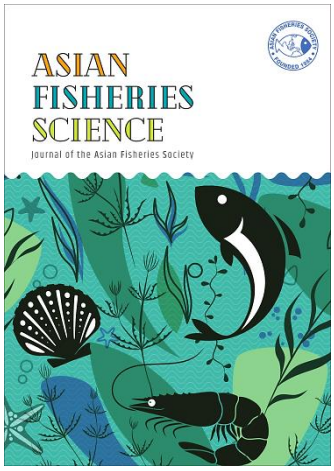

Effect of Storage Temperature on the Physicochemical and Sensory Properties of Green Mussel, *Perna viridis* (Linnaeus, 1758)

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Abstract

In the Philippines, the green mussel *Perna viridis* (Linnaeus, 1758), is an economically important aquatic commodity but there is a perceived lack of appropriate post-harvest technologies to preserve its freshness. Wet icing is normally employed, but it often leads to deteriorating texture. The present study assessed the influence of protease activities on the texture of mussels stored in different ice:mussel ratios (1:3, 1:4, and 1:5) over a 3-day icing period. Results showed that protease activities in mussel increased as the storage period. Significantly highest specific protease activity ($P < 0.05$) (17.53 ± 0.06 U.mg^{-1} protein) was detected in samples drawn from 1:5 ratio while soluble protein in mussel was significantly highest ($P < 0.05$) from 1:4 ratio (432.30 ± 2.28 mg.L^{-1}). Moisture content did not significantly vary among treatments, and the pH for all treatments was within the slightly acidic range. Results also showed that measured parameters in sensory evaluation and texture profile analysis were highest in mussel samples from 1:3 and 1:4 ratio. However, mussel from 1:5 ratio obtained the highest scores for flavour and texture acceptability. Findings revealed that addition of less ice leads to higher enzyme activity and mussel flesh degradation. However, it was more acceptable in terms of texture and flavour.

Keywords: enzyme activity, texture profile analysis, chilling, soluble protein

Introduction

In the Philippines, the green mussel *Perna viridis* (Linnaeus, 1758), which exhibits fast growth rate, is one of the commercially important seafood commodity; thus, making it a viable aquaculture organisms (Soon and Ransangan, 2014). The green mussel is known for its proteinaceous meat and as an excellent source of n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), along with essential minerals, amino acids, and vitamins (Orban et al., 2002; Astorga-Espana et al., 2007; Chakraborty et al., 2014).

The quality of mussels heavily depends on the temperature at which they are stored or transported. Live mussels may be transported without ice, but icing could minimise stress, increase quality, and extend the value of the mussel (Harding et al., 2004; Barrento et al., 2013). The usual practice of live mussel transport is in the chilled form with a temperature range of 7 - 10 °C although, there are also varying accounts of transporting it at 0 °C (Binsi et al., 2007; Barrento et al., 2013). Chilling, however, does not guarantee long-term preservation of mussel, as degradation can still occur; primarily attributed to the hydrolytic reactions catalysed by endogenous enzymes. The presence of proteolytic or collagenolytic enzymes especially in the digestive organs of the mussel plays a major role in this textural change, wherein the myofibrillar and extracellular matrix proteins that are degraded, could result in the softening of the muscle tissue during iced storage (Simpson et al., 2012; Sriket, 2014). Further, the capacity of the muscle tissue to hold water is compromised which in turn would provide an environment which is conducive for the growth of spoilage microorganisms (Hernandez-Herrero et al., 2003).

Generally, the texture may be defined as a sensory property and a functional manifestation of structural, mechanical, and surface properties of food detected through the senses (i.e. vision, hearing, touch, and kinesthetics) (Szczesniak, 2002). The deformation, disintegration and flow of the food upon application of force is usually related to the texture (Bourne, 2002). Instrumental texture analysis is essentially designed to provide such objective measurements that reflect attributes of sensory evaluation made by taste panels (Trinh and Glasgow, 2012). Using an instrumental texture analysis can provide important comparison with sensory assessments by involving discrete, well-defined physicochemical properties. Whereas, sensory perception is not often discrete, and various stimuli (within or across different sensory modalities) interact at both physiological and psychological levels. Establishing a correlation between the instrument and sensory analysis is thus important to ascertain that important perceived sensory characteristics of the product are as intended in the consumer goods (Kilcast, 2013). Texture profile analysis (TPA) has been used in a wide range of food products but has not yet been used in studies on bivalves, particularly in the mussel.

Thus, this study primarily aims to assess the changes on the physicochemical and sensory properties of green mussel subjected to storage at different ice to mussel ratios (1:3, 1:4, and 1:5). Specifically, this study intends to determine the characteristics of the mussels through sensory evaluation and TPA and relate these properties to the total protease activities and soluble protein content of the mussel during iced storage.

