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Quality Indices of Squid (*Photololigo duvaucelii)* and Cuttlefish (*Sepia aculeata*) Stored in Ice

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The objective quality indices of squid (Photololigo duvaucelii) and cuttlefish (Sepia aculeata) stored in ice were compared with the subjective counterparts. Sensory (overall quality rating, quality index method [QIM], and multisample difference test), microbiological (total viable count [TVC], psychrophilic count), chemical (trichloroacetic acid-soluble peptide [TCA-soluble peptide], trimethylamine nitrogen [TMA-N], total volatile bases nitrogen [TVB-N], ammonia content, and protein pattern), and physical analyses (expressible drip, color, and texture) were determined in both species during 16 days of iced storage. As storage time increased, TCA-soluble peptide, TVB, ammonia content, and expressible drip were increased ($p < 0.05$). TMA content was markedly increased after 10 and 8 days of storage in squid and cuttlefish, respectively. Both TVC and psychrophilic count increased as the storage time increased ($p < 0.01$). Myosin heavy chain was degraded with coincidental decrease in shear force and sensory texture during storage ($p < 0.05$). According to the overall rating score, shelf life of both species in ice was estimated to be 6 days. The increases in ammonia content and expressible drip were highly correlated with the decrease in overall quality rating and increase in quality index score of squid and cuttlefish ($p < 0.01$).

Keywords squid, cuttlefish, freshness, sensory evaluation, chemical analysis

Introduction

Seafood products have become an important income generator for Thailand. Cephalopods such as cuttlefish, squid, and octopus are exported to several countries, and cephalopod products are of great consumer demand in the Mediterranean and Oriental countries. The state of freshness of seafood is an important factor determining its commercial value. As the freshness of seafood declines, the acceptability of quality attributes—including appearance, taste, flavor, and texture—becomes lower. Currently, the most wholesome way of describing freshness of fish seems to be by means of sensory analysis. However, sensory assessment requires trained personnel, is somewhat time consuming, and may

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therefore be considered costly and not always practical for large-scale commercial purposes (Nilsen and Esaiassen, 2005). Some of the quality indices used to assess freshness or degradation in fish have shown complications when applied to cephalopods. Freshness indicators that are not appropriate for cephalopod species include *k* value, total volatile basic nitrogen (TVB-N), pH, and polyamines (except agmatine; Ohashi et al., 1991; Márquez-Ríos et al., 2007). The contents of agmatine (Yamanaka et al., 1987; Ohashi et al., 1991; Vaz-Pires and Barbosa, 2004) and octopine (Vaz-Pires and Barbosa, 2004), as well as psychrophilic count (Paarup et al., 2002), were reported as the potential freshness indices for squid. At present, no objective method of freshness evaluation for squid (*Photololigo duvaucelii*) and cuttlefish (*Sepia aculeata*) species caught in Thailand has been developed.

The objective of this study was to determine the chemical, microbiogical, physical, and sensory changes of whole squid and whole cuttlefish during iced storage and to investigate an appropriate objective method equivalent to the sensory method for freshness evaluation.

Materials and Methods

Chemicals/Microbial Media

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (BME), glycerol, bovine serum albumin, high molecular weight markers, Folin-Ciocalteu's phenol reagent, and Coomassie Blue R-250 were purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid and ethanol were obtained from Fisher Chemicals (Morris Plains, NJ, USA). Acrylamide, *N,N,N',N'*-tetrametyhlethylenediamine (TEMED), bis-acrylamide, urea, and plate count agar (PCA) were obtained from Fluka (Buchs, Switzerland). All chemicals and medias were of analytical grade.

Raw Materials

Whole squid of the size 20–28 g/squid and whole cuttlefish of the size 80–150 g/cuttlefish, caught by cast net from the Songkhla coast along the Gulf of Thailand and off-loaded 48 h after capture, were purchased from a dock in Songkhla province. The squid and cuttlefish were placed in ice with an ice/squid ratio of 2:1 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h.

