Seasonal variations of nutritional components in cockles (*Tegillarca granosa*) processed from the Southern Coast of Korea

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Thanh Tri Nguyen1,2, Yong-Jun Choi1, Zuliyati Rohmah3, Seok-Bong Jeong4, Doo-Jin Hwang5, Yong-Gil Jung6 and Byeong-Dae Choi1*

Abstract: Cockles (Tegillarca granosa) species that found throughout the Indo-Pacific region and this study was conducted in South Korea from late autumn (November 2015) through early spring (April 2016). The proximate composition, mineral content, free amino acid, and fatty acid (FA) profiles and the nutritional quality of the cockles were studied at different stages during the processing. The contents of protein (11.7–13.9/100 g), lipids (1.1–2.5/100 g) and ash (1.6–2.7/100 g) varied significantly (\( p < 0.05 \)) based on seasonal variations and processing steps. Taurine, glutamic acid, lysine and arginine were the most abundant amino acids in the amino acid profiles. All samples contained limited concentrations of Cr, Pb, Cd, As, and Co. The FA profiles showed that n-3 polyunsaturated FA (PUFA) were the major fatty acids (28.7–37.0% of total FA, which was predominantly DHA and EPA (7.9–17.4%). Saturated FA (SFA) and monounsaturated FA (MUFA) levels were also observed throughout the experiment, as well as n−3/n−6 and PUFA/SFA ratios. The data obtained from this study may be useful to indicate the periods of the harvest season more suitable for consumption and the importance of processing chain on quality of cockle.

ABOUT THE AUTHOR
Byeong-Dae Choi is a seafood chemist. He completed his MSc degree in seafood chemistry from Pukyung National University. Thanh-Tri Nguyen is a food chemist; he completed his MSc degree in food chemistry from Montpellier II University, Yong-Jun Choi is a seafood chemist from Gyeongsang National University, Zuluyati Rohmah is a fish biologist from Gadjah Mada University, Seok-Bong Jeong is a food engineer from Gyeongsang National University, Doo-Jin Hwang is a marine engineer from Hokkaido National University and Yong-Gil Jung is a mechanical engineer from Pukyung National University. At present, they are preceding government project. They have carried out extensive research work on shellfish processing, food quality control and related mechanical engineering aspects.

PUBLIC INTEREST STATEMENT
The cockle is marine bivalve molluscs, is widely distributed throughout the Indo-Pacific region. In South Korea, cockles are called “kkomak”, harvested from lately autumn to early spring along the western and southern shores of the peninsula, though the Yeojwa bay, which is surrounded by Goheung and Yeosu peninsula due to its environmental conditions. Due to their taste and high nutritive value such as hemoglobin, iron and variety of nutrients, cockles have been used as food and traditional medicine and received attention for the treatment of anemia and inflammation. The aim of this work was to study the nutritional quality of cockles harvested in different periods and at different steps of the processing chain (harvest, washing, sorting, and marketing). The data obtained demonstrates that both seasonal variation as steps of processing may interfere with the quality of cockle, useful to indicate the periods of the harvest season more suitable for consumption.
1. Introduction

Cockles (*Tegillarca granosa*) are very popular edible shellfish found in Korea and available as fresh, frozen, or processed in the market. The reproductive cycle of *T. granosa* can be divided into five successive stages: early stage (March to May), late active stage (April to June), ripe stage (May to July), spent stage (July to August) and recovery and resting stage (September to March) (Kim, Moon, Shin, & Park, 2009) (Figure 1), so they are harvested from late autumn in November to early spring in April along the western and southern shores of the peninsula. Besides their culinary value, the products of these marine bivalve molluscs are regarded to have nutraceutical value due to their proteins, lipids, carbohydrates and other components that are beneficial to human health (antioxidants, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), etc.) (Grienke, Silke, & Tasdemir, 2014). Proteins are generally the most abundant component of the meat, followed by carbohydrates (Okumuş & Stirling, 1998). Although mussels and cockles have a rather low lipid content, they are known to contain a wide variety of structural fatty acids (FAs), including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAbs), and polyunsaturated fatty acids (PUFAs) (Martínez-Pita, Sánchez-Lazo, Ruiz-Jarabo, Herrera, & Mancera, 2012). The most well-known FAs to have high nutritional value are EPA (C20:5n-3) and DHA (C22:6n-3); these are considered as fatty acids that are known to benefit human health (Taylor & Savage, 2006). The yields and the biochemical composition of the cockle meat determine its organoleptic characteristics and hence its commercial value. The quality of seafood products from cockles is not only influenced by parameters such as water temperature, food intake, and geography but also by the different processing steps from harvest to markets (Almonacid, Bustamante, Simpson, & Pinto, 2014; Irisarri, Fernández-Reiriz, & Labarta, 2015).