Materials and Methods

Collection and preparation of samples

Live green mussels *P. viridis* were collected from mussel-growing areas in Barangay Buntod, Municipality of Panay, Capiz. Mussel samples were cleaned on-site with byssal threads kept intact, placed in clean large low density polyethylene (LDPE) plastic bags and packed inside insulated boxes with ice at the bottom. This was to keep the samples at a temperature of 15 - 20 °C during the 3–4 h transport to the laboratory in University of the Philippines Visayas, Miagao, Iloilo. Fresh mussels were cleaned using filtered seawater. Only live mussels were used in the icing experiment. The average length, width and dry weight of mussels (n = 30) used in the study were 6.79 ± 0.24 cm, 3.14 ± 0.13 cm and 0.84 ± 0.14 g, respectively. Samples were then stored in insulated containers with ice to mussel ratios of 1:3, as the control (Warwick, 1984), 1:4, and 1:5 over a period of 3 days.

Chilling conditions

Each insulated polystyrene box initially containing 10 kg of live green mussels packed in polyethylene (PE) bags (1 kg in each PE bag) were placed below the layers of ice, making sure that the ice and live mussels were not touching. This was done in triplicates for each ice to mussel ratio. Re-icing was done every morning to maintain the ratio (internal temperature of the shellstock at 9.61 ± 0.94 °C, 1:3 ratio; 12.05 ± 0.96 °C, 1:4 ratio; 13.07 ± 0.45 °C, 1:5 ratio). The experiment was carried out for 3 days with the initial sampling at 0 h and at every 24 h intervals after that. From each lot, PE bag of mussel was selected at random and were used for total protease assay, soluble protein content, moisture content, pH determination, TPA and organoleptic tests. There were 10 randomly selected samples from the 1kg PE bag that were used for the crude enzyme extraction, and about 30 pieces were also taken for TPA and sensory evaluation.

Moisture content and pH analyses

The moisture content of each sample was measured following the standard oven method (AOAC, 2016) using 5 g of the homogenised mussel meat. For pH analysis (pHasion C-73X pH meter, Asone Co., Japan) about 10 g of homogenized sample was used. Measurements were carried out at sample temperatures ranging from 18.7–25.8 °C.

Preparation of crude enzyme extracts

The extraction method for crude enzyme followed the protocol of Tizon et al. (2012) with slight modification. Fresh samples were macerated using a homogeniser (WiseTis HG-15D, Witeg, Germany) and transferred into a pre-chilled mortar and pestle. It was then washed with cold 50 mM, pH 7 citrate-phosphate buffer, added to the mussel at 1:30 (w/v). The samples were then transferred in centrifuge tubes and were centrifuged at 4000 ×g for 15 min. The supernatant was filtered through Whatman No. 1 filter paper, and the filtrate was used as the crude enzyme extract. All procedures were performed at 4 °C unless otherwise indicated.

Determination of protease activity

Conditions for routine total protease activity determination were established by performing linearity assay with time and crude enzyme concentration. The total protease was measured using casein as a substrate and L-tyrosine as the standard. The reaction mixture consisted of 1 mL 1 % casein dissolved in 0.01N NaOH, 1.7 mL phosphate buffer, pH 7, and 0.3 mL enzyme extract in a final volume of 3 mL. Assays were done at ambient temperature (30 ± 2 °C). The reactions were allowed to proceed for 30 min and were stopped by adding 1

mL of 5 % ice-cold TCA. The reaction mixture was filtered through a Whatman No. 1 filter paper and amount of tyrosine in the solution was measured by reading the absorbance at 280 nm. One unit of protease activity is the amount of enzyme required to liberate 1 µmol of tyrosine from casein per min per mL at 30 °C, while specific activity was expressed as Units of protease activity per mg of protein in the sample (U.mg^{-1}).

$$Specific\ activity\ (U.mg^{-1}) = \frac{U.mL^{-1}}{Concentration\ of\ protein \times Volume\ of\ enzyme\ sample}$$

Determination of soluble protein content

The soluble protein of the mussel was measured following the protocol of Lowry et al. (1951) which was modified by UIUC (2004) using bovine serum albumin as standard. The determination was done for all crude enzyme preparation.

Texture profile analysis (TPA)

Mussel samples for this analysis were steamed for 5 min prior to measurement. Objective measurement of texture was done through automated analysis using CT3 Texture Analyser (Brookfield AMETEK, USA) through puncture method with the use of a penetrating needle type probe (TA3/100) and a fixture (TA-RT-KIT). The attributes being measured for TPA was modified to give an accurate analysis that is suitable for the steamed mussel sample. First cycle measured hardness, deformation at hardness, hardness work done, recoverable deformation, recoverable work done, and total work done. The second cycle measured hardness, hardness work done, cohesiveness, recoverable deformation, recoverable work done, total work done, springiness, springiness index, gumminess, chewiness, chewiness index, corrected cohesiveness, corrected gumminess, and corrected chewiness. This was set at: target, 10 mm; trigger load, 5 g; test speed, 1 mm. s^{-1}; return speed, 1 mm.s^{-1} and number of cycles, 2.

