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REGULAR ARTICLE

Comparative evaluation of the quality changes in squid (*Ommastrephes bartrami*) during flake and slurry ice storage

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ABSTRACT

Raw squid suffers a rapid quality loss which occurs after catch immediately and during ice storage. This research is a comparative study between the effect of Slurry ice (SI), a binary mixture consists of 40% ice and 60% filtered seawater (salinity: 3.3%), and Flake ice (FI) on the quality parameters of squid (*Ommastrephes bartrami*) during storage period. Different analysis such as salinity, moisture, TVB-N, myofibrillar protein, microbiological, total sulphur (SH), and ATPase activity, which are related to the quality changes, were analyzed during the storage period (15 days). The rapid decline in the initial temperature with SI (0.83 °C min^{-1}) resulting in a significant reduction of total aerobic bacteria, extend the shelf life, and better control of water content. It was found that the squid stored on SI has significantly higher content of myofibrillar protein (30 mg ml^{-1}) compared with the samples stored on FI (24.26 mg ml^{-1}) at the end of storage time. Additionally, the storage of squid on SI retard the formation of TVB-N, where the samples content of TNB-N after 15 days of storage were (13.26 mg $N(100g)^{-1}$), while in the FI samples TVB-N content reached to (30 mg $N(100g)^{-1}$). Similarly, the $Ca2^{+}$-ATPase activity, and total sulfhydryl (SH) content in squids treated with SI were significantly ($P < 0.05$) higher than the FI samples. Our results indicate that, the application of SI to squid is advisable to achieve better quality maintenance during storage and distribution.

Keywords: Chemical quality; Microbiological quality; Shelf life; Slurry ice, Squid.

INTRODUCTION

Cephalopods constitute an important part of the marine resource and most suitable for human consumption (Jeyasekaran et al., 2010). Squid, belonging to the class of Cephalopod, is one of the most common seafood dishes at many parts of the world. It is considered low in Saturated Fat and Sodium and a good source of Niacin, Zinc, Protein, Riboflavin, Vitamin B_{12}, Phosphorus, Copper, Manganese and Selenium (Vleeming et al., 1999). However, the raw squid suffers a rapid quality loss by producing various off-odor components, that mainly caused by trimethylamine-N-oxide (TMAO) reduction and microbial contamination which occur immediately after the catch and during storage in ice (Sungsri-in et al., 2011; Ramirez-Suarez et al., 2008; Gou et al., 2010). Therefore, the refrigeration immediately after catch is required, to slow down the susceptibility of squid to spoilage and quality loss. Traditionally, flake ice, refrigerated sea water, modified atmospheres, brine solutions, the incorporation of chemical preservative agents and slurry ice have been used for the preservation of fresh aquatic food products (Múgica et al., 2008).

Slurry ice, a binary mixture of small spherical ice crystals surrounded by seawater at subzero temperature, has been reported to be a promising technique for the preservation of aquatic food products (Rodríguez et al., 2006; Huidobro et al., 2002). Slurry ice has many features including (I) fast cooling rate resulting from the large heat transfer surface area created by its numerous particles, (II) the sub-zero storage of the seafood material (III) the latent heat of fusion of its ice crystals led to a high energy storage density (IV) spherical particles of the slurry ice play an main role in reducing the physical damage of seafood surface,

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and thus reduces dehydration and oxidation events (V) the complete coverage of the seafood surface (Piñeiro et al., 2004; Aubourg et al., 2007; Kauffeld et al., 2010).

Several studies suggest that slurry ice is a promising technology to improve the quality for a wide range of fish species (Piñeiro et al., 2004). For example, European Hake (*Merluccius merluccius*) (Losada et al., 2004), horse mackerel (*Trachurus trachurus*) (Losada et al., 2005), salmon (*Oncorhynchus kisutch*) (Rodríguez et al., 2008), seabass (*Dicentrarchus labrax*) (Cakli et al., 2006) and pink shrimp (*Parapenaeus longirostris*) (Huidobro et al., 2002).

