are very important and conservative elements of bio-membranes. The amount of essential FA may serve as an indicator for the preferred diet (Dalsgaard *et al*., 2003; Soudant *et al*., 1999). For example, high levels of n-3 PUFA (37.02%–40.27% of total fatty acids) is important in the human diet for platelet anti-aggregating and blood pressure-reducing properties (Orban *et al*., 2006; Karnjanapratum *et al*., 2013). However, Karnjanapratum *et al*. (2013) reported the levels of (n-3) PUFA was 28.70% in *M. meretrix* in the viscera, and this value was much lower than that in *C. gigas*. The relatively high n-3/n-6 PUFA ratio indicated the high proportion of essential n-3 fatty acids. These aspects contribute to a positive evaluation of the lipid quality of *C. gigas* from the Rushan Bay. In consideration of the high lipid quality, an increment of the consumption of *C. gigas* is recommended by the current dietary guidelines (Simopoulos, 2003).

Like other bivalve mollusks, *C. gigas* may be considered as a good source of nutritionally important minerals. In addition, as a filter-feeder, *C. gigas* can enrich the dissolved trace elements from the diet and aquatic environment. Therefore, they are well established as indicators to evaluate the marine pollutions (Neuberger-Cywiak *et al*., 2003; Özden *et al*., 2009). The essential minerals, such as Ca, Mg, Zn, Fe, Cu, and Se play a key role in keeping good condition of physiological fluid (Orban *et al*., 2004). Ca is responsible for the bone development and maintenance, as well as blood clotting and heartbeat regulation. Mg is a co-enzyme and essential to many biochemical reactions in the tissues (Karnjanapratum *et al*., 2013). Ca concentration in analyzed oyster samples was in a range from 3.94 to 5.16 $g\,kg^{-1}$, while Mg concentration ranges between 2.92 and 3.71 $g\,kg^{-1}$ in the samples. Dietary Reference Intakes (RDI) of Ca and Mg are 1000 and 400 mg every day, respectively, maximizing over sex and age groups (FDA, 2012). Oliveira *et al*. (2011) studied the mineral composition of geoduck clams harvested in Southest Alaska, in which S, K and P were the most abundant minerals in both mantle and siphon tissues, whereas Ca and Mg were only found at low concentrations. Fe is an essential mineral and RDA of Fe is 7–27 $mg\,d^{-1}$ (Institute of Medicine, 2001). *C. gigas* in the present study possessed a higher Fe content (0.56–1.31 $g\,kg^{-1}$) than hard clam (0.70 $g\,kg^{-1}$) and *Chamelea gallina* (0.11 $g\,kg^{-1}$). The Cu level of *C. gigas* showed a similarity with the previous study of Futagawa *et al*. (2011), while a higher concentration than *C. gallina* (Usero *et al*., 2005). Zn was detected in almost all of the samples with a concentration ranging from 0.82 to 1.64 $g\,kg^{-1}$. Zn is necessary for normal growth and development, and arginal Zn deficiency among children would produce serious problems, such as a retarded growth, an increase in infectious diseases, and impaired cognitive function (Rosado, 1998). Cu, as a catalytic cofactor, plays a pivotal role in cell physiology in the redox chemistry of enzymes for proteins that conduct fundamental biological functions required for growth and development (Camara, 2005). Se is an essential trace element for humans and plays a vital role as an antioxidant in proper organ function and development (Kubachka *et al*., 2017). The Se content varied between 8.97 $mg\,kg^{-1}$ and 11.18 $mg\,kg^{-1}$ in oyster samples. However, the level of Se is 0.60 $mg\,kg^{-1}$ in *C. gallina* collected from the cen- tral Adriatic sea (Orban *et al*., 2006), and is 0.29 $mg\,kg^{-1}$ in *Mizuhopecten yessoensis* cultured around Zhangzi Island (Hao *et al*., 2015).

The variation in mineral composition of oysters could be attributed to species, season, the location and characteristics (Özden *et al*., 2009; Futagawa *et al*., 2011). In this study, the difference of most of the mineral contents in the shell color strains and commercial population varied notably depending on the shell color of *C. gigas*. In general, shell color is due to the presence of biological pigments, which are produced by the mantle and incorporated into the shell along the growing edge. Tetrapyrrole is one of the main shell pigments in Mollusca. They exist as cyclic structures known as porphyrins, or linear structures known as bile pigments. Some evidences showed that the colors of tetrapyrrole pigments may differ when they are associated with metal ions (McGraw, 2006). The colorful oyster pearls derive some of their color from both free porphyrins and porphyrins complexed with metal ions (Williams, 2016). Additionally, Zou *et al*. (2015) reported that the difference of mineral contents in prismatic layer and nacreous layer of shells from four shell color strains in pearl oyster (*Pinctada fucata*) resulted in the color difference in different individuals. Thus we postulate that the oysters may present different shell color when porphyrins are combined with different contents of metal elements. It is obvious that the oyster shell color was closely associated with the content and type of metal ions.

5 Conclusions

In conclusion, the contents of glycogen, amino acid, fatty acid and minerals differed significantly between different shell color strains, as well as between shell color strains and commercial population. These results will

provide useful information on selective breeding of different shell pigmentation and comprehensive utilization of *C. gigas.*

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