Sensory evaluation

Sensory evaluation was performed by 10 semi-trained panellists. The sensory scorecard and acceptability score sheet were modified to suit the parameters measured in the TPA. The evaluation includes assessment of flavour, texture, and acceptability of flavour and texture of the samples from each chilling ratio. A 5-point scale was used for the sensory scorecard with the following characteristics evaluated: (1) flavour of the meat with a score of five for sweet/natural taste to one for off-flavour taste, (2) texture of the meat in terms of firmness with five for firm/plump to one for very soft, and (3) texture of the meat in terms of hardness with five having a very chewable characteristic to one for very gummy. For the 7-point hedonic scale for acceptability, the following scale system was used: 7-like very much, 6-like moderately, 5-like slightly, 4-neither like nor dislike, 3-dislike slightly, 2-dislike moderately and 1-dislike very much.

Statistical analysis

The significant difference of the mean from the different measured parameters were determined using two-way analyses of variance (3 × 3 factorial in completely randomised design) at 0.05 level of significance with the use of IBM SPSS Software Package ver. 23. There were three treatments with differing ice to mussel ratio (1:3, 1:4, and 1:5) within a 3-day icing period, thus relationship and interactions of the treatments across the 3-day icing period at a certain parameter were measured. One-way ANOVA was run to confirm statistical significance and Univariate ANOVA was also measured to test between-subjects effects (ratio, days, and ratio-day interaction). Levene's test was used to test for the homogeneity of the variances and ANOVA contrasts was done for the statistically significant values to determine statistically significant interactions in the treatment or day given a certain parameter ($P < 0.05$).

Results

pH and moisture content of mussel over the 3-day icing period

The initial pH value showed that the green mussel samples were acidic at pH 5.87 (Table 1). After days of icing, the pH of mussels stored in the different ice:mussel ratio significantly increased ($P < 0.05$), although it still remained in slightly acidic pH range (6.23 to 6.25). No significant differences ($P > 0.05$) were observed among the three treatments.

The initial moisture content of the mussel prior to icing was 86 % (Table 1). Icing brought about significant increases ($P < 0.05$) in the moisture content of mussel for 3 days. Highest mean moisture content (88.11 %) was found in mussel stored in 1:3 ice to mussel ratio although no significant differences were found among the values obtained in the different treatments.

Table 1. Mean pH and moisture content of green mussel *Perna viridis* at initial 0 day and stored in 1:3, 1:4 and 1:5 ice:mussel ratio for 3-day period.

Ice:Mussel ratio	pH	Moisture content (%)
Initial	5.87 ± 0.05^{a}	86.00 ± 0.43^{a}
1:3	6.23 ± 0.06^{b}	88.11 ± 0.68^{b}
1:4	6.24 ± 0.07^{b}	87.99 ± 0.76^{b}
1:5	6.25 ± 0.03^{b}	87.90 ± 0.88^{b}

Values represent the mean scores ± SD from replicate determinations (n = 10). Values sharing the same superscript were not considered significantly different ($P > 0.05$).

Total protease activities and soluble protein content in the mussel after the 3-day icing period

It was observed that the highest initial (day 1) specific protease activity was found in mussel samples from 1:3 ratio (7.58 U.mg^{-1} protein) while the lowest activity was from mussel samples in the 1:5 ratio (5.04 U.mg^{-1} protein) (Fig. 1). After 3 days, however, the highest activity was found in mussel samples from 1:5 ratio at 17.53 U.mg^{-1} protein while the lowest was from the samples stored in 1:3 ratio at 12.79 U.mg^{-1} protein. Statistical analysis revealed significant differences ($P < 0.05$) in mussel total protease activities across treatments and across icing periods.

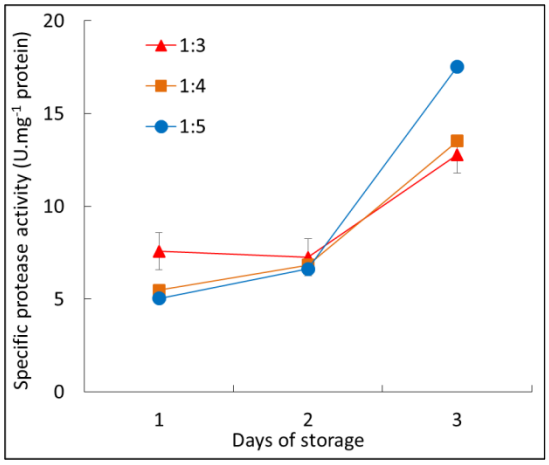

Fig. 1. Changes in specific protease activities of green mussel *Perna viridis* stored from day 1 to day 3 in 1:3, 1:4 and 1:5 ice:mussel ratio.