Sample Preparation

The cuttlefish and squid were kept in an insulated plastic box containing crushed ice, with an ice/sample ratio of 2:1 (w/w). The box was kept at room temperature (28–30°C) and the temperature inside the box ranged from 0 to 4°C. To maintain the ice content, melted ice was removed and replaced with an equal amount of ice every 2 days. The samples were taken every 2 days until 16 days for analyses.

Chemical Analysis

Determination of Trichloroacetic Acid-Soluble Peptide (TCA-Soluble Peptide). TCA-soluble peptides were determined according to the method described by Morrissey et al. (1993). Squid muscle (3 g) was homogenized with 27 mL of 5% TCA (w/v) using an Ultra Turrax homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 12,000 rpm for

30 min in an ice bath. The homogenate was kept in ice for 1 h and centrifuged at 5000 × *g* for 5 min. Soluble peptides in the supernatant were measured and expressed as μmole tyrosine/g muscle.

Determination of Total Volatile Bases Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) Contents. TVB-N and TMA-N contents were determined using the Conway microdiffusion assay according to Hasegawa (1987). Squid meat (2 g) was homogenized with an Ultra Turrax homogenizer at a speed of 12,000 rpm for 2 min with 8 mL of 4% TCA (w/v). The mixtures were filtered using Whatman no. 41, the fastest ashless filter paper for coarse particles or gelatinous precipitates; and the filtrate was used for analysis. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the sample. TVB and TMA were released after the addition of saturated K_2CO_3 and diffused into the boric acid solution. The titration of solution was performed, and the amount of TVB-N or TMA-N was calculated.

Determination of Ammonia Content. The ammonia content was determined using a distillation assay according to Parris and Foglin (1983). The ground sample (10 g) was placed in a 500 mL Erlenmeyer flask with 200 mL of distilled water, 10 g of carbonate-free MgO, and several drops of antifoam. The mixture was distilled, and the distillate (100 mL) was collected in 20 mL of 0.1 N HCl for titration by 0.05 N NaOH with methyl red as the indicator. Ammonia content was calculated and expressed as mg/g sample.

Determination of pH. The determination of pH was carried out according to Dublán-Garcia et al. (2005). Five grams of muscle was homogenized with an Ultra Turrex homogenizer with 45 mL of distillated water at a speed of 12,000 rpm for 2 min. The pH was measured on a digital pH meter (Sartorius Docu-pH meter, Goettingen, Germany).

Analysis of Protein Patterns. The protein pattern of squid and cuttlefish muscle taken every 4 days of storage was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli (1970). To prepare the protein sample, 27 mL of 5% (w/w) SDS solution was added to the sample (3 g). The mixture was then homogenized using an Ultra Turrax homogenizer at a speed of 12,000 rpm for 1 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged at 3500 × *g* for 20 min to remove undissolved debris. Protein concentration was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard. The samples (24 μg protein) were loaded into the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were fixed and stained for 3 h in 0.02% Coomassie Blue R-250 in 50% methanol and 15% glacial acetic acid. Gels were destained for 15 min with destaining solution I (50% methanol and 7.5% glacial acetic acid) and overnight with the destaining solution II (5% methanol and 7.5% glacial acetic acid).

Microbiological Analysis

Determination of Total Viable Count (TVC) and Psychrophilic Counts. TVC and psychrophilic counts were determined following the method of Speck (1976). Mantle muscle with skin (25 g) was weighed into a stomacher bag containing 225 mL of sterile normal saline water (0.85 g NaCl in 100 mL water). Blending was done in a Stomacher (M400,

Seward, UK) at low speed for 2 min. Decimal dilutions were prepared according to the estimated contamination. Pour inoculation was made in plates containing Plate Count Agar, which were incubated at 4°C for 7 days, and TVC were incubated at 37°C for 2 days.

Physical Analysis

Determination of Shear Force. Texture was measured using a Texture Analyzer model TA-XT2i (Stable Micro System, Surrey, England) equipped with a Warner-Blatzler shear apparatus, according to Dublán-Garcia et al. (2005). The squid and cuttlefish mantle was taken every 4 days of storage, and the central part was cut into a dimension of 1 cm × 3 cm. The shear force, perpendicular to muscle fibers, was determined in at least 5 replicates for each species. The peak of the shear force profile was regarded as the shear force value.