The nutritional value of cockles in Korea, it is a great relevance to identify their biochemical compositions to determine the most favourable season for harvesting as well as their nutraceutical value during each step of the processing chain. The aim of this work was to study the nutritional quality of cockles harvested in different seasons and at different steps of the processing chain (harvest, washing, sorting, and marketing). This study would also be useful to assess the prospects of cockles as a key ingredient for the development of functional foods or for pharmaceutical uses.
2. Materials and methods

2.1. Materials
Cockles (T. granosa) were obtained from Yeosu, Korea (Figure 2). The samples (35–40 mm length, 25–30 mm width, and 25–30 mm height) were gathered at every step of the processing chain (harvesting, washing and sorting, and marketing) (three each weighing about 800 g to 1.0 kg) on harvest season from late autumn to early spring in Korea (Figure 3); three periods were chosen: November 2015, January 2016, and April 2016. The cockle samples were recorded and labelled and then immediately transported to the Nutrition Chemistry laboratory, Gyeongsang National University. The cockles were manually shucked. The muscles were then divided into two portions, with one part of the samples (three each weighing about 100–200 g) ground for moisture, lipid, amino acid, mineral and ash analysis. The remaining muscle (three each weighing about 50–100 g) was then freeze-dried (Vacuum freeze drier, SFDSF06, Samwon Freezing Engineering Co., Seoul, Korea), milled and kept in moisture-free cabinets until further analysis.

2.2. Chemical and reagents
All standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). In the case of the fatty acid methyl ester (FAME) standards, FAMEs from Menhaden fish oil (47116-analytical standard) was obtained from Supelco (Bellfonte, PA, USA). All solvents and reagents were of analytical or HPLC grade as required.

2.3. Proximate composition analysis
Moisture, total lipid, total protein, and ash analysis were performed to determine the cockle proximate compositions. The moisture content was determined using a halogen moisture analyser (WBA-110 M, DAIHAN Scientific Co., Ltd, Wonju, South Korea). The protein (N × 6.25) and ash contents of the cockles were determined according to AOAC methods No. 955.04 and 938.08 (Horwitz, 2000), respectively. The total lipid content was extracted by the Bligh and Dyer (1959) method. The total carbohydrate content was determined with modified phenol–sulphuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).
2.3.1. Free amino acid analysis
The amino acid profiles were determined according to Ozols (1990) with some modifications. Briefly, cockle muscles were hydrolysed with 6 N HCl at 110°C for 24 h, and the hydrolysates were then filtered, concentrated at 40°C and filtered through a 0.4 μm membrane. The filtrate was then diluted with deionized water up to 10 mL. The amino acid composition was analysed with an amino acid analyser (Biochrom 20 Plus, Harvard Bioscience Co., Cambridge, UK).

2.3.2. Mineral and heavy metal composition analysis
To determine the cockle mineral and heavy metal compositions, AOAC method No. 968.08 (Horwitz, 2000) was employed. Cockle samples (0.5 ± 0.0 g) were added to 5 mL of concentrated HNO₃. The mixture was heated at 120–130°C for 16 h, treated with H₂O₂ and then diluted with deionized water up to 50 mL. An aliquot of the sample was diluted 1:1 with deionized water. The analysis was done using an inductively coupled plasma spectrophotometer (Atomscan 25, Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4. Fatty acid analysis and atherogenic, thrombogenic, and hypocholesterolemic/hypercholesterolemic index calculation
Fatty acids analysis was done by converting fatty acids to fatty acid methyl esters (FAMEs), which were then separated by gas chromatography according to AOCS-Ce 1b-89 method (AOCS, 1998). In this study, analyses of FAMEs were performed by gas chromatography (Perkin-Elmer, Buckinghamshire, England) with the following temperature program: The initial oven temperature was set at 180°C and held there for 8 min. It was then raised to 230°C at a rate of 3°C/min and held for 15 min. Fatty acids were identified by comparing sample peak retention times to those of authentic standards. Relative quantities were expressed as weight percent of fatty acid to total fatty acids in each sample. The ratios of DHA/EPA, PUFA/SFA, and omega-3 to omega-6 fatty acids were calculated.

The indices of atherogenicity (IA) and thrombogenicity (IT) are important values for estimating the risk of food contributing to coronary heart disease development. The indices were calculated based on the sample’s fatty acids content (Ulbricht & Southgate, 1991) as follows:

$$IA = \frac{12:0 + (4 \times 14:0) + 16:0}{\Sigma MUFA + \Sigma PUFA (n - 6) + (n - 3)}$$

$$IT = \frac{(14:0 + 16:0 + 18:0)/(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma PUFA (n - 6)) + (3 \times \Sigma PUFA (n - 3)) + (n - 3)}{(n - 6)}.$$  

The hypocholesterolemic/hypercholesterolemic index of the sample was evaluated according to the Santos-Silva, Bessa, and Santos-Silva (2002):
3. Results and discussion

3.1. Proximate composition

The cockle proximate compositions resulted for various seasons and production steps are shown in Table 1. There were no differences in cockle moisture contents from different steps of production. On the other hand, seasonal variation influenced the moisture content significantly ($p < 0.05$). In this study, the protein content ranged from 11.7 to 13.9/100 g, with the highest content belonging to a sample collected in November (HC-11), whereas the lowest (11.7%) belonged to cockles marketed in January (MC-1). The total lipids varies among all the samples. The highest lipid content was found in a sample gathered in April (HC-4, 2.5 ± 0.1/100 g; MC-4, 2.3 ± 0.2/100 g). This was significantly higher ($p < 0.05$) than those from November (HC-11, WC-11, MC-11) and January (HC-1, WC-1, MC-1). Meanwhile, ash contents ranged from 1.6/100 g in MC-11 to 2.7/100 g in HC-1. The results of this study are in agreement with those reported by Karnjanapratum, Benjakul, Kishimura, and Tsai (2013), which stated that Asian hard clams ($Meretrix lusoria$) harvested from the coast of the Andaman Sea had moisture 76.23–84.22%, protein 9.09–12.75%, carbohydrate 0.32–7.89%, fat 1.58–6.58% and ash 1.23–2.58%. The results are also shown that protein and lipid content of cockle samples are higher than the striped venus clam ($Chamelea gallina$) from the Adriatic Sea which content protein 8.55–10.75/100 g, total lipid 0.73–1.59/100 g (Orban et al., 2006) and mussels ($Mytilus galloprovincialis Lmk.$) which had protein, lipid content ranged from 6.5–10.0/100 g, 1.4–2.1/100 g respectively (Fuentes, Fernández-Segovia, Escriche, & Serra, 2009).