Although these advantages and applications of slurry ice are well known, few studies are available on the ability of slurry ice to extend the shelf-life of squid and enhance the quality parameters (*Ommastrephes bartrami*). Therefore, the objective of this study was to compare the effects of slurry ice and flake ice treatment on the microbiological, and chemical parameters of Squid (*Ommastrephes bartrami*).

MATERIALS AND METHODS

Preparation of flake ice (FI) and slurry ice (SI)

An ice system (RF-1000-SP, JiangSu, China) was used to prepare the slurry ice (SI). The ice slurry mixture was consisted of 70% ice and 30% filtered seawater (salinity 3.0%).The temperature of the SI mixture ranged from -2.0 °C to -2.5°C. Flake ice (FI) was prepared using freshwater with a compact device (SM-F140AY65, HITACHI, Japan); the temperature of the FI ranged from 0 to 0.8°C.

Fish material, processing, and sampling

Fresh squid *(Ommastephes bartrami)* of approximately 10 to 15 cm body length and approximately 70.0 g body weight were provided by Zhejiang XingYe Company (Zhoushan, China). Squids were transferred to the laboratory within 30 min into aseptic bags and stored in zero degrees using portable refrigeration box. Upon arrival in the laboratory they were divided into two groups and placed in SI or FI at a ratio of 13 (fish to ice), before being stored in a refrigerated room at 0°C for 15 days. The FI and SI were renewed every day during the storage period. To determine the cooling curves for squid stored in SI and FI the temperature of different parts of squid was measured by multiplex temperature tester (JK-24U, JiangSu, China) every 0.5 min for 30min.

For each chilling treatment, three carcasses without skin and cartilage were studied separately throughout the whole experimental period. Every three days, the samples were subjected to chemical and microbial analyses.

Determination of salinity and moisture content

To determine the salinity level in the squid muscles, about 10.0 g minced squid were mixed with 100 ml water (70 °C) and boiled for 15 min with shaking. The samples were homogenized for 1.0 min (high speed) before cooled to room temperature. Then the salinity content was measured using salinometer (YSI EC300, America) as described by Chinese National Standard GBT 12457-2008.

Moisture content was determined by Automatic moisture meter (HG63, METTLER, Switzerland) according to Chinese National Standard (GB 5009-2010). 5.0 g minced squid were placed on the specimen disc with zero clearing, the moisture contents were measured directly and recorded, each sample was measured five times.

Determination of total volatile basic nitrogen (TVB-N)

Total Volatile Bases Nitrogen (TVB-N) was measured by automatic kjeldahl apparatus (FOSS-8400, Sweden). The results were expressed as mg N (100g)-1muscle. Squid samples prepared as described by (Aubourg et al., 1997) with slight modification. briefly, 10 g of squid sample was homogenized with 90ml of 0.6M perchloric acid solution for 1 min using a laboratory homogenizer. The homogenate was centrifuged at 10000 rpm for 10 min (4°C) and the supernatant was filtered through Whatman No. 1 filter paper.

Microbiological analyses

The total bacterial load was determined using pour-plate method. Samples (25.0 g) were transferred to a sterile blender Jar containing 225 ml of sterile 1% peptone water (PW) and homogenized for 2 min using a stomacher. Serial dilutions of each homogenate were carried out with 0.1% PW. Appropriate dilutions (1 ml) were plated onto plate count agar (GB/T 4789.1-2010, China). The plates were incubated at 30 °C for 48 h to enumerate the total bacteria count.

Myofibrillar protein analyses

To isolate myofibrils from muscle, about 10.0g minced flesh was homogenized in 100 ml of 20 mM Tris-maleic acid solution (containing 0.05 mM KCL, pH7.0). The homogenate was centrifuged at 10000rpm for 10 min (-4°C), the supernatant discarded and the sediment, containing most of myofibrillar was resuspended in 100ml of 20 mM Tris-maleic acid solution (containing 0.6 mM KCL, pH7.0) and centrifuged again for 5 min at 9000 rpm (-4 °C). The resulting supernatant that contains total myofibril protein solution, was measured by biuret method (Gornall et al. 1949).