For the cooked sample, squid and cuttlefish mantle were cooked in a water bath at 95°C, and the core temperature of the sample was 70°C for 1 min, and then immediately cooled in ice water for 5 min. The sample was drained for 10 min at room temperature and subjected to analysis as described above.

Determination of Color. The color of the deskinned squid and cuttlefish mantle was measured using a colorimeter (Model Color Flex, Hunter Associates Laboratory, Reston, VA, USA) and reported in the CIE color profile system as L^* value (lightness), a^* value (redness/greenness), and b^* value (yellowness/blueness). The hue angle (h°) and chroma (C^*) were calculated (Hunter Associates Laboratory, 2002) as follows:

$$h^\circ = \tan^{-1}(b^*/a^*),$$

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2}.$$

Determination of Expressible Drip. Expressible drip of squid and cuttlefish mantles was measured according to Hasegawa (1987). The mantle was cut into a size of 1 cm × 3 cm, weighed (x), and wrapped in Whatman No. 1. The 5 kg standard weight was placed on top of the samples for 2 min, then the sample was removed and weighed again (z). The expressible drip was calculated as follows:

$$\text{Expressible drip (\%)} = \frac{x - z}{x} \times 100.$$

Sensory Analysis

Quality Index Method (QIM). Quality Index Method (QIM; Vaz-Pires and Seixas, 2006) was carried out by 12 trained panelists. The sensory analyses were performed for raw squid and cuttlefish focusing on skin: appearance, color, odor, and mucus; flesh: texture; eyes: appearance; and mouth region: color, odor, and mucus. QIM schemes for squid (*Illex coindetii)* and cuttlefish (*Sepia officinalis*) were used to classify the sample from each storage time. Training consisted of 3 sessions (2 h each session) until the panelists knew the constituted quality of squid and cuttlefish and were trained in differentiating appearance, odor, and texture of QIM score.

Overall Quality. Overall quality (Lawless and Heymann, 1998) was carried out by 12 trained panelists. The sensory analyses were performed for raw squid and cuttlefish focusing on appearance, odor, and texture. A point structured scale with a value of 1 for *match* (seaweedy and fresh marine odor, bright and well-defined pigments, iridescent, and firm texture) and 10 for *reject* (strong ammonical, putrid odor, undesirable discoloration, and soft flaccid texture) was used. Training consisted of 3 sessions (2 h each session) until the panelists knew the constituted quality of squid and cuttlefish and were trained in differentiating odor (according to a method of the U.S. Food and Drug Administration, 2005), appearance, and texture of squid and cuttlefish.

Texture Score. The hand-felt firmness of squid and cuttlefish mantles was carried out by the Multisample Difference Test (Meilgaard et al., 1999), using a point-structured scale with a value of 1 for *very soft* and 10 for *very hard*. Eight to 10 squids or cuttlefish were sampled every 4 days of storage and evaluated by 12 trained panelists. The panelists were trained over 3 sessions (1 h each) in differentiating the texture of squid and cuttlefish kept for different storage times.

Sensory testing was held in a clean, well-lighted, and well-ventilated room.

Statistical Analysis

All experiments were run in duplicate using two different lots of cephalopod. Completely Randomized Design (CRD) was used. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by the Duncan's Multiple Range Test at a significance level of $p < 0.05$. Analysis was performed using SPSS statistic program (version 6.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Chemical Analysis

Changes in TCA-Soluble Peptide Content. TCA-soluble peptide content of squid and cuttlefish during iced storage are shown in Figure 1. At day 0, squid meat contained higher TCA-soluble peptide content than did cuttlefish meat ($p < 0.05$). This was possibly caused by the differences in small peptides or free amino acids presented in both species. Ruiz-Capillas et al. (2002) showed that total free amino acid content varied to a great extent depending on the species. As seen in Figure 1, TCA-soluble peptides in both samples increased throughout 16 days of iced storage ($p < 0.01$), indicating that hydrolysis of peptide bonds occurred during the extended storage. Squid generally had the higher increase in TCA-soluble peptides, compared with cuttlefish. This result suggested that squid might contain higher proteinase activity and a higher bacterial load. After death, cephalopods undergo very rapid protein degradation due to endogenous and bacterial enzymes. Such high proteolytic activity produces an increase in muscle-derived nitrogenous degradation products, hence favoring proliferation of degenerative flora and rapid decomposition (Vaz-Pires and Barbosa, 2004). Proteinases play a major role in the degradation of octopus muscle proteins, and the proteolytic activity is higher than that of various species of fish (Hurtado et al., 1999). Our results are in agreement with the studies of black tiger and white shrimps (Sriket et al., 2007) and lizardfish (Benjakul et al., 2003), whose TCA-soluble peptides and amino acids increased throughout storage times in ice, suggesting the autolytic degradation of protein. In addition, Lapa-Guimarães et al. (2005) also reported that the

