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Effect of season and processing steps in nutritional components and bioactivities of blue mussels (*Mytilus edulis*)

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Abstract

Blue mussels (*Mytilus edulis*) from Tongyeong, South Korea were evaluated for seasonal variation in terms of proximate composition, fatty acid (FA) profile, antioxidant activities (DPPH radical scavenging, superoxide anion radical scavenging, and reducing power), and alcohol dehydrogenase (ADH) activity. The effect of different processing steps on blue mussels (from harvest to market) was also assessed. Samples were taken from late autumn to early spring (November 2015 - April 2016). Moisture (79.4 - 82.9%), lipid (1.3 - 2.2%), and protein (8.9 - 10.5%) components varied significantly according to season and processing stage; while ash content remained relative (2.1 - 2.4%). The lowest glycogen (34.2 - 36.7 mg/g) content was found in mussels sampled in January, signifying its usage to generate energy and combat cold stress in winter. Meanwhile, an inverse relationship was observed in mussel lipid (highest, 2.0 - 2.1%) and protein (lowest, 8.9 - 9.3%) contents in April, signifying the conversion of protein to lipid in preparation for spawning in summer. The main FA from lipid class profiling were *n*-3 polyunsaturated FAs (PUFA, 39.0 - 45.1%), and primarily DHA (14.8 - 16.9%). Atherogenic (AI), thrombogenic (TI), and hypocholesterolaemic/hypercholesterolaemic (h/H) indices, as well as PUFA/SFA and DHA/EPA ratios remained relative throughout the duration of the study. Freshly-harvested mussels exhibited significantly greater radical scavenging and ADH activities than the marketed mussels which emphasise the importance of keeping the bivalve's freshness. April was determined as the most suitable time for utilising blue mussels as food, food ingredient, and/or for pharmaceutical development. This is because it is during its harvest season where the proximate composition is neither too high nor too low, and omega-3 PUFA content (28.7 - 30.9%) and omega-3/omega-6 PUFA ratio (6.5 - 7.2) are at their peak which support better antioxidant benefits.

Introduction

Antioxidants, which can either be consumed from our diet or produced in the body are used to counter reactive oxygen and nitrogen species that are generated *in vivo*, and cause damages to DNA, lipids, proteins, and other biomolecules (Halliwell, 1996). Synthetic antioxidants have been formulated, tested for acute toxicity, and proposed to supplement naturally occurring antioxidants. However, recent reports revealed that these man-made antioxidants could be linked to toxic and carcinogenic effects (Zhang *et al.,* 2010). With the increasing demand for healthier lifestyle and longer lifespan, consumers are getting more interested in nutraceuticals and functional foods rich in natural bioactive compounds (Fung *et al.,* 2013). This means that society is becoming more health-conscious, thus seeking more natural and organic sources for their consumption. Marine natural sources are no exception. Among the most widely consumed and enjoyed seafood options are the bivalves such as clams, oysters, scallops, and mussels.

The demand for marine mussel species as a food commodity gradually increased over the years. In South Korea, blue mussel (*Mytilus edulis*) consumption reached around 57,000 tons per year, and is the second most cultivated shellfish next to oysters. Despite being ranked 9th out of the world's mussel producers, Korea still imports large quantities

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from China, New Zealand, and Chile with an average of 23,079 tons over the last three years (FOC, 2017) due to high demand. *Mytilus edulis*, along with other commercially available blue mussel species are served as live, frozen, or processed seafoods. Additionally, they are considered as a potential source for proteins, lipids, and carbohydrates, and have been studied for their good influence on human health (Grienke *et al.,* 2014). Mussel components also play a vital role in the development of functional foods (food with specific beneficial health effect beyond simple nutrition), or nutraceuticals (nutrition + pharmaceuticals) (Haller, 2010; Bernal *et al.,* 2011).

Mussel species exhibit antimicrobial, antioxidant, and anti-inflammatory activities. An example is the anti-inflammatory supplement, Lyprinol®, which contains ingredients that prevents and treats progressive arthritis. One of its components is the lipid extract from green mussels, *Perna canaliculus* (Grienke *et al.,* 2014). Similarly, blue mussels have demonstrated specific activities on alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) as reported by Ok *et al.* (2014).

Studies on seasonal and geographical variation in nutritional quality and biochemical composition of different mussel species have been conducted previously (Zandee *et al.,* 1980; Fernández *et al.,* 2015; Bongiorno *et al.,* 2015; Gallardi *et al.,* 2017). However, not much is known about the seasonal variation in blue mussels' antioxidant and alcohol dehydrogenase activities, especially during its harvest time.

The present work was conducted to investigate the most suitable period during harvest season for consuming blue mussels as food, food ingredient, and/or for use in pharmaceutical development. To create nutritional component baseline data for blue mussels in the temperate waters of South Korea, we investigated the seasonal variation and effects of processing stages (from harvest to market) on the nutritional value and bioactivity of those obtained from Tongyeong, Korea. We examined the biochemical composition, antioxidant, and alcohol dehydrogenase activities of blue mussels in each season from November to April during harvest time, and also checked for each different stage of processing. The data obtained from the present work could be used to boost the potential of blue mussels in developing functional foods, food ingredients, and nutraceuticals.

