RESEARCH ARTICLE

Quality Changes in the Adductor Muscle of Ezo Giant Scallop *Mizuhopecten yessoensis* (Jay, 1857) during Refrigerated Storage

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Abstract

Recently, the popularity of scallops consumption and the preference to eat them raw have been increasing worldwide. Therefore, maintaining its freshness and quality is important. It is necessary to investigate the changes in quality, particularly umami-related component parameters and perform a comprehensive evaluation to assess scallop quality over time. In this study, the distinction in the abundance of microorganisms, *K* value, pH, color value, glycogen content, and ATP-related compound levels (i.e., ATP, ADP, AMP, IMP, HxR, Hx, and glutamic acid levels) were investigated to determine the quality of Ezo giant scallops. The parameters were evaluated every day for six days at 4°C post mortem of the scallops. The total viable aerobic count of marine bacteria increased from 1 to 3 log CFU/g over six days, and the *K* value increased sharply from 18% on day 2 to 66% on day 4. The pH decreased from 7.0 on day 0 to 6.0 on day 3, but the color value did not change during the six days of observation. The AMP content increased over three days and then decreased during the last three days of storage. IMP was not detected; meanwhile, the glycogen and glutamic acid levels were stable during the observation. Based on these results, the best recommendation is to serve the refrigerated scallops as sashimi for not more than two days and cook by the third day to preserve the quality.

Keywords: scallop, marine product, umami components, seafood, adductor muscle

Introduction

In recent years, the consumption of marine products has been increasing along with food distribution and the need for a highly proteinaceous diet. The global demand for marine products is expected to grow with the increasing population worldwide (Fisheries Agency, 2016). Japanese marine products are high-quality and highly valued globally (Ministry of Agriculture, Forestry, and Fisheries, 2014). The most exported Japanese marine product is scallops (84,000 tons in 2019) (Policy Research Institute, Ministry of Agriculture, Forestry and Fisheries, 2020). In 2013, the Hokkaido Federation of Fisheries Cooperatives scallop "hanging ceremony" and "digit net fishing" fisheries' export volume in 2013 was approximately 60,000 tons. It increased to 85,000 tons in 2018 (Hakodate Customs, 2019). Scallops are consumed in several countries, and their export is expected to increase. Currently, most marine products are exported as "frozen," but in recent years, opportunities to export these products "raw and refrigerated" are increasing. Quality assessment is critical when exporting "raw" products; consequently, accurate quality evaluation is necessary to confirm quality assurance.

To accurately evaluate the quality of marine products, especially scallops, it is necessary to investigate the alteration in quality-related parameters and perform a comprehensive evaluation over time. Such quality-related parameters include the abundance of microorganisms, *K* value, pH, color value, odor, and degree of oxidation. As the lipid content in the Ezo giant scallop *Mizuhopecten yessoensis* (Jay, 1857) is low, the changes in hardening, pH, *K* value, arginine and octopine levels over time are considered important for the quality evaluation (Kimura, 2003). However, as scallops are a popular food due to their "deliciousness," investigating the changes in umami-related components and analyzing quality-related parameters over time is necessary.

The umami-related components in scallop are glycogen, inosinic acid (IMP), adenosine monophosphate (AMP), and glutamic acid (Glu)

(Yamanaka, 2007). In particular, a moderate positive correlation between the detection threshold and taste preference associated with Glu and IMP has been reported, at 0.46 and 0.36, respectively. Besides, the range of preferred Glu and IMP concentrations is 3.13 to 200 mM and 0.313 to 20 mM, respectively (Chamoun et al., 2019).

Therefore, this study investigated the distinction in the abundance of microorganisms, ATP-related compounds, *K* value, pH, color value, and glycogen and Glu levels to evaluate the quality of scallops during a 6-day storage trial at 4 °C. Raw scallops could be contaminated by photogenic bacteria such as (*Vibrio* sp/*Salmonella* sp/*Listeria* sp). Hence, health hazards and food safety issues become a concern for raw consumption purposes. In the current study, the shift in total viable aerobic count (TVAC) and total viable count (TVC) of marine bacteria were investigated to determine the scallop quality.