The results show that cockles could be a rich source of nutrients, such as proteins, fats and carbohydrates. Moisture, protein, lipid and ash contents will vary depending on the season, the phase of energy accumulation and utilisation cycle, and interactions between food availability and environmental conditions (Fernández et al., 2015). Moreover, phytoplankton growth parameters such as the quantitative sunlight or temperature of the sea surface would change the rate of biomass production as the seasons changed, in turn affecting the diet of cockles and mussels (O’Boyle & Silke, 2010). In addition, Yurimoto, Kassim, and Man (2014) suggested that seasonal variations food availability are one of the major factors defining the spawning time of blood cockles in the Matang mangrove estuary. They further added that highest spawning period of this cockle is from November to February, which parallels the peak season of phytoplankton availability in that water. Marine bivalves store their carbohydrate in the form of glycogen. The highest glycogen content in the muscle would be in line with the spawning and phytoplankton boom periods (Fernández et al., 2015).

\[
\frac{h}{H} = \frac{(18:1n - 9 + 18:2n - 6 + 20:4n - 6 + 18:3n - 3 + 20:5n - 3 + 22:6n - 3)}{(14:0 + 16:0)}. 
\]

Table 1. Proximate composition of seasonal variation in cockles ($T. gransosa$) treated (g/100 g wet weight)

| Sample (n=3) | Moisture | Crude protein | Carbohydrates | Lipids | Ash |
|-------------|----------|---------------|---------------|--------|-----|
| HC-11       | 75.6 ± 0.0c | 13.9 ± 0.0ac | 6.5 ± 0.3a    | 1.6 ± 0.3ac | 1.9 ± 0.0bc |
| WC-11       | 76.8 ± 0.6c | 13.5 ± 0.0a  | 5.9 ± 0.4a    | 1.5 ± 0.1bc  | 2.1 ± 0.2ab |
| MC-11       | 76.3 ± 1.8c | 12.6 ± 0.1bc | 6.3 ± 0.4a    | 1.8 ± 0.1bc  | 1.6 ± 0.2cc |
| HC-1        | 79.4 ± 0.5ac | 12.8 ± 0.0a  | 3.6 ± 0.7ac   | 1.1 ± 0.1ac  | 2.7 ± 0.5ac |
| WC-1        | 80.4 ± 0.9a  | 12.5 ± 0.0ac | 3.1 ± 0.6a    | 1.4 ± 0.1ac  | 2.4 ± 0.3ac |
| MC-1        | 80.9 ± 0.5a  | 11.7 ± 0.0ac | 3.5 ± 0.4ac   | 1.5 ± 0.1ac  | 2.1 ± 0.0ac |
| HC-4        | 78.1 ± 0.1a  | 12.0 ± 0.0ac | 4.5 ± 0.6a    | 2.5 ± 0.1ac  | 2.5 ± 0.2ac |
| WC-4        | 79.4 ± 0.1ac | 12.5 ± 0.1ac | 4.1 ± 0.5ac   | 1.6 ± 0.2ac  | 2.2 ± 0.1ac |
| MC-4        | 79.3 ± 0.9ac | 12.5 ± 0.0ac | 3.5 ± 0.8ac   | 2.3 ± 0.2ac  | 2.2 ± 0.1ac |

Notes: HC = Harvested (before washing) cockle; WC = Washed and sorted cockle; MC = Marketed cockle; 11, 1 and 4 = Sampled time on November, January and April, respectively. Values are shown as mean ± standard deviation of triplicates. Data in the same column no sharing the same superscript are significantly different ($p < 0.05$).
influences of the season on the carbohydrate and lipid content were also observed by Lin, Jiang, Xue, Zhang, and Xu (2003) on mussels collected on the coast of Qingdao. They recorded that the highest lipid levels were prior to spawning time and the lowest values were right after that process. The protein content decreased during the spring spawning season. Meanwhile, the lipid content decreased after spawning and increased again as the gametes matured. This trend was also observed in *M. galloprovincialis* as reported by Çelik, Karayücel, Karayücel, Öztürk, and Eyüboğlu (2012).