Mussels stored in both 1:4 and 1:5 ratios showed consistent increases in enzyme activities as storage time progresses with a sharp increase on the 3rd day observed in samples from the 1:5 lot. On the other hand, mussels kept in 1:3 ratio only showed a significant increase ($P < 0.05$) in total protease activity on the 3rd day, although it started with the highest activity among treatments on the 1st day.

Highest soluble protein content after one day of ice storage was found in mussel from the 1:3 ratio at 426.88 mg.L^{-1}, while the lowest was found in samples taken from 1:5 ratio at 399.31 mg.L^{-1} (Fig. 2). At the end of the 3-day icing period, mussel samples from 1:4 ratio had the highest soluble protein content (432.30 mg.L^{-1}) while mussels from 1:5 ratio was consistently lowest (386.79 mg.L^{-1}). Soluble protein content in samples stored in 1:3 and 1:4 ice ratio increased in the first 2 days of storage and decreased thereafter. Mussels taken from the 1:5 ratio showed a decrease in soluble protein content after 2 days and an increase on the 3rd day but was still significantly lower ($P < 0.05$) than those found in other treatments.

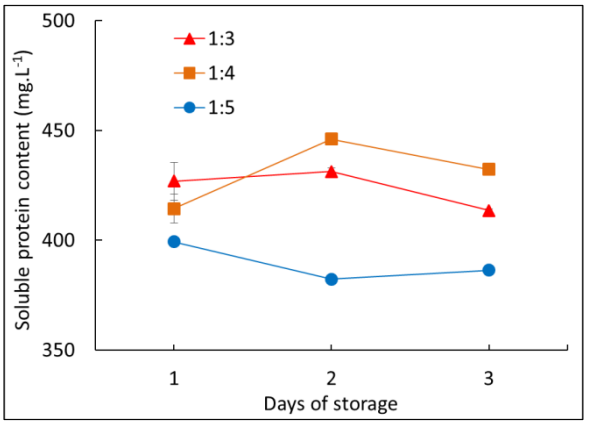

Fig. 2. Changes in soluble protein content of green *Perna viridis* mussel stored from day 1 to day 3 in 1:3, 1:4 and 1:5 ice:mussel ratio.

Textural changes in mussel through sensory evaluation and texture profile analysis

Sensory evaluation

Results of the sensory evaluation show that there were significant differences ($P < 0.05$) in the mean of firmness, flavour acceptability, and texture acceptability while no significant differences ($P > 0.05$) were found in flavour and hardness of the samples (Table 2). Further statistical analysis revealed that hardness and flavour attributes were not significantly affected ($P > 0.05$) by ice ratio and icing period.

Mussel samples from 1:5 and 1:3 ratio obtained the highest and the lowest scores for flavour (between bland and slightly sweet), respectively. In terms of hardness, the highest value was found in mussels from 1:3 ratio (chewable) while the lowest scores were from samples stored in 1:5 ice ratio (moderately chewable) at the end of the 3-day icing period. In both attributes, decreasing sensory scores with increasing storage time were observed.

Firmness was significantly highest ($P < 0.05$) in samples from 1:3 ratio and lowest in mussel samples taken from 1:5 ratio in the initial sampling. At the end of the storage period however, mussels from the 1:3 ratio obtained the lowest scores while the highest was found from mussel samples found in 1:4 ratio but was with statistically insignificant ($P > 0.05$). The firmness of mussel stored at the lowest temperature (1:3 ratio) significantly increased ($P < 0.05$) on the 2nd day of storage and significantly decreased ($P < 0.05$) on the last day. Mussel samples from both 1:4 and 1:5 ratios have observable but not significant decreases ($P > 0.05$) in firmness scores.

Table 2. Sensory evaluation scores and acceptability of green mussels *Perna viridis* stored in 1:3, 1:4 and 1:5 ice:mussel ratio during the 3-day icing period.