Materials and methods

Materials and reagents

Blue mussels from the coastal area of Tongyeong, Korea were collected and immediately stored at -20°C while being transported to the laboratory. Sampling was done in November 2015, January 2016, and April 2016. Around 5 kg of mussel samples were taken from each processing stage: after harvest (labelled HM); after seawater mechanical washing (labelled WM), and at the market (labelled MM). After manual shucking, half of the mussel flesh were homogenised and used for analyses of moisture, lipid, glycogen, and ash contents. The remaining portion was freeze-dried (Samwon SFDSF06 Freeze-dryer, Samwon Engineering Co., Korea), ground, and stored at -20°C for other analyses. All standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). The fatty acid methyl ester (FAME) standards composed of standard mixed FAMEs and Menhaden fish oil were purchased from Supelco Ltd. (Bellefonte, PA, USA). All solvents and reagents used were of analytical and/or HPLC grade.

Proximate composition

Moisture content was determined using a halogen moisture analyser (WBA-110M, DAIHAN Scientific Co. Ltd, Korea). Ash content was determined using modified AOAC method 18.025 (AOAC, 2000). Residues from moisture analysis were used and ignited at 550°C overnight in the furnace (F-12, WiseTherm®, DAIHAN Scientific Co. Ltd., Korea) until a light grey ash colour occurred. Samples were then transferred to a desiccator and allowed to cool to room temperature before weighing. Ash content was calculated from the loss in weight. Crude protein was determined using the semi-micro Kjeldahl method.

Glycogen content

Glycogen concentrations in blue mussel were determined using the method described by Leyva *et al.* (2008) with minor modifications. Samples (25 mg dry weight) were hydrolysed with 15 mL of 33% potassium hydroxide in a water bath at 100°C for 15 min. After cooling, 0.5 mL of hydrolysed sample was mixed with 50 µL of saturated sodium sulphate solution (Merck, Darmstadt, Germany) and 2 mL of ethanol (96%, Sigma-Aldrich Co., St. Louis, Mo, USA). Samples were then placed in an ice bath for precipitation (~30 min) and centrifuged (14,000 *g*, 8 min). The precipitate was dissolved in 0.5 mL of distilled water,

re-precipitated with 1 mL of ethanol (30 min), and centrifuged (14,000 *g*; 8 min). Later, 0.5 mL of distilled water and 3 mL of anthrone-reagent were added to the precipitate, and the resulting mixture was heated at 90°C for 20 min. Absorbance was measured at 620 nm with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Glycogen standard from Sigma-Aldrich Co. (St. Louis, Mo, USA) was used. Anthrone-reagent was prepared using 38 mL of concentrated sulphuric acid (Sigma-Aldrich Co., St. Louis, Mo, USA), 15 mL of distilled water, and 0.075 g of anthrone (Sigma-Aldrich Co., St. Louis, Mo, USA).

Fatty acid composition and free fatty acid nutritional quality assessment

The total lipid content was extracted by using the Bligh and Dyer's (1959) method. Fatty acids (FA) were converted to methyl ester forms (FAME), and separated by gas chromatography following the AOCS Ce 1b-89 official method (AOCS, 1998). FAME analyses were performed on Clarus 600 gas chromatographer (PerkinElmer Ltd, Bridgeville, PA, USA) with flame ionisation detector (GC-FID). Samples were injected at 1.0-µL volume into Omegawax-320 fused silica capillary column (30 m × 320 µm × 0.25 µm; Supelco Ltd., Bellefonte, PA, USA) with helium as carrier gas at 1 mL/min flow rate. Both injector and detector temperatures were set to 250°C, with injector in split mode (100:1). The oven temperature was programmed to start at 180°C for 8 min, with final temperature of 230°C at 3°C/min ramp rate and 15 min holding time. FAs were identified by computing the ECL values from the retention time and comparing them with the values calculated from the standards (Sigma-Aldrich; St. Louis, MO, USA).

The atherogenic (AI) and thrombogenic (TI) indices are used to estimate the probability of developing coronary heart diseases from food. For each mussel sample, the AI and TI were estimated according to Ulbricht and Southgate (1991) using Eqs. 1 and 2:

$$AI = \frac{[C12:0 + (4 \times C14:0) + C16:0]}{[\Sigma MUFA + \Sigma PUFA\ (n-6) + \Sigma PUFA\ (n-3)]} \quad \text{(Eq. 1)}$$

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma PUFA\ (n-6)) + (3 \times \Sigma PUFA\ (n-3)) + \frac{\Sigma PUFA\ (n-3)}{\Sigma PUFA\ (n-6)}]} \quad \text{(Eq. 2)}$$

The hypocholesterolaemic/hypercholesterolaemic index (h/H) was determined according to Santos-Silva *et al.* (2002) using Eq. 3:

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

Two ratios related to FA content were also calculated, namely DHA/EPA (docosahexaenoic acid/eicosapentaenoic acid) and PUFA/SFA (polyunsaturated FA/saturated FA), as well as monounsaturated FA (MUFA) and omega-3 to omega-6 FA ratio.