Determination of Ca^{2+}-ATPase activity

ATPase activities were determined as described by (Ooizumi and Xiong, 2004). To prepare the reaction

mixture the following chemicals were added: 0.5 M Tris-maleic acid-containing buffer (pH 7.0), 0.1 M $CaCl_2$, H_2O, 20 mM ATP solution and myofibrillar proteins. The reaction mixture was incubated for 5 min at 30 °C in a water bath, and terminated by adding 1.0 ml of chilled 15% (w/v) trichloracetic acid (TCA) solution. The blank group was mixed with 1.0 ml 15% TCA solution to denature proteins before the reaction. 1.0 ml sulfuric acid molybdate, 0.5 ml metal and 2.5 ml water were added to 1.0 ml reaction mixture followed by incubated for 45 min at room temperature and then measured at 640 nm, 0.5 mM KH_2PO_4 was used as a standard. the following formula has been used to calculate Ca^{2+}-ATPase

$$Ca^{2+}\text{-ATPase} = (A\text{-}B)/(t\times C)$$

Where (A) refer to 1.0ml phosphoric acid generated in the reaction solution (μM) ; (B) is phosphoric acid generated in the Blank (μM); (T) is the reaction time (min) and (C) is The content of enzyme in 1.0 ml reaction solution.

Determination of total sulphur (SH)

The method described by (Zhang et al., 2015) has been used to measure total SH content, briefly, 1.0 ml myofibril protein was added to 9.0 ml 0.2 M Tris-HCL buffer containing 8.0 M carbamide, 2% (w/v) sodium dodecyl sulfate (SDS) and 10 mM EDTA, (pH6.8). Thereafter, 4.0 ml of this mixture was added into 0.4 ml of 0.1% (w/v) DTNB solution (containing 0.2 M Tris-HCl, pH8.0) and further subjected to incubation at 40°C for 25min in a water bath. Then the absorbance of the mixture was measured at 412 nm using a spectrophotometer (U2800, HITACHI, Japan). A blank was prepared by replacing the sample with 0.6 M KCl. Total SH content was calculated by the following formula

$$CO = (A\times D)/(C\times B)$$

Where CO SH molar concentration; A absorbance value on 420nm; D dilution ratio; C protein concentration ($mgml^{-1}$) and B molecular absorption coefficient 13600 (M^{-1} cm^{-1});

Statistical analyses

All experiments were performed in triplicate, and the data were presented as means ± standard deviation (SD). Statistical analyses were performed with the Excel and SPD7.05. In all cases, *P*-values with (P < 0AE05) were considered statistically significant.

RESULTS AND DISCUSSION

Cooling curves for squid placed in SI or FI are shown in (Fig. 1). The results indicated that the SI cooled the squid quite rapidly than the FI. The FI cooled the squid core temperature to -0.4~-0.5 °C in approximately 30 min, while the SI could cool the squid core temperature to -1.1~-1.3 °C within the same time. The cooling rates were 0.83 °C min^{-1} and 0.50 °C min^{-1} for SI and FI, respectively. This rapid cooling rate is desirable as degradation by bacteria and enzymes is quickly suppressed at low-temperature and that leading to extending shelf-life and kept the squid fresh (Leelapongwattana et al. 2008, Wang et al. 2003, Gou et al. 2010). In general, slurry ice was produced by mixing crushed ice with seawater (salinity 3.0%), resulting in a freezing point below 0.0°C (Gao 2010).

Moisture content and salinity analyses

Significant differences (P <0.05) were observed in moisture content between the squid stored in SI and FI (Fig. 2). Fresh squid samples had water contents of 83.72%, which increased gradually to 86.64% at the end of storage period in SI. While in the sample stored in FI the water content decreased to 78.35% after 15 days. The losing in water content refers to disruption of the muscle structure and denaturation of myofibrillar proteins and sarcoplasmic, resulting in to a decreasing water holding capacity of the protein fraction (Castrillón et al., 1996; Rodríguez et al., 2008).