Icing period (Days)	Ice:Mussel ratio	Sensory attributes			Acceptability	
		Flavour	Firmness	Hardness	Flavour	Texture
1	1:3	4.30 ± 0.62	4.32 ± 0.60^{a,d}	4.37 ± 0.47	6.32 ± 0.34^{a,d}	6.40 ± 0.42^{a,d}
	1:4	4.21 ± 0.37	4.00 ± 0.41^{ab,d}	4.17 ± 0.50	5.59 ± 0.50^{b,d}	6.00 ± 0.67^{a,d}
	1:5	3.89 ± 0.65	3.68 ± 0.35^{b,d}	4.10 ± 0.48	5.78 ± 0.26^{ab,d}	6.40 ± 0.43^{a,d}
2	1:3	3.63 ± 0.23	3.00 ± 0.42^{a,e}	4.02 ± 0.37	5.11 ± 0.58^{a,de}	5.63 ± 0.53^{a,e}
	1:4	3.4 ± 0.53	4.31 ± 0.52^{b,d}	4.08 ± 0.53	5.18 ± 0.27^{a,d}	5.49 ± 0.50^{a,d}
	1:5	3.60 ± 0.61	3.73 ± 0.29^{b,d}	4.02 ± 0.59	5.31 ± 0.54^{a,d}	5.53 ± 0.48^{a,e}
3	1:3	3.47 ± 0.46	3.61 ± 0.49^{a,f}	4.23 ± 0.26	4.93 ± 0.82^{a,e}	5.47 ± 0.45^{a,e}
	1:4	3.49 ± 0.50	3.71 ± 0.36^{a,d}	4.12 ± 0.38	5.40 ± 0.45^{a,d}	5.66 ± 0.40^{a,d}
	1:5	3.56 ± 0.46	3.62 ± 0.37^{a,d}	4.00 ± 0.36	5.50 ± 0.70^{a,d}	6.12 ± 0.45^{a,de}

Values represent the mean scores ± SD from replicate determinations (n = 10). Values sharing the same superscript (a-c, difference across treatments; d-f, difference across days of storage) were not considered significantly different ($P > 0.05$).

For flavour acceptability, an initial significant difference ($P < 0.05$) in the score of mussel from the 1:4 ratio was observed. In all treatments, the flavour acceptability of mussel samples decreased over time. In the last day of storage, there were no significant differences in the flavour acceptability of mussel in all treatments but the highest score was obtained by samples from the 1:5 ice ratio. Mussels collected from 1:3 ratio showed a significant decrease ($P < 0.05$) in the score from the 1st and the last day of icing.

The acceptability of texture of mussels in the different treatments showed a generally decreasing trend from the initial day to the last day of storage. There are no observed significant differences ($P > 0.05$) in the perceived texture acceptability of the samples across treatments. For mussel stored in the 1:3 ice ratio, scores significantly decreased after 1 day of storage. In higher ice ratios, texture acceptability scores showed no significant differences in the initial and third day of storage although a significant increase in the score ($P > 0.05$) was observed on the second day for mussel from the 1:5 ratio.

Texture profile analysis

Table 3 shows that attributes chosen for the TPA were comparable with that of the attributes from the sensory analysis as well as appropriate parameters for the texture of the mussel samples were included. It can be observed that all attributes, except corrected cohesiveness, were found to have significant differences ($P < 0.05$).

Statistical analysis showed that the highest value for hardness in the 1st day was in mussel samples taken from 1:3 and 1:4 ratio and were significantly higher ($P < 0.05$) than the mussels collected from 1:5 ratio. In the last day, the highest values were still found in samples from the 1:3 and 1:4 ratios while the lowest was consistently found in the mussels from 1:5 ratio. Generally, the hardness of samples across treatments increased in the second day but decreased again on the last day of storage.

Resilience values of the samples did not show significant differences ($P > 0.05$) across treatments during the initial and final storage period. On the last day, the highest resilience value was found in mussel samples from 1:4 ratio while mussels from 1:5 ratio was consistently lowest. A significant increase ($P < 0.05$) in resilience for mussels taken from both 1:4 and 1:5 ratios were observed in the 3rd day, whereas mussels from 1:3 ratio showed no significant changes ($P > 0.05$).

Values obtained for springiness index of the mussel sample from the three treatments showed no significant differences ($P > 0.05$) in the first 2 days of storage. On the 3rd day however, springiness index was significantly lower in the mussel from 1:5 ratio. Furthermore, despite a fluctuating trend in values, mussels taken from 1:3 and 1:4 ratios showed an increase in springiness index at the end of the icing period while values from the 1:5 ratio showed a significant decrease ($P < 0.05$).

Corrected chewiness showed a range of values from 0.33 to 1.27 mJ. Highest and lowest values were consistently found in the 1:3 and 1:5 ratio, respectively, during the 1st and last days of icing. Significant differences ($P < 0.05$) among treatments

Table 3. Texture profile of green mussels *Perna viridis* stored in 1:3, 1:4 and 1:5 ice:mussel ratio during the 3-day icing period.