### 3.2. Free amino acid compositions

The free amino acid (FAAs) compositions of cockles collected during different seasons and production steps were observed and are presented in Table 2. Taurine, glutamic acid, lysine and arginine were the dominant amino acids of the profiles. The profiles showed comparable concentrations of amino acids regardless of the seasonal variation, except for proline, glycine and leucine, which increased steadily from November and reached their highest concentrations in April. The average concentration of proline was 246.2 mg/100 g, 278.7 mg/100 g, and 301.1 mg/100 g for HC-11, HC-1, and HC-4 respectively. Meanwhile, the average concentrations of glycine were 462.2 mg/100 g, 517.1 mg/100 g, and 540.6 mg/100 g and those of leucine were 364.1 mg/100 g, 357.8 mg/100 g, and 484.9 mg/100 g for HC-11, HC-1, and HC-4 respectively. On the other hand, serine decreased from 309.7 mg/100 g to 293.7 mg/100 g from HC-11 through HC-1 to HC-4. Sokolowski, Wolowicz, and Hummel (2003) had reported that glycine fluctuated significantly with the reproductive cycle in the Baltic clam (*Macoma balthica*). However, the processing steps also the concentration of FAAs, as shown in Table 3. Amino acids are known to serve as energy sources during reproduction, growth, and hypoxic conditions. Their concentrations in aquatic bivalves depend not exclusively on environmental conditions but will also be influenced by the developmental and physiological conditions of the animals (Rosa, Calado, Andrade, Narciso, & Nunes, 2005). The variation of amino acid compositions of various bivalves is associated with seasonal shifting as suggested by Kube, Sokolowski, Jansen, and Schiedek (2007), who also worked on Baltic clams. The amino acid concentrations in Baltic clams, especially glycine and taurine, varied based on the season, with high concentrations recorded in winter and low concentrations in spring and summer; moreover, southern Atlantic and Mediterranean mussels showed a decrease in glycine and taurine in winter, demonstrating that the observed seasonal patterns of alanine, glycine and taurine values related to environmental factors (salinity, temperature) and physiological parameters (glycogen content, gonadal index) (Kube et al., 2007).

### 3.3. Mineral contents

Cockles (*T. granosa*), similar to other bivalve seafood products, contain nutritionally important minerals. The mineral contents of cockles collected during different seasons and production steps were observed and are presented in Table 3. Concentrations of potassium, calcium, zinc and phosphorus fluctuated from 284.5, 141.2, 3.4 and 151.8 mg/100 g when harvested to 190.7, 85.1, 2.2 and 132.7 mg/100 g in marketed samples, respectively, but the content of iron, copper and manganese did not significantly differ between seasons and processing steps. Furthermore, the results showed that the magnesium and sodium concentrations of cockles were significantly higher in November than in January and April (*p* < 0.05), with the highest values at 84.1 mg/100 g and 298.7 mg/100 g, respectively. Cobalt and heavy metals were obtained and showed seasonal fluctuation in cockles (*T. granosa*), but were treated as a trace. This result is in accordance with those obtained by Oliveira et al. (2011), who reported the mineral composition of commercial geoduck clams (*Panopea abrupta*) harvested in Southeast Alaska, in which sulphur, potassium, and phosphorus were the most abundant of the macro minerals. Karnjanapratum et al. (2013) also reported that sodium (107.3–259.7 mg/100 g) and potassium (155.6–198.0 mg/100 g) were the most abundant macro minerals in all the Asian hard clam (*M. lusoria*) portions, followed by calcium (39.8–149.2 mg/100 g) and magnesium (36.4–57.9 mg/100 g). Furthermore, the results of this research showed higher concentrations of calcium, magnesium, iron and copper than in hard clams (*M. meretrix*) harvested from the coast of China, which had concentrations of 17.5, 2.9, 0.175 and 0.069 mg/100 g, respectively (Xie et al., 2012).
Table 2. Free amino acid compositions of seasonal variation in cockles (*T. granosa*) treated

| Amino Acid                        | Concentration (mg/100 g) (n = 3) |
|-----------------------------------|-----------------------------------|
|                                  | HC-11                             |
|                                  | WC-11                             |
|                                  | MC-11                             |
|                                  | HC-1                           |
|                                  | WC-1                           |
|                                  | MC-1                           |
|                                  | HC-4                           |
|                                  | WC-4                           |
|                                  | MC-4                           |
| O-Phospho-L-serine                | 60.5 ± 3.1                       |
| Taurine                           | 489.9 ± 29.1                     |
| Urea                              | 351.2 ± 17.3                     |
| L-Aspartic acid                   | 288.5 ± 9.2                      |
| L-Threonin                         | 229.3 ± 6.8                      |
| L-Serine                          | 309.7 ± 15.0                     |
| L-Glutamic acid                   | 812.5 ± 24.5                     |
| L-Sarcosine                       | 122.9 ± 4.9                      |
| L-α-Amino apidic acid             | 24.2 ± 1.6                       |
| L-Proline                          | 246.2 ± 12.5                     |
| Glycine                           | 462.2 ± 18.2                     |
| L-Alanine                          | 357.6 ± 12.3                     |
| L-Citrulline                       | 265.1 ± 10.1                     |
| L-α-Amino iso-n-butyric acid      | 24.1 ± 0.9                       |
| L-Isoleucine                       | 130.2 ± 4.6                      |
| L-Leucine                          | 364.1 ± 10.7                     |
| β-Alanine                          | 115.3 ± 3.8                      |
| L-Phenylalanine                   | 172.6 ± 6.9                      |
| D,L-β-Amino iso-butyric acid      | 115.6 ± 3.0                      |
| L-Homocystine                      | 8.6 ± 0.6                        |
| γ-Amino-n-butyric acid            | 12.7 ± 0.4                       |
| Ethanoamine                        | 21.9 ± 0.9                       |
| Ammonium Chloride                 | 433.6 ± 15.4                     |
| δ-Hydroxylysine                   | 21.6 ± 0.9                       |
| L-Ornithine                        | 22.3 ± 10.7                      |
| Cystathionine                     | 19.2 ± 0.6                       |
| L-Prolinle                         | 157.9 ± 5.2                      |
| L-Valine                           | 361.3 ± 15.2                     |
| L-Cystine                          | 52.3 ± 20.0                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |
| β-Alanine                          | 111.3 ± 3.8                      |
| L-Methionine                       | 143.6 ± 5.1                      |
| L-Homo-cysteine                    | 22.3 ± 10.7                      |
| L-Proline                          | 157.9 ± 5.2                      |
| L-Lysine                           | 246.2 ± 12.5                     |
| L-Leucine                          | 357.6 ± 12.3                     |
| L-Tyrosine                         | 196.8 ± 9.7                      |