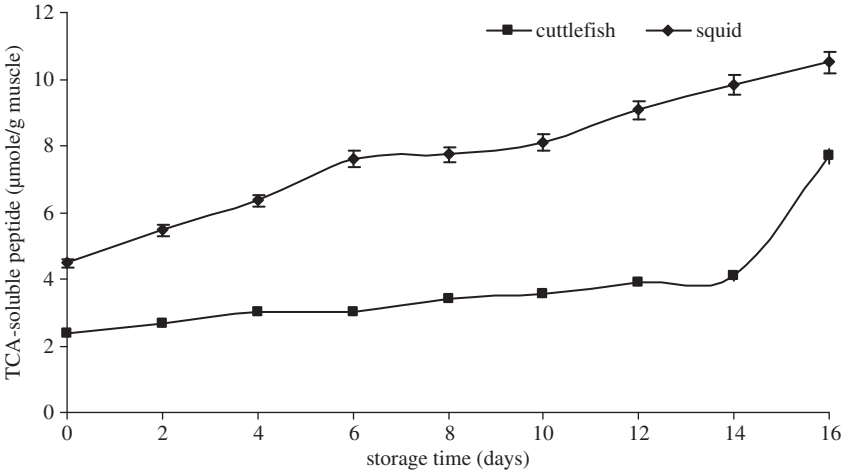

Figure 1. Changes in TCA-soluble peptides of deskinned squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

increase of non-protein nitrogen (NPN) and free amino acid nitrogen (FAA-N) content of squid muscle (*Loligo plei*) during iced storage could be a consequence of endogenous or microbial proteolytic enzymes.

Changes in TMA-N, TVB-N, and Ammonia Contents. Changes in TMA-N, TVB-N, and ammonia contents of squid and cuttlefish during iced storage are shown in Figures 2a, b, and c, respectively. TMA-N of both samples were formed after 6 days of storage and reached 1 mg/100 g sample at day 10. Lapa-Guimarães et al. (2005) also found that TMA-N of squid mantle (*Loligo plei*) was detected after 12 days of storage in ice. In most newly captured cephalopods, the TMA-N levels are equal to or lower than 1 mg of N/100 g. These TMA-N levels are indicative of an initial high freshness in these cephalopods. Similar results were reported by Márquez-Ríos et al. (2007), who showed that the steady increase in TMA-N of giant squid (*Dosidicus gigas*) mantle was observed from an initial value of 1.5 ± 0.1 mg/100 g to a final value of 4.5 ± 0.5 mg/100 g muscle at day 15. Nevertheless, significant differences among squids were governed by the different variables studied (anatomical zone, sex, and sexual stage; Ruiz-Capillas et al., 2002). However, the values increased sharply after 12 days of storage. Trimethylamine oxide nitrogen (TMAO-N) is broken down to TMA by psychrotrophic bacteria enzymes during iced storage, resulting in off-odor and off-flavor (Gram and Huss, 1996). Thus, our results suggested that the spoilage of squid and cuttlefish caused by bacteria with TMA-O reducing activity occurred when storage time increased.