Material and Methods

Sample Collection

In September 2020, twenty farmed Ezo giant scallops, *M. yessoensis* (diameter 12–15 cm), were obtained from Hokkaido. After harvest, the scallops were transported to the laboratory in cold storage and shelled; thereafter, the adductor muscles were separated from other tissue and stored at 4 °C. Three adductor muscles were assessed daily, with microbiological and chemical parameters measured from arrival to day 6. Samples that were harvested at the same places and periods were considered the same sample.

Changes in Viable and Marine Bacterial Counts

The changes in the TVAC and TVC of marine bacteria were analyzed according to Seki, Nakazato and Hamada-sato (2017) with minor modifications. Briefly, the scallop adductor muscle (2.5 g) was finely chopped and placed in a 50 mL centrifuge tube. Sterile NaCl solution (0.9 %; The Salt Industry Center of Japan) was added to adjust the final volume to 25 mL. The supernatant was appropriately diluted with 0.9% sterile NaCl solution, and 100 µL of the diluted sample was poured onto standard agar medium (5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, and 15 g agar/1.0 L pure water; Eiken Chemical Co., Ltd.) to determine the TVAC. Subsequently, 100 µL of the diluted sample was poured onto a standard agar medium containing 1% NaCl to determine the TVC of marine bacteria. The standard agar medium was incubated at 35 °C for 48 h, and the standard agar medium containing 1% NaCl was incubated at 20 °C for 168 h.

Changes in ATP-related Compound Levels and *K* value

Only ATP-related compound levels and *K* value were measured in this study following the method of Seki et al. (2016) with minor modifications. Approximately 2.5 g of scallop adductor muscle was placed in a 15-mL centrifuge tube, and 4 mL of 10% perchloric acid (Fujifilm Wako Pure Chemical Corporation) was added to extract ATP-related compounds. The solids were removed by centrifugation (11,000 × *g*, 10 min, 5 °C) (MX 201; Tomy Seiko Co., Ltd.), and the supernatant volume was adjusted 10 mL with 10% perchloric acid. One milliliter of this solution was neutralized with KOH (Kanto Chemical Co., Inc.). The precipitate was removed by centrifugation (12,000 × *g*, 5 min, 5 °C), and the volume of the supernatant was adjusted to 5 mL with purified water. This solution was filtered through a 0.22-µm filter (Shanghai Fenghan Industrial Co., Ltd.), and the level of ATP-related compounds was measured by high-performance liquid chromatography (HPLC; Column: Cosmosil Packed Column 5C18-PAQ, 4.6 mm I.D. × 150 mm, mobile phase: 20 mM KH_2PO_4 (Fujifilm Wako Pure Chemical Corporation) solution (pH 7), flow velocity: 1.0 mL/min, temperature: 40 °C, detector: U.V., wavelength: 260 nm, injection volume: 20 µL) (10A series; Shimadzu Corporation).

The *K* value was calculated using the following formula:

$$K \text{ value } (\%) = \frac{(Hx + HxR)}{(ATP + ADP + AMP + IMP + HxR + Hx)} \times 100$$

where HxR, inosine (Tokyo Chemical Industry Co., Ltd.); Hx, hypoxanthine (Fujifilm Wako Pure Chemical Corporation); ATP, adenosine triphosphate (Fujifilm Wako Pure Chemical Corporation); ADP, adenosine diphosphate (Oriental Yeast Co., Ltd.); AMP, adenosine monophosphate (Oriental Yeast Co., Ltd.); IMP, inosine monophosphate (Tokyo Chemical Industry Co., Ltd.).

Changes in pH and Color Values

The pH of samples was measured by setting a piercing pH electrode (PE-06HDA; Sato Shouji Inc.) on a digital pH meter (YK-21SP; Sato Shouji Inc.) and piercing the electrode into the scallop adductor muscle. The color value was measured using a colorimeter (CR-13; Konica Minolta, Inc.) directly on the surface of the scallop adductor muscle and measuring the L*a*b*

value using the Hunter Lab system, where L* indicates lightness, a* redness, and b* blueness (Konica Minolta, 2021).