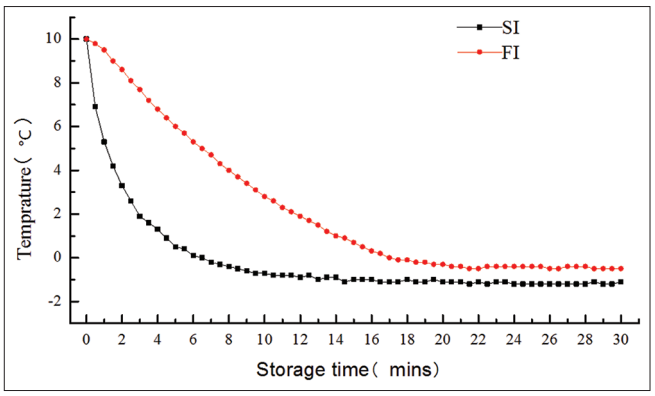

Fig 1. Cooling curves for squids during 30 min of pre-cooling in slurry ice (SI) and flake ice (FI). The temperature of refrigerator room set to 4 ˚C.

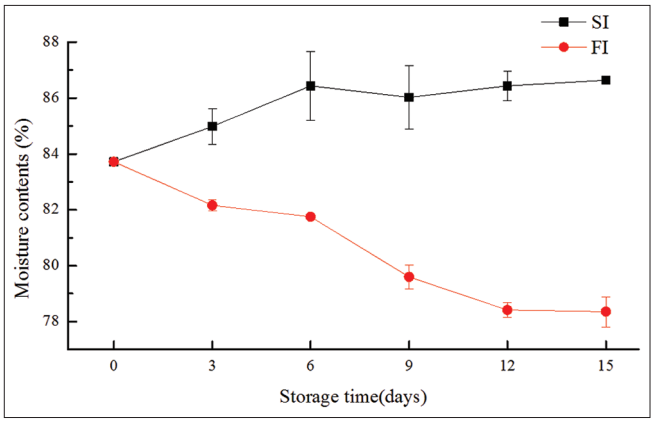

Fig 2. Water content (%) of squid during storage in slurry ice (SI) and flake ice (FI). Average of three replicates and standard deviation are presented.

The salinity content of the squid stored in SI increased from 0.52 to 2.81‰ at the end of the storage period (15 days), while the squid stored in FI changed slightly and the senility content was 0.58‰ after 15days (Fig. 3). The absorption of NaCl into the fish during the storage period in slurry ice was found to be slow (Losada et al., 2005; Rodríguez et al., 2008; Losada et al., 2004). The increasing in salinity can enhance the water holding capacity and inhibit the growth of microbial during the storage period of marine species (Lei Y. et al., 2007). It is worth mentioning that the increasing in salinity of squid stored in SI would not affect the quality properties.

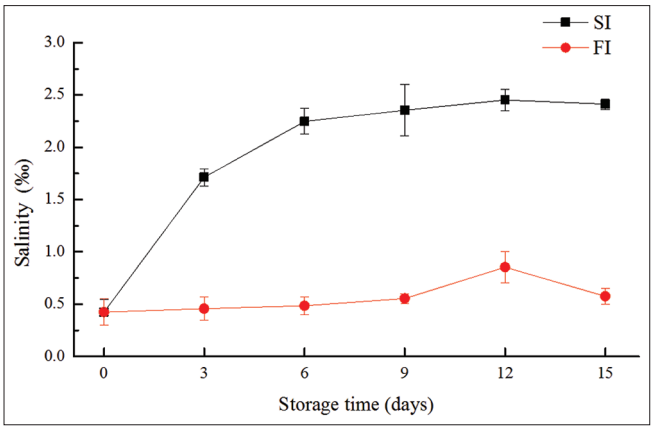

Fig 3. Salinity content (%) of squid during storage in slurry ice (SI) and flake ice (FI). Average of three replicates and standard deviation are presented.

Total volatile basic nitrogen (TVB-N) analyses

The TVB-N content normally produced as a result of microbiological activity during the chilling storage (Lei Y. et al., 2007; Rodríguez et al., 2008), and the TVB-N contents should not be more than 30 mg N $(100g)^{-1}$ for fresh and frozen aquatic products according to Chinese National Hygienic Standard (GB2733-2005).