Icing period (Days)	Treatments Ice:Mussel ratio	Characteristics				
		Hardness (g)	Resilience	Springiness index	Corrected cohesiveness	Corrected chewiness (mJ)
1	1:3	$24.33 \pm 2.08^{a,d}$	$0.037 \pm 0.015^{a,d}$	$0.62 \pm 0.036^{a,d}$	0.42 ± 0.12	$0.60 \pm 0.17^{a,d}$
	1:4	$22.33 \pm 0.58^{a,d}$	$0.033 \pm 0.0058^{a,de}$	$0.70 \pm 0.047^{a,d}$	0.59 ± 0.34	$0.60 \pm 0.00^{a,d}$
	1:5	$15.00 \pm 0.00^{b,d}$	$0.020 \pm 0.0100^{a,d}$	$0.63 \pm 0.040^{a,d}$	0.51 ± 0.11	$0.47 \pm 0.12^{a,d}$
2	1:3	$31.00 \pm 2.65^{a,e}$	$0.050 \pm 0.0173^{a,d}$	$0.62 \pm 0.082^{a,d}$	0.45 ± 0.11	$0.83 \pm 0.15^{a,d}$
	1:4	$27.00 \pm 4.36^{ab,d}$	$0.030 \pm 0.0100^{b,d}$	$0.59 \pm 0.076^{a,d}$	0.47 ± 0.03	$0.63 \pm 0.21^{a,d}$
	1:5	$19.00 \pm 4.58^{b,d}$	$0.020 \pm 0.0100^{b,d}$	$0.74 \pm 0.052^{a,d}$	0.51 ± 0.18	$0.70 \pm 0.26^{a,d}$
3	1:3	$30.33 \pm 3.79^{a,de}$	$0.047 \pm 0.0058^{a,d}$	$0.76 \pm 0.006^{a,e}$	0.58 ± 0.10	$1.27 \pm 0.06^{a,e}$
	1:4	$33.33 \pm 3.79^{a,e}$	$0.050 \pm 0.0100^{a,e}$	$0.70 \pm 0.154^{a,d}$	0.41 ± 0.17	$0.60 \pm 0.10^{b,d}$
	1:5	$16.00 \pm 1.00^{b,d}$	$0.043 \pm 0.0058^{a,e}$	$0.49 \pm 0.040^{b,e}$	0.43 ± 0.09	$0.33 \pm 0.12^{c,d}$

Values represent the mean scores ± SD from replicate determinations (n = 6). Values sharing the same superscript (a–c, difference across treatments; d–f, difference across days of storage) were not considered significantly different ($P > 0.05$).

were only observed on the last day. Furthermore, only mussels taken from 1:3 ratio exhibited a significant change ($P < 0.05$) from the 1st to the last day, showing a constantly increasing trend.

Discussion

In the present study, several factors that may contribute to the change in physicochemical, sensory qualities and integrity of the green mussel *P. viridis* flesh such as pH, moisture content, proteolytic enzyme activity, sensory evaluation, and TPA, were investigated and measured. Measurement of pH is important in post mortem studies because it indicates the extent of glycogen conversion into lactic acid and the degradation of muscle components such as proteins and nucleotides. In the present study, there was an increase in pH of the mussel tissues which may indicate that the low temperature preservation of the samples during 3-day iced storage retarded decomposition of glycogen. Similarly, in a study conducted by Erkan (2005), the pH in shucked mussels (*Mytilus galloprovincialis* Lamark, 1819) during 6-day cold storage did not change significantly. This finding is comparable to the results of the present study wherein significant increase only occurred between the initial and after one day of iced storage with no significant changes occurring within 3 days among treatments.

There are certain digestive enzymes like trypsin that are activated by higher pH and favours optimal hydrolytic activity (Hultmann and Rustad, 2004). For fish and shellfish, serine collagenolytic enzyme is relatively stable within pH range of 6–11 (Aoki et al., 2003; Klomklao, 2008; Sriket et al., 2012). Spoilage in molluscan shellfish can be measured through the decrease in pH since it is deemed to be reliable and objective, although in other seafood, spoilage is indicated by the level of volatile nitrogen bases. A pH of 6.5 to 5.9 is considered to be good; however a pH of 5.8 and below is no longer acceptable for consumption in terms of palatability (Jay et al., 2005). This therefore indicates that the pH of the mussel in all treatments across a 3-day icing period in this study was within the range that is still palatable for the consumers. The findings are supported with the results of the sensory evaluation wherein the flavour acceptability of the mussel in the different treatments although variable, were consistently within the acceptable level. This also suggests that the mussels did not undergo further autolysis within the observed icing period that would have resulted in a decline of pH to 5.4–5.8 in the tissues (Huff-Lonergan and Lonergan, 2005).