Changes in the Glycogen Content

Glycogen content was evaluated according to the methods described by Ishihara, Kodama, and Yasuda (1966) and Bennett, Keirs, Peebles, and Gerard (2007) with minor modification. Approximately 0.1 g of scallop adductor muscle was placed in a 15-mL centrifuge tube, and 2 mL of 10% perchloric acid was added to it for glycogen extraction. The solid matter was removed by centrifugation (12,000 × *g*, 5 min, 5 °C), and the supernatant was moved to another tube. Thereafter, 5 mL of ethanol (Fujifilm Wako Pure Chemical Corporation) was added to the supernatant, the mixture was stirred, and the precipitated glycogen was centrifuged (12,000 × *g*, 5 min, 5 °C). The supernatant was removed, and the precipitate was resuspended in 50 mL of purified water. Two hundred microliters of the sample solution, 12 µL of phenol (Fujifilm Wako Pure Chemical Corporation) and 500 µL of sulfuric acid (Fujifilm Wako Pure Chemical Corporation) were added into a new tube. The solution was incubated at 25 °C for 10 min and then shaken briefly. After incubating for another 20 min, the glycogen content was measured using a plate reader at 490 nm (iMark; Bio-Rad Laboratories, Inc.) and calculated using a glycogen standard (Fujifilm Wako Pure Chemical Corporation) curve.

Changes in Glutamic Acid Content

Glutamic acid extraction was performed following the method used to determine the ATP-related compound levels and *K* value as previously described (Seki et al., 2016). The labeled Glu in the sample solution was obtained by mixing 40 µL of the sample solution with 70 µL of ethanol, 20 µL of triethylamine (Fujifilm Wako Pure Chemical Corporation), and 20 µL of phenyl isothiocyanate (Kanto Chemical Co., Inc.). Hereafter, the mixture was allowed to react at 25 °C for 30 min (Hanzawa et al., 2001). Subsequently, 500 µL of acetate–sodium acetate (Fujifilm Wako Pure Chemical Corporation) buffer (50 mM, pH 6.0) and acetonitrile (Fujifilm Wako Pure Chemical Corporation) (97:3 v/v) were added to the sample mixture. The mixture was filtered through a 0.22-µm filter, and the Glu content was measured using the HPLC method. The HPLC column (Cosmosil Packed Column 5C18-MS-II, 4.6 mm I.D. × 150 mm) was injected with 20 µL of the sample at a 1.0 mL/min flow velocity. HPLC detection was at a wavelength of 254 nm at the temperature of 40 °C. The mobile phase conditions were as follows: (eluent A was composed of 50 mM acetate–sodium acetate buffer (pH 6.0):acetonitrile (97:3); eluent B was composed of acetonitrile:water (6:4); Gradient: eluent B was increased from 5-100% between 0 to 16 min, decreased from 100-5% for 4 min and was kept steady for another 5 min. The glutamic acid content was calculated using the standard curve of a glutamic acid (Fujifilm Wako Pure Chemical Corporation).

Statistical Analysis

Data were obtained based on Fisher's three principles. Data change by time was analyzed using the one-way analysis of variance, and the post hoc analysis was performed to determine the difference between mean values using Microsoft Excel. Results with $p < 0.05$ were considered statistically significant.

Results and Discussion

Changes in the Viable and Marine Bacterial Counts

Figure 1 shows the changes in the TVAC and TVC of marine bacteria on the scallops during six days of refrigerated storage. Almost no TVAC was recorded on scallops during the six days of storage; however, the number of marine bacteria decreased from 1.9 log CFU/g on day 0 to 1.8 log CFU/g on day 1 ($p > 0.05$). It increased to 2.0 log CFU/g on day 2 ($p > 0.05$) and to 3.4 log CFU/g on day 6 ($p < 0.05$).

In this study, almost no general viable cell count was recorded during the six days of observation (Figure 1). The viable bacterial count of scallops acclimatized overnight in artificial seawater at 10 °C after fishing increased from approximately 3.0 log CFU/g on day 0 to 3.0–4.0 log CFU/g on day 6 when stored at 5 °C (Yanagiya, Shimada, Yamabi, Koizumi, & Nagamine, 1999). Meanwhile, the viable bacterial count of scallops stored in a refrigerator for 1–2 days has been reported to be 2.69–2.75 log CFU/g (Narita et al., 2018). These values are higher than those observed in this study, where no bacteria were observed in standard agar. Moreover, bacteria were not observed on the surface of the scallop adductor. Bacteria do not exist in muscles when marine organisms are alive, and they move from the internal organs to the surface post-mortem (Seki et al., 2017). Here, hygienic practices were employed during scallop preparation using all sterilized tools, and not all samples were rinsed with fresh water after separating the adductor muscles. It is possible that the adductor muscles were still in a sea water-like environment for six days, and bacteria that are not resistant to salt could not have grown on the