(Continued)
| Amino Acid          | HC-11 ± 20.4 | WC-11 ± 19.7 | MC-11 ± 15.6 | HC-1 ± 15.2 | WC-1 ± 17.2 | MC-1 ± 16.3 | HC-4 ± 18.6 | WC-4 ± 17.9 | MC-4 ± 15.3 |
|---------------------|--------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| L-Lysine            | 450.8 ± 20.4 | 448.2 ± 19.7 | 440.2 ± 15.6 | 432.5 ± 17.2 | 436.7 ± 19.4 | 409.0 ± 16.3 | 438.1 ± 18.6 | 435.2 ± 17.9 | 426.1 ± 15.3 |
| 1-Methyl-L-histidine| 116.4 ± 3.1  | 112.3 ± 4.2  | 107.4 ± 4.1  | 113.5 ± 3.6  | 102.1 ± 4.6  | 95.6 ± 2.9  | 115.2 ± 5.1  | 109.1 ± 4.8  | 112.4 ± 3.6  |
| L-Histidine         | 110.2 ± 5.1  | 109.8 ± 4.6  | 101.7 ± 4.1  | 108.9 ± 3.9  | 107.7 ± 5.2  | 104.1 ± 4.1  | 113.9 ± 4.7  | 116.3 ± 4.8  | 110.1 ± 4.2  |
| 3-Methyl-L-histidine| 21.1 ± 0.6   | 19.4 ± 1.1   | 15.2 ± 0.5   | 23.2 ± 1.1   | 25.8 ± 1.2   | 22.0 ± 0.9   | 21.4 ± 0.7   | 19.2 ± 0.5   | 17.2 ± 0.8   |
| L-Arginine          | 435.7 ± 21.1 | 415.9 ± 19.5 | 406.1 ± 20.3 | 457.4 ± 19.6 | 427.2 ± 18.4 | 431.0 ± 15.4 | 460.4 ± 18.2 | 436.3 ± 16.7 | 438.9 ± 19.4 |
| Total               | 7,165.8      | 7,220.7      | 6,802.4      | 7,330.7      | 7,103.8      | 6,835.9      | 7,552.4      | 7,323.5      | 6,833.8      |

Notes: HC = Harvested (before washing) cockle; WC = Washed and sorted cockle; MC = Marketed cockle; 11, 1 and 4 = Sampled time on November, January and April, respectively. Values are shown as mean ± standard deviation of triplicates.
Table 3. Mineral contents of seasonal variation in cockles (*T. granosa*) treated

| Mineral | HC-11    | WC-11    | MC-11    | HC-4     | WC-4     | MC-4     |
|---------|----------|----------|----------|----------|----------|----------|
| K       | 228.0 ± 1.5 | 238.3 ± 4.6 | 252.2 ± 1.4 | 284.5 ± 1.1 | 279.0 ± 3.9 | 237.8 ± 3.2 | 241.0 ± 2.7 | 223.9 ± 1.6 | 190.7 ± 2.3 |
| Ca      | 141.2 ± 0.4 | 142.2 ± 1.0 | 134.0 ± 1.5 | 113.9 ± 0.7 | 112.3 ± 4.3 | 85.1 ± 1.3 | 126.4 ± 0.6 | 123.8 ± 0.6 | 101.9 ± 0.8 |
| Mg      | 81.5 ± 0.4  | 84.1 ± 0.9  | 82.2 ± 0.2  | 83.5 ± 0.2  | 84.0 ± 1.0  | 79.4 ± 0.9  | 80.6 ± 0.8  | 79.0 ± 0.4  | 80.6 ± 0.9  |
| Na      | 284.6 ± 1.5 | 288.6 ± 6.5 | 298.7 ± 2.1 | 254.6 ± 1.5 | 253.2 ± 7.9 | 243.8 ± 4.3 | 256.7 ± 3.2 | 263.6 ± 2.2 | 284.5 ± 3.2 |
| Cu      | 0.1 ± 0.0   | 0.1 ± 0.0   | 0.2 ± 0.0   | 0.1 ± 0.0   | 0.2 ± 0.0   | 0.1 ± 0.0   | 0.1 ± 0.0   | 0.1 ± 0.0   | 0.1 ± 0.0   |
| Zn      | 3.1 ± 0.0   | 3.4 ± 0.0   | 2.2 ± 0.1   | 3.4 ± 0.1   | 2.7 ± 0.0   | 2.4 ± 0.0   | 3.2 ± 0.0   | 3.4 ± 0.0   | 3.1 ± 0.0   |
| Mn      | 1.2 ± 0.0   | 0.9 ± 0.0   | 0.9 ± 0.0   | 1.0 ± 0.0   | 1.1 ± 0.0   | 1.2 ± 0.0   | 1.1 ± 0.0   | 0.9 ± 0.0   | 0.9 ± 0.0   |
| Fe      | 8.5 ± 0.1   | 8.4 ± 0.1   | 8.3 ± 0.0   | 8.9 ± 0.4   | 8.6 ± 0.5   | 7.3 ± 0.0   | 8.6 ± 0.1   | 8.6 ± 0.2   | 8.0 ± 0.0   |
| P       | 151.8 ± 2.7 | 148.6 ± 2.5 | 141.4 ± 2.6 | 143.6 ± 4.9 | 142.1 ± 0.8 | 140.3 ± 2.1 | 149.6 ± 2.2 | 147.0 ± 2.8 | 132.7 ± 0.4 |
| Co      | Trace      | Trace      | Trace      | Trace      | Trace      | Trace      | Trace      | Trace      | Trace      |
| Heavy metal (mg/100 g) |
| Pb      | 0.019 ± 0.002 | 0.019 ± 0.001 | 0.016 ± 0.004 | 0.018 ± 0.001 | 0.017 ± 0.002 | 0.017 ± 0.002 | 0.016 ± 0.001 | 0.017 ± 0.001 | 0.015 ± 0.001 |
| Cd      | 0.034 ± 0.001 | 0.026 ± 0.000 | 0.041 ± 0.001 | 0.036 ± 0.001 | 0.036 ± 0.001 | 0.031 ± 0.001 | 0.032 ± 0.001 | 0.029 ± 0.001 | 0.031 ± 0.000 |
| Cr      | 0.018 ± 0.001 | 0.018 ± 0.000 | 0.017 ± 0.001 | 0.017 ± 0.001 | 0.018 ± 0.001 | 0.016 ± 0.000 | 0.019 ± 0.000 | 0.018 ± 0.000 | 0.013 ± 0.001 |
| As      | 0.193 ± 0.004 | 0.200 ± 0.007 | 0.194 ± 0.006 | 0.118 ± 0.002 | 0.197 ± 0.004 | 0.132 ± 0.004 | 0.111 ± 0.003 | 0.107 ± 0.003 | 0.148 ± 0.004 |