Fresh squid samples had a TVB-N content of 12.46 mg N $(100g)^{-1}$, and the TVB-N formation in the squid stored in FI was significantly ($P < 0.05$) higher than in SI (Fig. 4). At the end of storage period, the TVB-N concentration was 13.26 mg N $(100g)^{-1}$ in squid muscles from SI batches, while these values rose above 30mg N $(100g)^{-1}$ after 9 days of storage in FI. Similar results have been obtained with different marine species such as horse mackerel (Rodríguez et al., 2005), turbot (Campos et al., 2006), and ray specimens (Múgica et al., 2008).

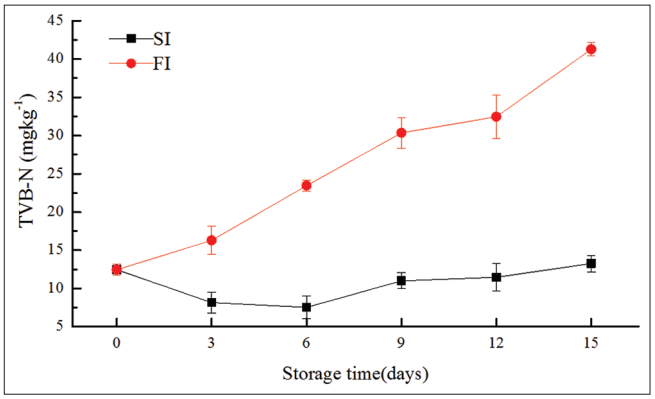

Fig 4. Total volatile base nitrogen (TVB-N) contents of the squids during storage in slurry ice (SI) or flake ice (FI). Bars represent the standard deviation.

Microbiological analyses

The comparative evaluation of microbial growth in squid during storage in SI or FI is displayed in Fig. 5, the use of SI significantly ($P < 0.05$) reduce the microbial growth when compared to FI. Where, the initial microbial load of 6 log $CFUg^{-1}$ was maintained after 12 days of storage in FI, and increasing to 6.61 log $CFUg^{-1}$ at the end of storage period. While the counts of total viable bacteria in squid muscle stored in SI were 4.74 log $CFUg^{-1}$ after 15 days of storage period. Previous reports have also described significantly lower bacterial growth in lobster stored in SI, as compared to conventional flake ice (Aubourg et al., 2007). Additionally, several studies showed a significant slowing down of microbial activity in other marine species, such as shrimp (Huidobro et al., 2002), sardine (Campos et al., 2006), horse mackerel (Rodríguez et al., 2005), and hake (Rodrìguez et al., 2004) during stored in SI. The reasons for the limited bacterial growth, that found in squid stored in slurry ice, definitely are subzero temperature achieved with storage on SI, and the surface wash caused by the liquid phase of the slurry ice. (Rodríguez et al., 2005).

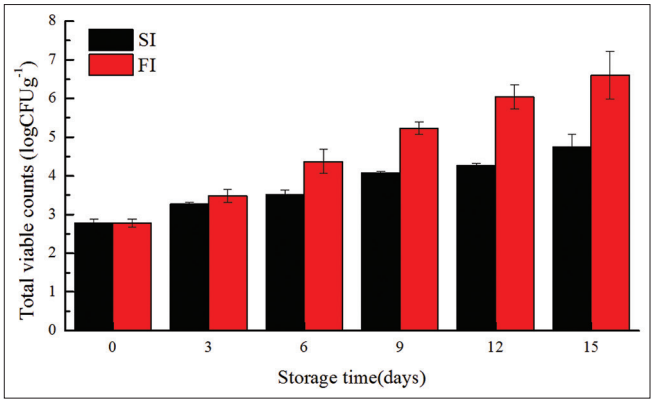

Fig 5. Total microbial count of squids during storage in slurry ice (SI) or flake ice (FI). Bars represent the standard deviation.