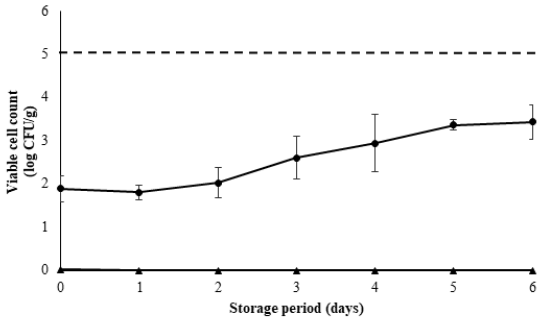

Figure 1. Changes in the TVAC and TVC of marine bacteria during storage. Error bars denote the standard deviation of the mean (n = 6). The dashed line indicates the maximum (allowable) TVAC at 5.0 log CFU/g (Ichinohe et al., 2015). ▲: TVAC, ●: TVC of marine bacteria

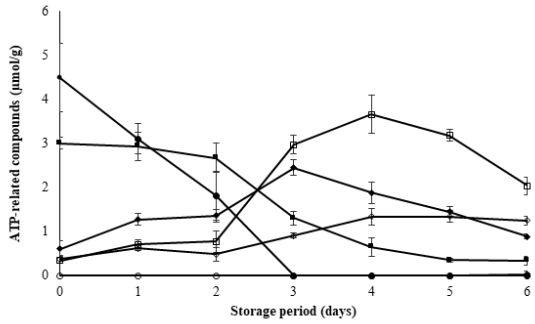

Figure 2. Changes in the level of ATP-related compounds in scallops during storage. Error bars denote standard deviations of the mean (n = 5). ●ATP, ▪ADP, ♦AMP, ○IMP, □HxR, ◇ Hx.

surface of the adductor muscle. Therefore, the internal organs of marine organisms should be removed immediately after post-mortem.

In addition, compared with the findings of previous studies (Kimura, Narita, Nomata, Fukushi, & Takahashi, 1998; Kimura, Narita, Imamura, Ushiro, & Yamanaka, 2000), the small number of bacteria might be due to immediate sample collection post mortem of the scallops. The abundance of general viable bacteria in scallops processed in a cleanroom is reportedly less than that in scallops not processed in a cleanroom (Yanagiya et al., 1999). In the present study, almost all scallops were processed in hygienic environments, which could be a reason for the low general viable cell count.

In contrast, the number of marine bacteria evaluated in this study was approximately 2.0 log CFU/g on day 0. The number was increased over the six days. In similar studies, the abundance of marine bacteria in scallops increased from approximately 3.0 log CFU/g on day 0 to approximately 4.0 log CFU/g on day 6 when stored at 5 °C (Kimura et al., 1998, Kimura et al., 2000). These results were considered lower than the changes in the TVC of marine bacteria observed in the current study. Although the changes in the present study were relatively high, the TVC of marine bacteria after six days of storage was similar to that of previous studies (Kimura et al., 1998, Kimura et al., 2000). Several microorganisms reported in marine products, such as scallops, are halophilic and mainly detected once cultivated in a salt-containing medium (Onodera, Miyamoto, Ishikawa, & Nakaya, 1997; Yanagiya et al., 1999). Nozawa et al. (2004) reported that *Vibrio* is the predominant microbe in the scallop adductor muscle. In general, *Vibrio* was not detected in a standard agar medium, as it requires salt for growth, but some *Vibrio* species may grow well in 0% NaCl medium (U. S. Food & Drug Association, 2004).

Changes in ATP-Related Compound Levels and *K* value

Figure 2 shows the changes in the levels of ATP-related compounds over time. The ATP level was 4.5 μmol/g on day 0, and then sharply decreased to 0.0 μmol/g on day 3 ($p < 0.05$). The ADP level was 3.0 μmol/g on day 0, and then slightly decreased to 2.7 μmol/g on day 2 ($p > 0.05$) and to 0.35 μmol/g on day 6 ($p < 0.05$). The AMP level was 0.61 μmol/g on day 0, increased to 2.5 μmol/g on day 3 ($p < 0.05$) and decreased to 0.89 μmol/g on day 6 ($p < 0.05$). IMP was not detected throughout the 6 days. The HxR level was 0.35 μmol/g on day 0, increased sharply to 3.7 μmol/g on day 4 ($p < 0.05$), and decreased to 2.0 μmol/g on day 6 ($p < 0.05$). The Hx level was 0.38 μmol/g on day 0 and increased to 1.3 μmol/g on day 4 ($p < 0.05$); no change was observed during the remaining days ($p > 0.05$).