Notes: HC = Harvested (before washing) cockle; WC = Washed and sorted cockle; MC = Marketed cockle; 11, 1 and 4 = Sampled time on November, January and April, respectively.
Values are shown as mean ± standard deviation of triplicates.
Table 4. Fatty acid profiles of seasonal variation in cockles (T. granosa) treated (% of total fatty acids)

| Fatty acids | HC-11 | WC-11 | MC-11 | HC-4 | WC-4 | MC-4 |
|-------------|-------|-------|-------|------|------|------|
| SFA         |       |       |       |      |      |      |
| C14:0       | 3.6 ± 0.2 | 3.8 ± 0.1 | 3.6 ± 0.1 | 3.6 ± 0.1 | 3.4 ± 0.1 | 3.5 ± 0.1 |
| C15:0       | 0.9 ± 0.0 | 0.9 ± 0.0 | 0.9 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 |
| Iso 16:0     | 1.8 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.0 | 1.2 ± 0.0 |
| Pritstanate  | 2.1 ± 0.2 | 1.8 ± 0.1 | 1.6 ± 0.2 | 1.6 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 |
| C16:0       | 12.4 ± 0.3 | 11.9 ± 0.1 | 12.6 ± 0.1 | 11.4 ± 0.2 | 11.1 ± 0.1 | 11.3 ± 0.2 |
| C17:0       | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.9 ± 0.0 | 1.1 ± 0.0 | 1.1 ± 0.0 | 1.0 ± 0.0 |
| C18:0       | 5.9 ± 0.1 | 5.2 ± 0.0 | 5.4 ± 0.0 | 4.9 ± 0.0 | 4.6 ± 0.0 | 4.5 ± 0.0 |
| ∑SFA        | 27.7 ± 0.9 | 26.3 ± 0.5 | 26.3 ± 0.4 | 24.5 ± 0.3 | 24.0 ± 0.1 | 23.7 ± 0.0 |
| MUFA        |       |       |       |      |      |      |
| C16:1n-7    | 3.9 ± 0.1 | 4.7 ± 0.1 | 4.6 ± 0.0 | 6.3 ± 0.0 | 5.7 ± 0.0 | 4.7 ± 0.0 |
| C16:1n-5    | 1.2 ± 0.1 | 1.1 ± 0.0 | 0.9 ± 0.1 | 1.2 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| C18:1n-9    | 2.7 ± 0.0 | 2.9 ± 0.0 | 3.1 ± 0.0 | 3.5 ± 0.0 | 3.4 ± 0.0 | 3.7 ± 0.0 |
| C18:1n-7    | 2.3 ± 0.0 | 2.8 ± 0.0 | 2.0 ± 0.0 | 2.2 ± 0.0 | 2.2 ± 0.0 | 2.8 ± 0.0 |
| C20:1n-9    | 3.0 ± 0.0 | 2.6 ± 0.0 | 2.8 ± 0.0 | 1.9 ± 0.0 | 1.6 ± 0.0 | 1.5 ± 0.1 |
| C16:3n-4    | 1.2 ± 0.0 | 1.5 ± 0.0 | 1.4 ± 0.0 | 1.5 ± 0.0 | 1.7 ± 0.0 | 2.1 ± 0.0 |
| C16:4n-1    | 0.8 ± 0.1 | 0.8 ± 0.0 | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.2 | 0.8 ± 0.1 |
| C18:3n-1    | 4.1 ± 0.3 | 3.3 ± 0.1 | 3.4 ± 0.0 | 3.5 ± 0.0 | 3.6 ± 0.0 | 3.9 ± 0.0 |
| C20:3n-6    | 6.0 ± 0.0 | 5.8 ± 0.0 | 5.8 ± 0.0 | 5.1 ± 0.0 | 4.1 ± 0.0 | 3.9 ± 0.0 |
| ∑MUFA       | 25.4 ± 0.4 | 25.8 ± 0.2 | 25.3 ± 0.1 | 24.5 ± 0.0 | 23.3 ± 0.0 | 23.2 ± 0.0 |
| PUFA        |       |       |       |      |      |      |
| C16:2n-9    | 0.8 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 |
| C16:4n-1    | 4.1 ± 0.3 | 3.3 ± 0.1 | 3.4 ± 0.0 | 3.5 ± 0.0 | 3.6 ± 0.0 | 3.9 ± 0.0 |
| C16:4n-3    | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 |
| C18:2n-6    | 1.2 ± 0.0 | 1.5 ± 0.0 | 1.6 ± 0.0 | 2.3 ± 0.0 | 2.3 ± 0.0 | 2.3 ± 0.0 |
| C18:3n-3    | 3.8 ± 0.0 | 3.7 ± 0.0 | 3.7 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 |
| C20:4n-6    | 4.2 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 |
| C20:5n-3(EPA)| 11.3 ± 0.0 | 11.3 ± 0.0 | 11.3 ± 0.0 | 11.3 ± 0.0 | 11.3 ± 0.0 | 11.3 ± 0.0 |
| C22:4n-6    | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 |
Notes: HC = Harvested (before washing) cockle; WC = Washed and sorted cockle; MC = Marketed cockle; 11, 1 and 4 = Sampled time on November, January and April, respectively. Values are shown as mean ± standard deviation of triplicates. SFA = saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; IA, index of atherogenicity; TI, index of thrombogenicity; h/H, index of hypocholesterolemic/hypercholesterolemic.