Myofibrillar protein analyses

Myofibrillar proteins constitute as the major protein in the marine organisms muscle (about 70-80 %) and play an essential role in many functions (Benjakul et al., 2011). the decreases of water holding capacity, juiciness and changes in the textural attributes, resulted from the denaturation and

aggregation of myofibrillar proteins. This lead to a hard, dry and fibrous marine product with low eating quality. (Careche et al., 1998; Careche et al., 1999; Lu et al., 2012).

As shown in Fig. 6, the content of extractable myofibrillar proteins in squid muscle decreased significantly ($P < 0.05$) over the storage period. Thus, the myofibrillar content of squid samples stored in FI decreased from 62.64 mg ml^{-1} (Day 0) to 24.26 mg ml^{-1} on the 15th day. However, the SI treated samples were in good condition and the extractable myofibrillar content remained at 30 mg ml^{-1} at the end of storage time (15 days). The main changes in myofibrillar are reported to occur in myosin light-chain but actin and actinin also degrade during storage period (Careche et al., 1998; Kjaersgard et al., 2006; Schubring, 2005). Numerous studies attributed the degradation of myofibrillar to proteases such as cathepsins, as well as calcium-dependent proteases (Okitani et al., 1980; Zhang et al., 2015). In several marine species, cathepsins B, D, and L were considered as the enzymes playing the most important role in postmortem muscle softening (Ladrat et al., 2003). Our results could be explained based on a faster cooling rate of slurry ice treatment that leads to a lower temperature, thus, formation of large number of small ice crystals in muscle nuclei, preventing the irreversible destruction of the myofibrils by large ice crystals. More importantly, the concentrations of sodium chloride in the SI is similar to its concentration in marine water, that cause stabilization of the myofibrillar protein fraction implying larger yields during storage, and thus enhancing the quality parameters (Kauffeld et al., 2010).

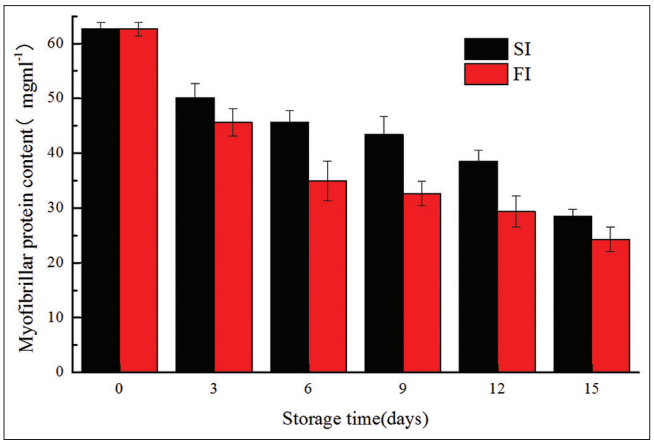

Fig 6. Myofibrillar protein content in squids during storage in Flake ice (FI), and slurry ice (SI). Bars represent the standard deviation.

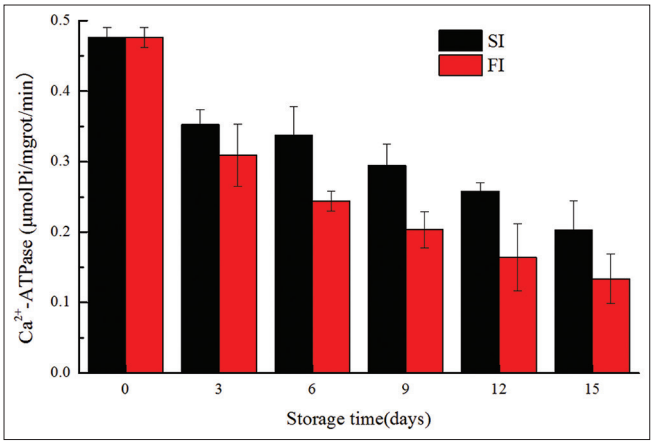

Fig 7. Ca^{2+}-ATPase activity of squids during storage in flake ice (FI), and slurry ice (SI). Error bars represent standard deviations.