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Antioxidant activity assays

The DPPH radical scavenging activity was determined using the method defined by Gu *et al.* (2009) with some modifications. Two sets of sample solutions (1.0, 2.0, and 3.0 mg/mL) were prepared. Equal volumes of DPPH solution (0.1 mM in ethanol) were added to each of the sample in one set. An equivalent volume of ethanol was added to the other set of sample solutions as control. A mixture of ethanol and DPPH (1:1, v/v) solution was used as the blank. All mixtures were placed in the dark at room temperature for 30 min. The absorbances were determined at 517 nm using a microreader plate (Molecular Devices, Sunnyvale, CA, USA). The DPPH radical-scavenging activity of samples was calculated using Eq. 4:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \{\frac{[1-(A_1-A_S)]}{A_0} \times 100\} \quad \text{(Eq. 4)}$$

where, A_1 = absorbance of the sample; A_s = absorbance of the control; and A_0 = absorbance of the blank. Ascorbic acid was used as the standard.

Superoxide anion radical scavenging activity was determined according to the method introduced by Nishikimi *et al.* (1972) with slight modifications. A mixture of 0.8 mL of potassium phosphate buffer (100 mM, pH 7.4), 0.1 mL of sample extract, 0.1 mL of 0.78 mM nitroblue tetrazolium (NBT), 0.1 mL of 2.34 mM NADH, and 50 mL of 60 mM phenazine methosulfate (PMS) were incubated in the dark at 25°C for 20 min. Absorbances were measured at 560 nm. A reaction blank was prepared for each measurement by replacing NADH with water. Ascorbic acid was used as the positive control.

Reducing power was determined using the

method by Oyaizu (1986). A volume (0.5 mL) of mussel extract was mixed with 2.5 mL of sodium phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min, and 2.5 mL of trichloroacetic acid (10%, w/v) was added afterwards. The mixture was centrifuged at 5,000 *g* for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and ferric chloride (1 mL, 0.1%). Absorbance was measured at 700 nm with the phosphate buffer (200 mM, pH 6.6) used as the blank.

Alcohol dehydrogenase activity

Alcohol dehydrogenase (ADH) activity of the mussel extracts was determined using a commercial assay kit (MAK053, Sigma-Aldrich Co., St. Louis, MO, USA). Isopropanol was used as substrate in the enzyme reaction. The absorbance of the resulting mixture was measured at 450 nm. The activity was one unit of ADH to the amount of enzyme that generated 1.0 µmol of NADH (reduced form of nicotinamide adenine dinucleotide, NAD) per minute (at pH 8.0, 37°C). ADH activity was calculated using Eq. 5:

$$ADH\ activity = \left(B \times \frac{sample\ dilution\ factor}{reaction\ time \times V}\right) \quad \text{(Eq. 5)}$$

where, B = amount (nmol) of NADH generated between $T_{initial}$ and T_{final}; reaction time = T_{final} - $T_{initial}$ in min; and V = sample volume (mL) added to the well.

Statistical analysis

All analyses were carried out in triplicates. The results were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed on the resulting data sets. The significant difference between means was determined by Duncan's multiple range test using MINITAB® version 16.2.0 software. The probability value ($p < 0.05$) was considered statistically significant.

Results and discussion

Nutritional components

Results from proximate composition analyses of mussel samples are shown in Table 1. Ash content of mussels remained relative throughout the duration of the study at 2.1 - 2.4%. Moisture content from January samples (81.7 - 82.9%; M-1) were slightly higher as compared to that of samples from April (79.4 - 80.5%; M-4) and November (79.6 - 80.4%; M-11). Crude protein in mussels collected in April (8.9 - 9.3%) were significantly lower ($p < 0.05$) than in both November (10.1 - 10.5%) and January (9.9 - 10.2%). Low protein content found in samples collected in April (late spring) might be caused by the spawning preparation activity of mussels as they use their protein fractions for egg development for spawning in summer (Zandee *et al.,* 1980). These data corresponded to the higher lipid content determined from samples in April (2.0 - 2.1%) than in November (1.3 - 1.4%) and January (1.4 - 1.6%), wherein the increase in lipid concentration indicated the approaching spawning activity as they built up their lipid reserves in the

Table 1. Proximate composition of processed mussel per season.

Sample	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)	Carbohydrate (mg/g of sample)
HM-11	80.4 ± 0.7^{b}	10.1 ± 0.3^{a}	1.4 ± 0.2^{b}	2.1 ± 0.2^{a}	52.2 ± 2.1^{a}
WM-11	80.8 ± 0.5^{b}	10.5 ± 0.0^{a}	1.3 ± 0.1^{b}	2.3 ± 0.2^{a}	49.3 ± 2.7^{a}
MM-11	79.6 ± 0.5^{b}	10.3 ± 0.2^{a}	1.4 ± 0.0^{b}	2.1 ± 0.2^{a}	52.8 ± 1.4^{a}
HM-1	82.9 ± 0.9^{a}	10.2 ± 0.0^{a}	1.4 ± 0.2^{b}	2.2 ± 0.1^{a}	34.2 ± 3.6^{b}
WM-1	82.5 ± 0.4^{a}	9.9 ± 0.2^{a}	1.6 ± 0.1^{b}	2.4 ± 0.1^{a}	35.3 ± 2.8^{b}
MM-1	81.7 ± 0.6^{a}	9.9 ± 0.2^{a}	1.5 ± 0.1^{b}	2.2 ± 0.3^{a}	36.7 ± 5.6^{b}
HM-4	79.4 ± 0.1^{b}	9.3 ± 0.1^{b}	2.1 ± 0.1^{a}	2.4 ± 0.6^{a}	52.2 ± 5.1^{a}
WM-4	79.8 ± 1.1^{b}	8.9 ± 0.4^{b}	2.0 ± 0.2^{a}	2.2 ± 0.2^{a}	50.8 ± 5.7^{a}
MM-4	80.5 ± 0.3^{b}	9.3 ± 0.2^{b}	2.2 ± 0.2^{a}	2.2 ± 0.2^{a}	49.4 ± 5.8^{a}

HM = freshly-harvested mussel; WM = washed and sorted mussel; MM = marketed mussel; and 11, 1, and 4 = sampling months of November, January, and April, respectively. Values are mean ± standard deviation of triplicates ($n = 3$). Means in the same column with different lowercase superscripts are significantly different ($p < 0.05$).

production of eggs (Prato *et al.,* 2010). The inverse relationship of protein and lipid concentrations observed in spring time demonstrated the conversion of protein fractions into lipids for gamete production (Zandee *et al.,* 1980).