The ATP level in this study was 4.5 μmol/g on day 0, but it decreased with time and was undetectable on day 3 (Figure 2). In similar studies, the ATP level on day 0 has been reported to be 5.8 μmol/g (Nagamine, Yamabi, Matsubara, & Fukuda, 1988) and 8.0 μmol/g (Kimura et al., 2000). Seasonal variations in the ATP level have been observed. The ATP level in scallops caught in September (the same harvested month as in the current study) was approximately 9.9 μmol/g (Shizukuishi, Onishi, Tanaka, & Narita, 2004) and 7.5 μmol/g (Kimura, 2003). The ATP values were higher than those recorded in this study. Further, the ATP level of 5.4 μmol/g on day 3 (Nagamine et al., 1988) and 5.0 μmol/g on day 4 (Kimura et al., 2000) were also higher than those observed in this study. The degradation of ATP-related compounds depends on temperature and time. The duration from harvest to sampling in the previous study was one day longer compared to that in current study. In addition, the ATP level sharply decreased from approximately 45% on

day 1 to 5.0% on day 3 in scallops stored at 5 °C in a previous study (Matsumoto, 1996). According to this study, the ATP level decreased from 50% to 0% from day 0 to day 3, showing the same trend as observed in a previous study (Kimura, 2003).

Based on the study, the ADP level was the highest on day 0; at the same time, the AMP level was the highest on day 3. Even in scallops stored at 5 °C, in which ATP degradation was observed in this study, the ADP level was the highest (30% of the total ATP-related compounds) on day 0. On the contrary, the AMP level was 20% of the total ATP-related compounds on day 0 and 5% of the total ATP-related compounds on day 1. This indicates a decrease after reaching approximately 20% of the total ATP-related compounds on day 3 (Matsumoto, 1996). In this study, the ADP level on day 0 was approximately 34% of the total ATP-related compounds, and the AMP level on day 3 was around 32%, indicating the same trend.

IMP was not detected throughout the six days of storage since the AMP deaminase was absent in scallops, and these results were similar to those of previous studies (Nishi & Nishida, 1976; Kawashima & Yamanaka, 1992; Kimura, Narita, Nomata, Kaneko, & Yamanaka, 1997; Kimura et al., 2000). It has been reported that AMP is degraded via two pathways, IMP and adenosine (Kawashima & Yamanaka, 1992). In consequence, a small amount of IMP has been detected in scallops (Nagamine et al., 1988, Matsumoto, 1996). AMP is decomposed by IMP when divalent metal ions are lost (Wei et al., 2020). However, as ATP is generally bound to Mg^{2+} in vivo (Kimura, 2003), the concentration of Mg^{2+} in muscle increases with ATP decomposition. Typically, IMP is not detected in that AMP is decomposed into adenosine due to the presence of Mg^{2+}

The HxR level increased sharply from days 2 to 3. Hardening of the scallop adductor muscle over time causes a reduction in the ATP level and increases the HxR level (Kimura et al., 1997). In this study, ATP was not detected, and the HxR level increased sharply on day 3. This means hardening is related to a sharp increase in the HxR level. Hx accounted for approximately 28% of the total level on day 6. In scallops stored at 5 °C, it has been reported to increase to approximately 50% on day 6 (Matsumoto, 1996), higher than the Hx level observed in this study. However, as the HxR level in this study was high, the decomposition of HxR to Hx was slow.