Water-holding capacity, defined as the capacity of fresh meat to retain its inherent moisture when subjected to external pressures such as compression or centrifugation, is used an indicator of freshness in fish and aquatic products (Kerry et al., 2002). Early post-mortem biochemical processes including a decline in pH contribute to the reduction of the water holding capacity of the meat, whereby low acidic pH causes denaturation or loss of functionality of protein (Huff-Lonergan and Lonergan, 2005). In the present study, the significant drop in pH observed in mussel samples from the initial and during storage did not lead to a reduction in moisture which may be due to the pH still being in the slightly acidic range. In fact, an increase in moisture was observed with iced

storage. This increase may be associated with the decrease in protein content as observed in several studies wherein an inverse relationship between protein and moisture content was established in mussel (Binsi et al., 2007; Chakraborty et al., 2016). These cytoskeletal proteins which have been shown to degrade in as early as 45 min up to 6 h post mortem in some muscles may possibly allow small quantities of water expelled from the intramyofibrillar spaces to be retained in the cell for a longer period of time (Kristensen and Purslow, 2001; Bee et al., 2004; Melody et al., 2004; Bertram et al., 2007). Generally, an increase in the moisture content indicates that water-binding capacity of proteins has weakened, thus the degradation of muscle (Suárez et al., 2005). However, it is also possible that the rapid degradation of the intermyofibril linkages leads to the creation of more "space" for water retention which in turn results in less water initially lost and an increase in water-holding capacity (Melody et al., 2004).

Mussels are primarily composed of carbohydrates which suggest that there may be an abundance of carbohydrases (Jay et al., 2005). In *Mytilus chilensis* Hupé, 1854, high carbohydrase activities and low protease activities were found, suggesting that carbohydrases play a more important role in catalysing extracellular and intracellular digestion (Fernandez-Reiriz et al., 2001). Despite having dominant carbohydrases in the digestive gland and crystalline style of mussel, active proteases in the muscle and digestive organ of fish and shellfish make their flesh prone to degradation especially during iced storage (Garcia-Carreno and Hernandez-Cortes, 2000; Sriket, 2014). Lysosomal enzymes such as purified cathepsins B, C, H, L, and S that has been characterised from fish and shellfish are known to be the major proteases which participate in intracellular protein breakdown, making the measurement of total proteolytic activity relevant (Pangkey et al., 2000; Aoki et al., 2004). In the current study, it was observed that despite having a low temperature condition there were increasing proteolytic activities in the mussel samples with increasing icing period in all ice to mussel ratios tested.

Hydrolysis of myofibrillar and connective tissue protein by the action of proteolytic enzymes during iced storage is responsible for the deterioration of the integrity of the mussel meat (Sriket, 2014). The present study showed that the samples from 1:5 ratio had the highest enzyme activity and lowest soluble protein content compared to other samples with higher ice addition. It is possible that proteolytic activities are mainly involved in the hydrolysis of myofibrillar and collagenous proteins since serine proteases responsible for these reactions are known to be active within the first 24 h post mortem and have an optimal pH at 5–7.5 (Camou et al., 2007; Sriket, 2014).

Hardness or firmness are important parameters that are often used to determine the freshness of the food (Chen and Opara, 2013). Textural changes in terms of hardness showed no significant differences ($P > 0.05$) in the sensory evaluation but were found to be significantly varied ($P < 0.05$) in the TPA. Nevertheless, both analyses showed similar trend wherein mussel samples from 1:5 and 1:30 ratio had the lowest and highest scores, respectively. Lower storage temperature results in a decrease in protease activity that can limit muscle softening, thus the higher temperature in 1:5 lot during storage could yield a shorter period in which rigour mortis was maintained. This could lead to further and faster degradation leading to the destruction of the integrity of its meat (Ando et al., 2007). This rapid proteolysis at a higher temperature might have been brought about by proteases degrading the key cytoskeletal proteins during post-mortem in pre- and post-rigour muscle (Melody et al., 2004). Mussel samples stored in 1:3 and 1:4 ratio have significantly higher hardness values ($P < 0.05$) than those from the 1:5 ratio based on the TPA, but sensory evaluation results did not significantly vary ($P < 0.05$) among the three lots.

Firmness is defined as the resistance of the food against deformation. This can easily be estimated by either Young's modulus or the breaking stress achieved from the force-displacement curve. Because modulus of elasticity is defined as the ratio of stress to strain, it is a measure of resistance to force or the firmness of a material (Trinh and Glasgow, 2012; Chen and Rosenthal, 2015). In the current study, this attribute was measured through sensory evaluation. At the end of the 3-day icing period, the values did not have any significant differences ($P > 0.05$) among the treatments, however highest firmness values were found in mussels taken from 1:4 ratio. In terms of texture acceptability, the mussels from 1:5 ratio scored the highest as it was the least hard compared to samples taken from 1:3 and 1:4 lots. Samples stored in lesser ice resulted in higher enzymatic activity leading to a more acceptable texture of slightly firm and moderately chewable compared to the very firm samples from other lots. The softening of the mussel tissues due to faster degradation did not seem to be a deterrent factor for the panellists because in consumer evaluation of meat quality, a meat that will give high acceptability has attributes that are tender, juicy, and low-fibrous (Huffman et al., 1996; Behrends et al., 2005). The result is in agreement with flavour scores and acceptability of mussel in which the highest scores was observed from the 1:5 ratio was characterised by sweet and natural flavour and the lowest from the 1:3 ratio having slightly sweet to bland flavour. Thus, the present study revealed that panellists lean more towards the mussel meat with a softer texture as it has a more desirable flavour.