Meanwhile, the lowest glycogen content was observed in samples from January (34.2 - 36.7 mg/g). This might be due to glycogen being the principal carbohydrate reserve in bivalves, and is mainly used for sustaining their condition under stressful situations (Bayne *et al.,* 1976; Patrick *et al.,* 2006). Anacleto et al. (2014a) observed a similar trend, and found a significant decrease in the native clam species *Ruditapes decussatus'* glycogen content in cooler temperature. During winter season (December to February), seawater temperature in Tongyeong area decreased to 7 - 14°C in the coastal areas. This could mean that cultured blue mussels from Tongyeong, South Korea used their glycogen reserves to combat stress from the cold during winter, resulting in the low concentrations of glycogen. This is consistent with studies in cold coastal waters (Gallardi *et al.,* 2017) and in Iwagaki oyster (*Crassostrea nippon*), wherein the glycogen content gradually decreased from May to September (summer to autumn, in which the sea water temperature starts to get colder) along with spawning (Okumura *et al.,* 2005). Overall, the variation in proximate composition at different processing steps is not as significant as that of the seasonal effect on the mussel samples.

Fatty acid profiles and nutritional quality assessment

Fatty acids (FAs) are vital structural components that make up some bioactive molecules and have functions related to reproduction, osmoregulation, and stress response (Makoto *et al.,* 1989). FA composition analysis of mussel samples is shown in Table 2.

A similar trend was observed in the FA profile of all samples with PUFAs dominating majority of the fractions at 39.0 - 45.1%, followed by SFAs at 25.7 - 28.9%, and MUFAs at 15.5 - 17.8%. In freshly-collected mussels, the total SFA content was 28.9, 25.7, and 28.8% in November, January, and April, respectively, with palmitic acid (C16:0) as the prominent SFA at 17.6, 15.3, 16.2%, respectively. In the same freshly-harvested mussel samples, the MUFA content ranged from 16.7 to 17.8% which was mainly composed of palmitoleic acid (C16:1 *n*-7) at 3.9 to 4.8%. PUFA content of fresh mussel samples were 41.9, 45.1, and 42.8% for November, January, and April, respectively. DHA (C22:6 n-3) was detected as the notable fraction at 16.8 - 16.9%, followed by EPA (C20:5 *n*-3) at 7.4 - 8.1%. In addition, omega-3 (*n*-3) PUFAs were observed to dominate the total FA profile with the highest concentration found in April samples at 28.7 - 30.9%. These results are comparable to the data found for New Zealand's green-lipped mussel, wherein the major components found were also *n*-3 PUFAs with 13% of EPA and 21% of DHA (Murphy *et al.,* 2002; Grienke *et al.,* 2014). Similar trend in FA profile was reported by Fernández *et al.* (2015) for the same species cultured in Ireland.

Interestingly, there was a slight increase in both alpha-linolenic acid (C18:3 *n*-3) and EPA (C20:5 *n*-3), while there was a slight decrease in both linoleic (C18:2 *n*-6) and arachidonic acid (C20:4 *n*-6) in the FA composition of mussel samples collected in April (M-4). This observation and other variation in FA composition of blue mussels in the present work could be attributed to the change in the temperature of seawater in the environment, and the reproductive cycle of the bivalves. PUFAs are also selectively utilised for energy and metabolic requirements in bivalves during periods of nutritional shortage (Freitas *et al.,* 2002). This could explain the reason for the lower C18:3 *n*-3 and EPA contents in the FA composition of blue mussels during the cold months (November to January).

The higher PUFA content observed in mussel samples collected in January might indicate a risk of oxidative stress of the species induced by high oxygen solubility in cold seawater during winter as PUFAs are prone to reactive oxygen species (ROS) driven oxidation (Abele and Puntarulo, 2004). The FA profile of bivalves might also demonstrate their food sources' availability. The high PUFA content observed in all samples might also be due to the abundance of phytoplankton, which are bivalves' major dietary source, and rich in linoleic (C18:2 *n*-6) and linolenic (C18: *n*-3) acids, as well as C20 and C22 PUFAs (Pirini *et al.,* 2007). In addition, the decrease in PUFA content from 45.1% in January to 42.8% in April demonstrated the building up and utilisation of PUFAs to generate egg biomass in the preparation for spawning (Fernández *et al.,* 2015).