Figure 3 shows the changes in the *K* value after six days of observation. The *K* value was 8.2% on day 0, increased to 15% on day 1 ($p < 0.05$), and then slightly increased to 18% on day 2 ($p > 0.05$). It increased rapidly to 66% on day 4 ($p < 0.05$) and gradually increased to 72% on day 6 ($p < 0.05$). Since the determination of *K* value is species-dependent, the maximum *K* value of refrigerated scallop was calculated to evaluate the other parameters such as Glu, glycogen, and ATP-related components. The *K* value of scallops has been reported to increase sharply from 20% on day 4 to approximately 60% on day 7 when stored at 5 °C (Kimura et al., 1997). Although the day on which the *K* value increased in this study was different from that reported in a previous study (Kimura et al., 1997), a rapid increase occurred in both studies. This rapid increase is associated with a decrease in pH because of hardening and the rapid decomposition of ATP (Nishi & Nishida, 1976; Kimura et al., 1997; Yamanaka, 2002). In this study, the ATP level decreased considerably from days 0 to 3 (Figure 4), suggesting that these factors rapidly increased the *K* value. The recommended *K* values are as follows; fresh fish, <10 %; sashimi, 10%–20%; moderately fresh fish, 20%–50%; raw material for processing, 35%–60%; and spoiled material, >60% (Choi et al., 2020). Based on the evaluation of *K* value and the findings of the current study, it might be

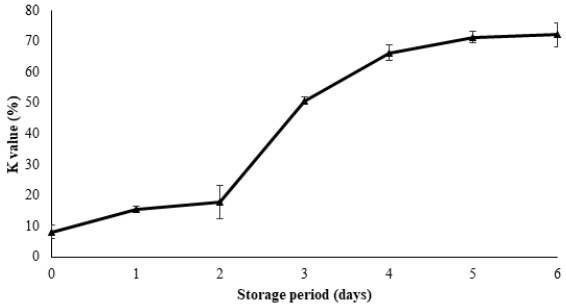

Figure 3. Changes in the *K* value of scallops over six days of storage. Error bars denote the standard deviations of the mean (n = 5). Fresh fish should have a value of <10 %, sashimi should have a value of 10%–20%, moderately fresh fish should have a value of 20%–50%, raw material for processing should have a value of 35%–60%, and spoiled material has a value of >60% (Choi et al., 2020).

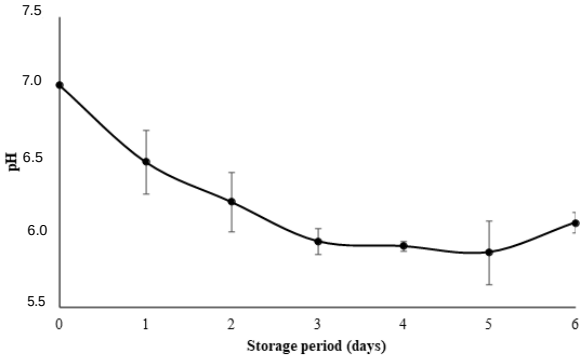

Figure 4. Changes in the pH of scallops during storage. Error bars denote the standard deviations of the mean (n = 8).

recommended that scallops can be consumed raw within two days and should be heated before consumption after three days of refrigerated storage.

Changes in pH and Color Values

Figure 4 shows the changes in pH over the six days. The pH was 7.0 on day 0; dropped to 6.0 on day 3 ($p < 0.05$), and continued slightly dropped to 5.9 on day 5 ($p > 0.05$). However, the value was insignificantly increased ($p > 0.05$) on day 6. The pH of marine organisms drops shortly post mortem (Koseki, Kitakami, Kato, & Arai, 2006). The pH of scallops stored at 5 °C gradually decreased over time, shown by the lessen to 6.9 on day 0 and 6.1 on day 6 (Kimura et al., 1997). The pH of scallop has been reported to be 7.1, which dropped to 5.9 on day 6 (Kawashima & Yamanaka, 1994; Kimura et al., 2000). These results are similar to those obtained in this study, whereas the pH of the scallop was 7.0 on day 0 and decreased to 5.9 on day 5.

The pH of scallops eases due to octopine and lactic acid accumulation and the increase in hydrogen ions associated with ATP decomposition (Kimura et al., 1997; Kimura et al., 2000). In this study, the ATP level and pH decreased over three days (Figs. 2 and 4), indicating the ATP decomposition caused the reduction in pH. Furthermore, the pH of scallops increased to 6.1 on day 6 of observation. The increase in pH has been reported to be caused by the accumulation of alkaline bio-transformers due to the accumulation of bacteria over time (Ocano-Higuera et al., 2006).

Figure 5 shows the changes in the L*a*b* value during the six days of storage. The L* value was 54 on day 0 and slightly decreased to 52 on day 6 ($p > 0.05$). The a* and b* values fluctuated between -0.70 and 0.27 and between 6.7 and 9.6, respectively, throughout the six days with minor changes ($p > 0.05$).