| Fatty acids                  | HC-11 | WC-11 | MC-11 | HC-1 | WC-1 | MC-1 | HC-4 | WC-4 | MC-4 |
|-----------------------------|-------|-------|-------|------|------|------|------|------|------|
| C22:5n-3                    | 1.1 ± 0.0 | 1.2 ± 0.0 | 1.1 ± 0.0 | 1.4 ± 0.0 | 1.2 ± 0.0 | 0.8 ± 0.0 | 0.9 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.1 |
| C22:5n-6                    | 1.4 ± 0.0 | 1.5 ± 0.0 | 1.4 ± 0.0 | 1.3 ± 0.0 | 1.1 ± 0.0 | 1.0 ± 0.0 | 1.2 ± 0.0 | 1.3 ± 0.1 | 1.4 ± 0.1 |
| ΣPUFA                       | 45.0 ± 0.7 | 46.8 ± 0.4 | 47.8 ± 0.8 | 46.8 ± 0.4 | 51.1 ± 0.5 | 54.0 ± 0.4 | 46.3 ± 0.3 | 46.3 ± 0.9 | 46.2 ± 1.3 |
| ΣPUFAn - 3                  | 28.7 ± 0.2 | 30.5 ± 0.1 | 31.5 ± 0.1 | 31.3 ± 0.2 | 34.8 ± 0.1 | 37.0 ± 0.2 | 32.9 ± 0.2 | 32.1 ± 0.3 | 32.9 ± 0.5 |
| ΣPUFAn - 6                  | 8.1 ± 0.0 | 8.5 ± 0.1 | 8.0 ± 0.1 | 9.2 ± 0.0 | 9.0 ± 0.1 | 8.8 ± 0.0 | 5.9 ± 0.0 | 6.4 ± 0.4 | 6.5 ± 0.3 |
| Σn-3/Σn-6                   | 35 ± 0.2 | 36 ± 0.1 | 39 ± 0.1 | 34 ± 0.2 | 39 ± 0.1 | 42 ± 0.2 | 5.6 ± 0.2 | 5.0 ± 0.3 | 5.1 ± 0.5 |
| Σn-6/Σn-3                   | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| DHA/EPA                     | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.0 | 0.5 ± 0.1 |
| PUFA/SFA                    | 1.6 ± 0.7 | 1.8 ± 0.4 | 1.8 ± 0.8 | 1.9 ± 0.4 | 2.2 ± 0.5 | 2.3 ± 0.4 | 1.6 ± 0.3 | 1.6 ± 0.6 | 1.7 ± 0.6 |
| IA                          | 0.4 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.4 | 0.5 ± 0.3 |
| IT                          | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.1 |
| h/H                         | 2.2 ± 0.2 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.5 ± 0.2 | 2.7 ± 0.1 | 2.7 ± 0.2 | 1.9 ± 0.2 | 2.0 ± 0.3 | 2.0 ± 0.5 |
3.4. Fatty acid profiles