The evaluated nutritional values, $\frac{PUFA}{SFA}$ $\frac{n-3}{n-6}$ PUFAs, $\frac{DHA}{EPA}$ ratios, atherogenic (AI), thrombogenic (TI), and hypocholesterolaemic/hypercholesterolaemic *(h/H)* indices of mussels are also shown in Table 2. The $\frac{PUFA}{SFA}$ ratio found in fresh mussels were 1.4, 1.8, and 1.5 for November, January, and April, respectively, while $\frac{n-3}{n-6}$ tratios computed were 4.8, 4.3, and 7.0, respectively. These results are comparable to the data obtained from native clam (*R. decussatus*) and invasive clam (*R. philippinarum*) species

Table 2. Fatty acid profile of processed mussel (%) per season.

Fatty acid	HM-11	WM-11	MM-11	HM-1	WM-1	MM-1	HM-4	WM-4	MM-4
C14:0	3.3 ± 0.0	3.0 ± 0.0	3.5 ± 0.0	2.5 ± 0.0	2.4 ± 0.1	3.6 ± 0.0	3.9 ± 0.0	3.6 ± 0.1	3.8 ± 0.1
C15:0	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0
Iso 16:0	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	0.9 ± 0.1	1.8 ± 0.1	1.5 ± 0.0	1.9 ± 0.1
Pristanate	1.9 ± 0.0	1.8 ± 0.1	1.9 ± 0.0	1.8 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	1.6 ± 0.0	1.5 ± 0.1	1.6 ± 0.1
C16:0	17.6 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.3 ± 0.0	15.3 ± 0.1	15.3 ± 0.1	16.2 ± 0.2	15.4 ± 0.0	15.5 ± 0.1
C17:0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
C18:0	3.7 ± 0.2	4.1 ± 0.3	3.8 ± 0.0	3.6 ± 0.1	3.5 ± 0.1	3.7 ± 0.0	3.7 ± 0.0	2.9±0.1	2.9 ± 0.0
∑SFA	28.9 ± 0.3	27.7 ± 0.2	27.7 ± 0.2	25.7 ± 0.3	25.8 ± 0.5	27.1 ± 0.2	28.8 ± 0.3	26.3 ± 0.3	27.2 ± 0.3
C16:1 n-7	4.8 ± 0.2	4.4 ± 0.0	4.1 ± 0.1	4.6 ± 0.1	4.4 ± 0.1	4.3 ± 0.0	3.9 ± 0.0	3.6 ± 0.0	3.2 ± 0.0
C16:1 n-5	1.5 ± 0.0	1.6 ± 0.0	1.4 ± 0.2	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
C18:1 n-9	1.7 ± 0.0	1.4 ± 0.1	1.4 ± 0.0	1.6 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	1.5 ± 0.0	1.3 ± 0.1	0.9 ± 0.1
C18:1 n-7	2.2 ± 0.1	2.1 ± 0.0	2.3 ± 0.0	1.7 ± 0.0	2.2 ± 0.0	2.1 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	1.4 ± 0.0
C20:1 n-9	2.0 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	2.1 ± 0.1	2.1 ± 0.0	1.7 ± 0.0	2.1 ± 0.1	2.2 ± 0.1	2.5 ± 0.0
C20:1 n-7	2.7 ± 0.1	2.9 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	2.7 ± 0.0	2.3 ± 0.0	2.9 ± 0.0	2.8 ± 0.1	2.5 ± 0.0
C22:1 n-9	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	1.5 ± 0.1	1.6 ± 0.0	1.8 ± 0.0
C22:1 n-6	2.4 ± 0.1	2.5 ± 0.0	2.2 ± 0.0	2.3 ± 0.1	1.9 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	2.2 ± 0.0
∑MUFA	17.8 ± 0.3	17.4 ± 0.3	16.3 ± 0.2	16.7 ± 0.3	16.6 ± 0.3	15.5 ± 0.1	16.7 ± 0.3	16.2 ± 0.2	15.7 ± 0.2
C16:2 n-9	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	0.9 ± 0.0
C16:2 n-4	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
C16:3 n-4	1.2 ± 0.0	1.1 ± 0.2	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.7 ± 0.1
C16:3 n-1	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.2	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	0.8 ± 0.0
C16:4 n-1	3.9 ± 0.1	4.4 ± 0.0	4.0 ± 0.4	5.9 ± 0.0	5.8 ± 0.0	4.2 ± 0.0	3.4 ± 0.0	3.5 ± 0.0	3.7 ± 0.0
C18:2 n-6	0.9 ± 0.0	0.9 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	2.0 ± 0.1	1.1 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.0
C18:3 n-3	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.0 ± 0.0	3.3 ± 0.1	3.5 ± 0.1	3.9 ± 0.1
C18:4 n-3	0.9 ± 0.1	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0.2	1.1 ± 0.1	1.4 ± 0.0	1.3 ± 0.3	1.2 ± 0.0	1.3 ± 0.0
C20:4 n-6	3.1 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	3.6 ± 0.0	3.4 ± 0.2	2.8 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.3 ± 0.1
C20:5 n-3	7.6 ± 0.0	7.5 ± 0.1	6.9 ± 0.1	7.4 ± 0.1	7.1 ± 0.0	6.8 ± 0.1	8.1 ± 0.1	7.8 ± 0.2	7.2 ± 0.1
C21:5 n-3	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.3 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0
C22:4 n-6	0.6 ± 0.0	0.6 ± 0.1	0.8 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
C22:5 n-3	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
C22:5 n-6	1.3 ± 0.1	1.4 ± 0.0	1.3 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.0
C22:6 n-3	16.9 ± 0.1	16.1 ± 0.1	15.6 ± 0.5	16.8 ± 0.0	16.1 ± 0.2	14.8 ± 0.3	16.8 ± 0.1	16.2 ± 0.1	14.8 ± 0.0
∑PUFA	41.9 ± 0.3	42.4 ± 0.3	41.5 ± 0.2	45.1 ± 0.2	44.5 ± 0.4	39.0 ± 0.3	42.8 ± 0.2	41.8 ± 0.4	39.8 ± 0.2
∑PUFA n-3	28.1 ± 0.2	27.3 ± 0.2	26.6 ± 0.2	28.9 ± 0.3	27.9 ± 0.2	25.7 ± 0.3	30.9 ± 0.2	30.4 ± 0.3	28.7 ± 0.2
∑PUFA n-6	5.9 ± 0.2	6.9 ± 0.1	7.1 ± 0.3	6.7 ± 0.2	7.3 ± 0.2	5.6 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.4 ± 0.1
∑n-3/∑n-6	4.8 ± 0.1	4.0 ± 0.1	3.7 ± 0.1	4.3 ± 0.2	3.8 ± 0.1	4.6 ± 0.1	7.0 ± 0.2	7.2 ± 0.1	6.5 ± 0.1
∑n-6/∑n-3	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
DHA/EPA	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.1
PUFA/SFA	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.8 ± 0.2	1.7 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	1.6 ± 0.2	1.5 ± 0.1
AI	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
TI	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
h/H	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.2