The L*a*b* value showed almost no change during the six days. The L* value ranged from 52 to 54 and showed no significant changes ($p>0.05$) in this study. This observation is similar to a previous study, where the L* value of scallops stored at 0 °C remained unperturbed over 15 days (Ocano-Higuera, Maeda-Martinez, Lugo-Sanchez, & Pacheco-Aguilar, 2006). In contrast, it has been reported that the L* value of oval squid (*Sepioteuthis lessoniana*) and Pacific Ocean perch (*Sebastes alutus*) increases after their shelf-life (Okamoto et al., 2008; Shamaila, Skura, & Nakai. 1995). This might be due to differences in flesh or tissue characteristics; when caught, these fish have transparent flesh but lose their transparency over time post mortem. Moreover, their L* values increase over time due to the presence of cloudiness. This phenomenon is not observed in the scallop adductor muscle based on the findings of this study and previous studies.

The a* value of scallops from Hokkaido was between -2 and 4 (Takeda, Nomata, Ohta, Kaneko, & Hashimoto, 1993), and the b* value was approximately 5 (Kaneko, Kimura, Nomata, Fukushi, & Nishi, 1996). The a* value of scallop in this study ranged from -0.70 to 0.27, which was in the range of the previous study. In contrast, the b* value of the scallop in the current study was 6.7 to 9.6 during the six days of observation, and it was outside the range reported previously. The L*a*b* values represent the following: L* for lightness, a* for hue, and b* for chroma, and these indicate hue and saturation. An L* value of 100 represents white, whereas a value of 0 signifies black. The a* value represents red in the + direction, green in the - direction, the b* value represents yellow in the + direction and blue in the - direction. In the current study, the scallop was white with a slight yellow tinge. It has been reported that because of rigor, scallops turn black with time. Still, the onset of rigor can be significantly delayed by wrapping the scallops with antibacterial sheets within five days (Kimura, 2003). The onset of rigor may be due to an increase in bacteria.

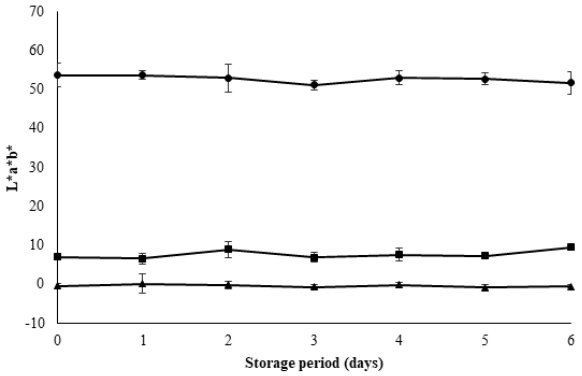

Figure 5. Changes in the color value of scallops during storage. Error bars denote the standard deviation of the mean (n = 3). : ● :L*value, ▲ :a* value, ■ :b* value.

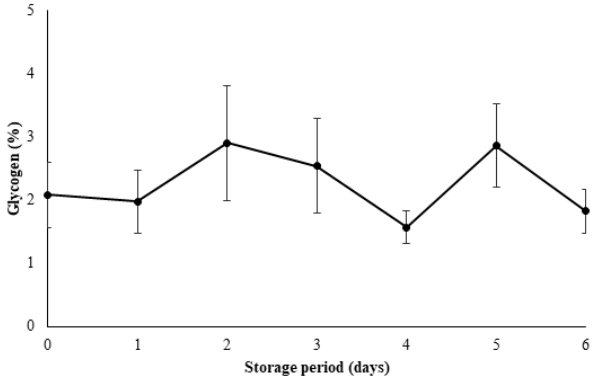

Figure 6. Changes in the glycogen content in scallops during storage. Error bars denote the standard deviation of the mean (n = 9).

In this study, the onset of rigor was not confirmed as no significant increase in the bacterial count was observed (Figure 1). In scallop, the color changes due to the Maillard reaction on heating both saccharides and amino acids. Since the scallops were not heated in this study, no significant difference in coloration was observed.

Changes in the Glycogen Content

The changes in the glycogen content over the 6-day storage period are shown in Figure 6. The glycogen content was between 2.0% and 3.0%, and no significant difference was observed during the six days of refrigerated storage. The glycogen content in scallops shows considerable seasonal fluctuations, decreasing below 0.5% from December to March, increasing from 3.0% to 4.0% from July to September, and decreasing to December (Kimura, 2003; Shizukuishi et al., 2004). This study was performed in September, and the glycogen content was almost the same as that previously reported.