Ca2 + -ATPase analyses

As a general trend, Ca^{2+}-ATPase activity in the squid muscle changed reversely with storage time ($P<0.05$) (Fig. 7). The initial Ca^{2+}-ATPase activity of fresh squid muscle (0 day) was 0.48 μ molPi mg^{-1} min-1, then the Ca^{2+}-ATPase activity significantly decreased to 0.1 μ molPi mg^{-1} min-1 for the FI ($P <0.05$) after 15 days of storage. While the Ca^{2+}-ATPase activity remained at 0.2 μ molPi mg^{-1} min-1, in the samples stored on SI at the same time (Fig. 6). The decreases in Ca^{2+}-ATPase activity was also observed with oxeye scad (*Selar boops*), shrimp scad (*Alepes djedaba*) and queen fish (*Chorinemus lysan*) during ice storage (Chantira et al., 2013). The aggregation as well as the changes of myosin globular head led to reduction in the ATPase activity (Reza et al., 2009). In addition, the rearrangement of protein via protein–protein interactions contributed mainly in the loss of ATPase activity (Benjakul and Bauer, 2000; Reza et al., 2009; Hossain et al., 2005). However, the SI samples maintained a comparatively higher activity of Ca^{2+}-ATPase, that caused by an increased stabilization of myofibrillar protein fraction, indicating larger yields during the storage and processing.

Total sulphur (SH) analyses

Total sulfhydryl (SH) content of squid at day (0) was about 4.53×10^{-5} mol g^{-1}. A sharp decline in SH content was found in the squid samples stored in FI group (1.53×10^{-5} $molg^{-1}$) over the storage period (Fig. 7). While, SI samples remained at $2.24\times10^{-5}molg^{-1}$ after 15 days (Fig. 8). It was also found that the content of SH in the tuna samples stored on slurry ice were significantly higher ($P <0.05$) than those sample stored on flake-iced (Zhang et al., 2015). In the other study, (Riebroy et al., 2007) reported that the formation of disulphide bonds through oxidation of SH groups or disulphide interchanges, resulted in decrease in total content of SH in muscle. In this study, the SI treatment allowed squid sample to reach subzero temperatures rapidly that resulted in decrease the enzymatic breakdown reactions and the oxidation, and thus reducing the polymerization denaturation degree of squid protein(Zhang et al., 2015).

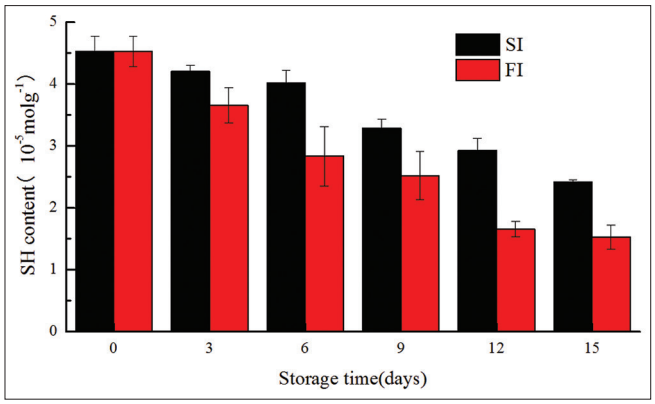

Fig 8. Total SH content of squids during storage in flake ice (FI), and slurry ice (SI). Error bars represent standard deviations.

SUMMARY AND CONCLUSION

The effect of slurry ice on the quality of squids during storage was investigated and compared to flake ice. The storage of squid samples on Slurry ice led to slow down the degradation of Myofibrillar proteins, decrease the of Ca 2 +-ATPase activity, total SH content, and total microbial count, and thus stability of tissue structures of squids. These results confirm that using of slurry ice can extend the shelf-life and improve the safety and quality of squid, and thus extend the commercialization of fresh squid.

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Author contributions

Pengxian Yuan, was responsible for performing the experiments, the data collection, and revisions materials and methods section. Shanggui Deng, designed the experiment, assembled input data, and supervised the whole study. Shaimaa Hatab, contributed with the study design, interpreted the results, and drafted the manuscript. Ning Yuan, contributed to the Data analysis and interpretation, and Drafting the article. Jiancong Huo contributed to the experimental design, chemical composition analysis.

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