HM = freshly-harvested mussel; WM = washed and sorted mussel; MM = marketed mussel; and 11, 1, and 4 = sampling months of November, January and April, respectively; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AI = atherogenic index; TI = thrombogenic index; and h/H = hypocholesterolaemic/hypercholesterolaemic index. Values are mean ± standard deviation of triplicates (n = 3).

reported by Anacleto *et al.* (2014b), with the highest $\frac{n-3}{n-6}$ ratio also found in spring. According to Her Majesty's Stationery Office (HMSO, 1994) of the United Kingdom's Department of Health, the minimum recommended value for PUFA/SFA ratio is 0.45 while for $\frac{n-3}{n-6}$ ratio is 4:1, which supports the nutritional worth and nutraceutical potential of Tongyeong, South Korea's blue mussels. AI and TI values in fresh mussel samples were relatively constant at 0.6, 0.5, 0.6 and 0.2, 0.2, 0.2 for November, January, and April, respectively. The observed low AI and TI denote the blue mussels' ability to be used in cardio-protective and antithrombogenic diets attributed to the strong platelet-aggregation-inhibiting effect via prostanoid PGI3 production (Ulbricht and Southgate, 1991). DHA/EPA ratios in blue mussels were found to be 2.1 - 2.3, which stayed comparatively constant all throughout the seasons together with the hypocholesterolaemic/hypercholesterolaemic (h/H) index values (1.5 - 1.8). This suggests that blue mussels could help lower cholesterol levels and aid in reducing inflammatory heart diseases.

Antioxidant activities - DPPH radical scavenging activity, superoxide radical scavenging activity, and reducing power

Antioxidants helps protect the body against ROS-induced damages. Reactive oxygen, usually in the form of free radicals, superoxide ($O_2^{\bullet-}$), or hydroxyl radical; and non-radical species, ($HO^{\bullet}$) hydrogen peroxide (H_2O_2) is a by-product of normal metabolism. ROS attacks biologically-important molecules such as lipids, proteins, enzymes, DNA, and RNA which leads to cell and even tissue impairment associated with degenerative diseases (Jung *et al.,* 1999). Excessive free radical production and lipid peroxidation could cause pathological conditions like atherosclerosis, aging, nephritis, diabetes mellitus, rheumatic diseases, cardiac and cerebral ischemia, cancers, and adult respiratory distress syndrome (Miyake *et al.,* 2000; Pulido *et al.,* 2000).

Results of the antioxidant activity assessment of mussel samples are shown in Figure 1. The DPPH radical scavenging activity (RSA) of mussel samples were evaluated in a concentration-dependent manner (1, 2, and 3 mg/ml; Figure 1) per season and per processing step. Significant variations ($p < 0.05$) were found in all concentrations of the sample. The results revealed that the freshly-harvested mussel (before washed and marketed) exhibited the strongest radical-scavenging activity at 43.5, 42.1, and 50.7% for 3 mg/mL of mussel extract in November, January, and April, respectively, with the peak DPPH RSA observed in April. On the other hand, the superoxide radical scavenging activity of the mussel samples showed relative to diverse variation in all concentrations. A linear trend could be observed as the superoxide RSA of each sample increased along with increasing concentration of the mussel extract, especially with 3 mg/mL concentrations which exhibited significantly higher RSA activity. However, processing steps and season seemed to have fairly significant effect as presented by the relative superoxide RSA values observed at each concentration. Meanwhile, mussel samples showed significant reducing power ($p < 0.05$) with the maximum value found in freshly-harvested samples collected in April (HM-4) at 1.81 OD for 3 mg/mL of mussel extract. The reducing power of mussel samples increased accordingly, with concentration and processing steps considerably affecting the mussels' reducing power regardless of concentrations. Similarly, the peak DPPH RSA was also found in mussel samples collected in April with the same trend of freshly-harvested mussels exhibiting the highest DPPH RSA than the washed and marketed counter-parts. These results display a direct relationship between DPPH RSA and reducing power as reported by Bougatef *et al.* (2010). A similar result was observed in hydrolysates of *Sardinella* fish by-products obtained by alcalase treatment (2.24 OD, 2 mg/mL) and crude enzyme preparation from *Bacillus licheniformis* (1.98 OD, 2 mg/mL; Bougatef *et al.,* 2010).