The glycogen content is related to the amount of feed consumed by the scallop. Generally, scallop feeding increases in the growing season from April and reaches the highest around September (Kimura, 2003); however, it fluctuates every year. Hereinafter, the amount of energy stored as glycogen also fluctuates, leading to the variation of glycogen content in the scallop. The difference in glycogen content in scallop up to 6% was observed between the same month of two different years (Miyazono & Kurata, 1995).

In this study, although minor changes in the glycogen content were observed each day, they were not significantly different ($p>0.05$). This could be because glycogen, being an energy source, is not used after post-mortem; hence, no changes in the glycogen content were observed (Figure 6). It has been reported that the taste level of hard clam soup correlates with the glycogen level (Yamamoto & Kitao 1993), indicating that the glycogen level is related to good flavors. Although the reference glycogen level in raw scallops acceptable for consumption has not been defined clearly, glycogen is an important factor influencing the taste of scallops. In this study, glycogen metabolism in the organisms stopped post mortem; consequently, the glycogen level did not change significantly for six days.

Changes in the Glutamic Acid Content

Figure 7 shows the changes in the glutamic acid content in the scallop during the 6-day refrigerated storage period. The glutamic acid (Glu) level varied between 41 and 58 mg/100 g throughout the six days

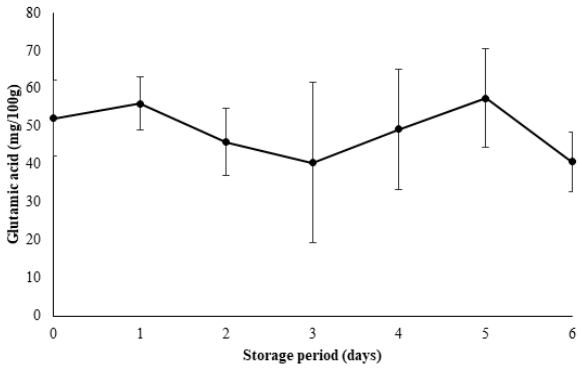

Figure 7. Changes in the glutamic acid level in scallop over storage time. Error bars denote standard deviations of the mean (n = 9).

and with no significant increasing or decreasing trend ($p > 0.05$). The Glu level in this study was generally in the range of the preferred concentration of above 46 mg/100 mL, as reported earlier (Chamoun et al., 2019). The Glu level varies with glycogen consumption during survival and ATP production (Kimura, 2003). After the post-mortem of an organism, glutamic acid metabolism stops. Therefore, its level did not change significantly during the six days in this study.

The Glu level in scallops was as high as 132 mg/100 g in May but decreased sharply after June, and it has been reported to be 35–40 mg/100 g from September to November (Kimura, 2003). The high level of Glu in May might be due to spawning and feeding effects. Furthermore, a survey in another area reported that the level was 100 mg/100 g or more in May–July but decreased in August (Shizukuishi et al., 2004). The current study was performed in September, and the results are similar to those reported previously (Kimura, 2003).

Conclusions

In this study, the quality of refrigerated Ezo giant scallops adductor was evaluated by investigating the umami-related parameters (such as glycogen, IMP, AMP, and Glu levels) and the changes of quality-related parameters (such as microorganisms abundance, *K* value, pH, and color). The quality-related parameters such as TVC of marine bacteria and the *K* value of scallops significantly increased during the refrigerated storage of 6 days. The *K* value of scallops has exceeded the acceptable level of 20% for sashimi consumption after two days of observation. Meanwhile, the pH of scallops slightly decreased until five days of observation and then relatively increased on the sixth day of observation. Although the umami-related parameters such as IMP, AMP, glycogen and Glu level varied, there

were no significant changes during refrigerated storage, except in the AMP level. Based on the findings in this study, refrigerated scallops can be consumed as sashimi for only two days post mortem, and further processing on the scallops is recommended after two days of storage. The fourth-day refrigerated scallops are unsuitable for human consumption. In the future, it is recommended to investigate the abundance of pathogenic marine bacteria such as *Vibrio* sp. for safety evaluation, analyze the changes in Mg2+ ion over time, perform sensory scallops assessment, and determine the correlation among umami-related components, quality, and sensory characteristics.

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