Other attributes relating to texture as measured by the texture analyser such as corrected chewiness,

resilience, springiness index, and corrected cohesiveness showed that mussel in 1:3 and 1:4 ratios obtained the highest values, except in corrected cohesiveness in which no significant difference ($P > 0.05$) in the treatments across the 3-day icing period was observed. The chewiness is measured as the energy needed to chew solid food until it is ready for swallowing (Trinh and Glasgow, 2012). In the present study, the chewiness of the mussel samples from 1:5 ratio is consistent with its hardness in obtaining the lowest values. This means that when mussels are stored in colder temperatures, it requires higher effort in masticating the solid mussel. This was deemed to be an undesirable characteristic for the panellists. Cohesiveness, on the other hand, is the strength of the internal bonds of the sample. At the end of the 3-day icing period, the highest value for cohesiveness was from the mussel samples stored in 1:3 ratio and the lowest was from 1:4 ratio. Although, based on the statistical analysis, the changes in the values did not have significant differences ($P > 0.05$) among treatments across the 3-day icing period. Despite fluctuating results during storage, results for cohesiveness was consistent with other textural attributes in mussels stored in 1:3 ratio which had the highest meat integrity. Results for the mussel samples stored in higher ice ratios showed a downward trend for these attributes which may be due to the proteolytic softening of muscle tissue (Simpson et al., 2012).

Resilience is the ability of a product to fight in order to regain its original height (Trinh and Glasgow, 2012). Similarly, springiness (originally called elasticity) is defined as the rate of the deformed sample to return to its original size and shape wherein rate is used as the ratio of the height between two distances. In the current study, high values from both resilience and springiness indices were found in mussels stored in 1:3 and 1:4 ratios, with low values observed in mussels stored in 1:5 ratio. The results are consistent with the other textural variables wherein the mussel samples from either 1:3 or 1:4 ratio has the highest value in terms of their objective textural attributes.

The present study serves as a preliminary study on the texture profile of the green mussel, thus TPA results from the needle penetrating probe used should be viewed sceptically as there might be a more appropriate probe. This is because a smaller diameter puncture probe typically does not work well for TPA test as this does not replicate the masticatory action well due to the size of compression probe. It should either be the same size or larger than the sample as to ensure that the forces registered in the TPA test are uniaxial compression forces and covers the whole sample piece studies (Pons and Fiszman, 1996). This is supported by studies from Zhu et al. (2011) and Dong et al. (2018) wherein they used a 50-mm flat plunger (probe P/50) and a P/100 probe (compression plate), respectively, and determined shearing force both using a heavy duty platform/blade set (HDP/BS) blade on abalone *Haliotis discus hanai* muscle. Since abalone and mussels are both molluscs, it can be inferred that the muscle structure of both organisms are similar, thus the probe used in their study might give a more meaningful result.

Conclusion

The current study determined the changes in the physicochemical and sensory properties of mussels kept in three ice to mussel ratio set-ups over a 3-day icing period. Results of the sensory evaluation using the 10 semi-trained panellists revealed that difference in icing ratio did not affect the flavour and hardness of the mussel but have a significant effect on the firmness, acceptability of flavour and of texture of the stored samples. Generally, mussel samples from 1:5 ratio had the lowest scores and samples from 1:3 ratio had the highest values for texture attributes. TPA using the texture analyser showed a similar trend in the values obtained corrected chewiness, resilience and springiness index. The low scores in texture indicated slightly firm and moderately chewable mussel meat wherein the relative soft texture from the 1:5 ratio was the most preferred texture of the panellists. This generally softer texture may be due to the faster textural degradation resulting from the higher total proteolytic activity found in mussels stored in the lowest ice ratio. The soluble protein content in the mussel from 1:5 ratio was also the highest which may be due to increase in moisture content. The pH of the mussel was affected by the chilling period but not due to the amount of ice added. The pH range within the 3-day chilling period is within the values considered fit for consumption. Findings of this study may serve as a preliminary or baseline data for TPA in mussel during iced storage, which cannot be found in any published literature. It is however recommended that different probes be used to compare the results depending on the objectives of specific texture analysis and establish its correlation with the sensory analysis.

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