Mussels are known to contain radical scavenging peptides and phospholipids that are rich in omega-3 fatty acids, which contribute to their antioxidant activity (Grienke *et al.,* 2014). Omega-3 (*n*-3) PUFAs in mussel samples assessed in the present work were also found to be the highest in April which might suggest its influence on their antioxidant capacity. However, further study should be done to investigate and confirm this connection. The lower DPPH RSA in samples collected in November and January could be due to the low seawater temperature during winter, as cold water conditions could cause reduced activity and lower metabolic rates in marine invertebrates (Abele and Puntarulo, 2004). In addition, higher oxygen solubility in seawater during winter also contributes to greater oxidative stress experienced by mussels (Abele and Puntaralo, 2004), as well as an increase in phospholipids (consequently, higher PUFAs) in the mantle of mussels. This makes them more prone to lipid oxidation, thus reducing their inherent antioxidant compounds upon harvest (Prato *et al.,*

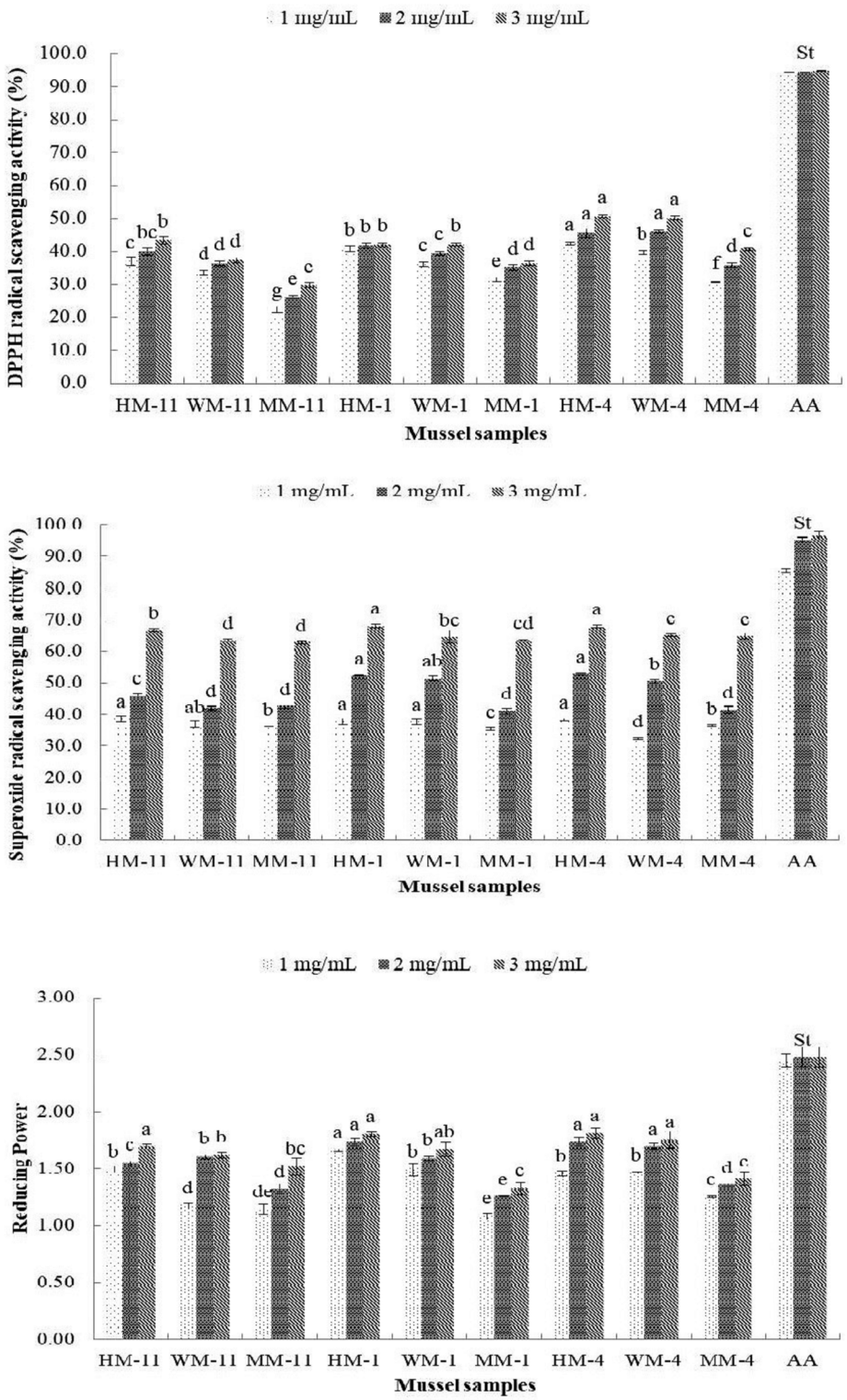

Figure 1. Antioxidant activities of processed mussel per season. HM = freshly-harvested mussel; WM = washed and sorted mussel; MM = marketed mussel; and 11, 1, and 4 = sampling months of November, January and April, respectively. Values are mean ± standard deviation of triplicates (n = 3). Bars having different letters are significantly different ($p < 0.05$).