The FAMES as a percentage of the total fatty acids mixture of cockles collected during different seasons and production steps were observed and are shown in Table 4. The total percentage of identified SFAs of cockles changed from 23.3 ± 0.4% of those marketed in January (MC-1) to 28.1 ± 0.1% with those harvested in April (HC-4), and from 22.3 ± 0.1% to 27.0 ± 0.2% during January for the MUFAs. For PUFAs, the cockles harvested in November (HC-11) showed 45.0 ± 0.7% of PUFAs, and the highest levels of PUFAs were the 54.0 ± 0.4% of those marketed in January (MC-1); the DHA (C22:6n-3) concentration slightly increased from November to January (10.2 ± 0.1% (HC-11) and 11.6 ± 0.1% (HC-1)) and sharply declined thereafter in April (HC-4) (7.9 ± 0.1%). EPA (C20:5n-3) content, was statistically significantly (p < 0.05) different between samples, with those harvested in April (HC-4) having the highest level of the major polyunsaturated fatty acid, peaking at 17.4 ± 0.1% of total fatty acids. Of the individual fatty acids, palmitic acid (C16:0), a saturated fatty acid, was the highest in the April samples (HC-4) (16.0 ± 0.1%), while stearic acid (C18:0) had the highest concentration in cockles harvested in November (HC-11) (5.9 ± 0.1%). These results are similar to those reported by Lin et al. (2003), where C16:0, EPA and DHA were the major fatty acids of the phospholipids in mussels (Mytilus edulis Linne) and where C16:0 was the main saturated fatty acid, ranging from 10.78 to 26.22%; EPA and DHA were also abundant, ranging from 5.25 to 23.10% and from 6.05 to 20.42%, respectively. Delaporte et al. (2005) reported that diet quality affects FA content in bivalves, as they are may affect by filtration rates and seasonal changes in food sources. Hendriks, van Duren, and Herman (2003) also showed that EPA and DHA are key nutritional constituents, which might come from the diet of algae consumed by bivalves. Pernet et al. (2012) reported that bivalve species consume nutrients from many material particles, such as resuspended benthic microalgae, and that phytoplankton is their primary food source, which propagates considerably during spring in accord with the high amounts of EPA and DHA during this period. Moreover, the temperature is also an important factor in the growth regulation of marine invertebrates including mollusc bivalves and affects fatty acid profiles; high levels of EPA and DHA are considered to contribute to the maintenance of membrane fluidity during periods of low temperature (Ezgeta-Balić, Najdek, Peharda, & Blažina, 2012).

To assess the nutritional value of fat, the results of the n – 3/n – 6 PUFA (3.4 ± 0.2 to 5.6 ± 0.2%), DHA/EPA (0.5 ± 0.1 to 1.1 ± 0.1%), PUFA/SFA (1.6 ± 0.7 to 2.3 ± 0.4%) ratios and the atherogenic (IA) (0.4 ± 0.0 to 0.5 ± 0.4%), thrombogenic (IT) (0.2 ± 0.1%) and hypocholesterolaemic/hypercholesterolaemic FA (h/H) (1.9 ± 0.2 to 2.7 ± 0.2%) indices of cockles collected during different season and production steps were recorded and are shown in Table 4. These are similar to results from a native clam (Ruditapes decussatus) and an invasive clam (Ruditapes philippinarum) species reported by Anacleto et al. (2014), which had n – 3/n – 6 ratios in the range of the recommended values, as well as a PUFA/SFA ratio well above 0.45, as recommended by the U.K. Department of Health (HMSO, 1994). As reviewed by Simopoulos (2008b), the beneficial health effects of omega-3 fatty acids, EPA and DHA, were related with a high seafood diet that has a higher ratio of n – 3/n – 6 PUFA and also have the most potent anti-inflammatory effects. Inflammation is at the base of many chronic diseases, including coronary heart disease, diabetes, arthritis, cancer, osteoporosis, mental health, dry eye disease and age-related macular degeneration. Simopoulos (2008a) also suggested that dietary intake of omega-3 fatty acids may prevent the development of diseases and that a lower n – 6/n – 3 ratio as part of a Mediterranean diet decreases vascular endothelial growth factor. The IA indicates the relationship between the sum of the major saturated fatty acids and that of the main classes of unsaturated acids, the former being considered pro-atherogenic and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases). The IT relates the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids. In our research, both these indices were lower than in other seafood products from previous studies, as raw roe of blue fin tuna (T. thynnus) and tuna botargo (T. thynnus) (Garaffo, 2011), orange fin pony fish (Leiognathus bindus) and sulphur goatfishes (Upeneus sulphureus) (Ghaeni, Ghafraroki, & Zaheri, 2013) and Mediterranean sea bass (D. labrax) (Smichi et al., 2017), which is mostly due to the low saturated fatty acid contents in these samples regardless of seasonal variation and freshness; suggest that these cockles can be...
used in a cardio-protective and antithrombogenic diet (Ulbricht & Southgate, 1991). The low values of the h/H index obtained from this research ranged between 1.9 in April and 2.7 in January sampling. This is similar to nutritional reports of lamb fatty acids by Santos-Silva et al. (2002), indicating that the regular consumption of these cockles causes a hypocholesterolemic effect.

4. Conclusions

The results showed that the proximate composition, amino acids and mineral content had high value and varied significantly with seasonal variation and production step. All research samples exhibited limited concentrations of heavy metal such as Cr, Pb, Cd, and As, as well as Co. The results also showed that cockle samples had high contents of PUFAs, especially EPA (C20:5n-3), which peaked in April, and DHA (C22:6n-3), which slightly increased from November to January, both of which are key nutritional constituents which may derive from the diet of algae consumed by bivalves. Moreover, from the standpoint of the nutritional quality of fats, the atherogenic (IA), thrombogenic (IT) and hypocholesterolemic/ hypercholesterolemic (h/H) indices remained low at all times studied, even through different processing conditions, suggesting that these food items can be used in a cardio-protective and hypocholesterolemic diet.