2010). Other components that might be related to antioxidant activity are carotenoids (pigment compounds) and other phenolic compounds present in the mussel meat, although this was not assessed in the present work. One factor is noticeable from the results though; preserving mussel's freshness from harvest to market should be emphasised in order to benefit the most from its antioxidant effects.

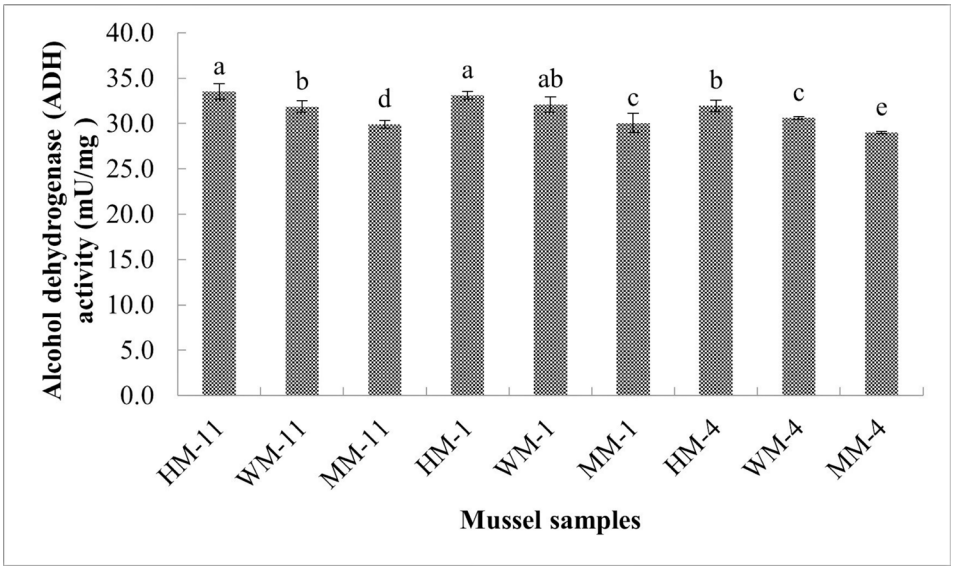

Figure 2. Alcohol dehydrogenase activity of processed mussel per season. HM = freshly-harvested mussel; WM = washed and sorted mussel; MM = marketed mussel; and 11, 1, and 4 = sampling months of November, January and April, respectively. Values are mean ± standard deviation of triplicates (n = 3). Bars having different letters are significantly different ($p < 0.05$).

Alcohol dehydrogenase activity

Alcohol dehydrogenases (ADH) are enzyme groups that catalyse the conversion of alcohols to aldehydes or ketones; wherein, nicotinamide adenine dinucleotide is reduced from NAD^+ to NADH which is related to alcohol detoxification. Approximately, 80% of alcohol absorbed in the body immediately after drinking is converted to acetaldehyde by ADH (EC 1.1.1.1) and oxidised to acetic acid by acetaldehyde dehydrogenase (ALDH, EC 1.2.1.10). The remaining 20% of alcohol is treated by microsomal oxidation (Lieber, 1991). ADH activity could be used to assess alcohol tolerance and help regulate vulnerability to alcoholism.

The evaluation of ADH activity of mussels per season is shown in Figure 2. There was no considerable seasonal variation in the data obtained from November to April (33.5, 33.1, and 32.0 mU/mg, respectively). However, treatment during processing reduced the ADH activity as displayed by the slight decrease in results (32.0, 30.6, and 29.0 mU/mg for HM-4, WM-4, and MM-4 samples, respectively). Mussel hot water extract is widely used in Korea as "*haejang-guk*" or soup that is taken to relieve hangovers. A comparative study of hangover soup's ADH activity was conducted by Ok *et al.* (2014). Arrowroot had the highest activity at 793.8%, followed by blue mussel (195.4%) for pure extracts. Blue mussel extract's ADH activity was double than that of mistletoe, shepherd's purse, bean sprout, and oriental raisin, and had synergistic effects when combined with the said plants/root herbs, thus proving why mussel soup is widely used in Korea for treating hangovers.

Conclusions

The collected data revealed that seasonal variation greatly affected mussel proximate composition, while the production steps had no significant effect on it. The most ideal time to consume mussels from Tongyeong, South Korea during harvest season is spring time (April) when proximate composition is average (not too high and not too low), and omega-3 PUFA content is at its peak, thus leading to greater antioxidant benefits. The highest antioxidant activities were observed in freshly-harvested mussels also in spring (April). Production steps also notably affected the antioxidant capacity of blue mussels which highlighted the importance of keeping the mussels' freshness during processing from harvest to market.

The atherogenic (AI), thrombogenic (TI), and hypocholesterolaemic/hypercholesterolaemic (h/H) indices remained relatively low all throughout the study, even under different processing conditions. From a nutritional fat quality perspective, the determined low AI, TI, and (h/H) values suggested that blue mussels could be utilised in a cardio-protective and low cholesterol diet. Moreover, the comparatively high ADH activities, which is associated with alcohol tolerance of mussel samples observed through the duration of harvest season, proved that mussels are a strong potential candidate

for seafood nutraceuticals production.

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