In squid muscle, TVB-N increased after day 6 of storage in ice, while cuttlefish had a progressive increase in TVB-N content throughout the storage. More pronounced formation of TVB-N compound was found in cuttlefish after 12 days, compared with squid ($p < 0.05$). Volatile bases nitrogen (VBN) has been considered useful as a spoilage indicator, having little use as a freshness index (Yamanaka et al. 1987; Ohashi et al., 1991; Civera et al., 1999; Lapa-Guimarães et al., 2005). Ke et al. (1984) proposed a classification to typify the quality of the cephalopod in accordance with TVB-N content, where a value higher than 45 mg/100 g is considered unacceptable. However, in the Japanese market, a TVB-N value above 15 mg/100 g is unacceptable (Ruiz-Capillas et al., 2002). TMA-N of

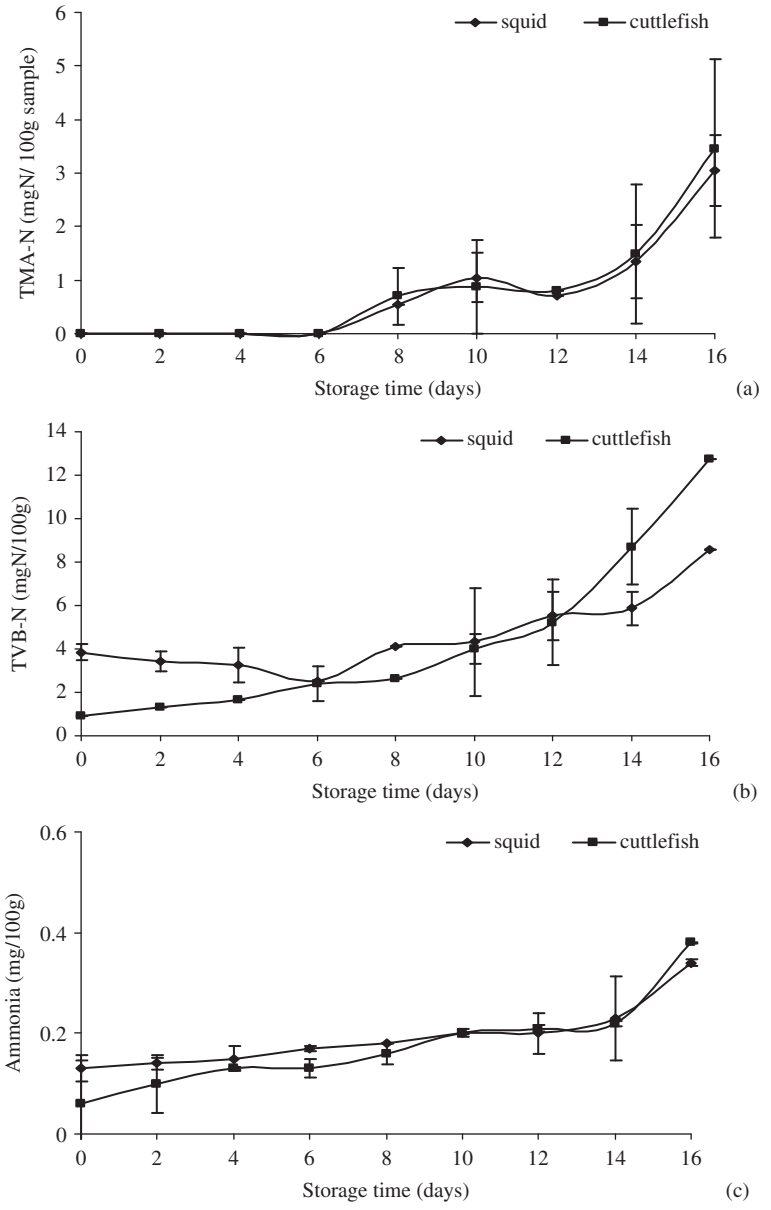

Figure 2. Changes in TMA-N (A), TVB-N (B), and ammonia (C) in deskinned squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

5 mg N/100 g in fish and shellfish have been reported as the limited level (Hebard et al., 1982).

Figure 2c shows that the ammonia (NH_3) content increased gradually in both samples when storage time increased. The sharp increase was noticeable after day 14 ($p < 0.05$). The results are coincidental with TVB-N content (Figure 2b). TVB-N consisting of TMA, dimethylamine (DMA), and NH_3 are particularly involved in the smell of fish (Fujii and

Okuzumi, 2000). Lapa-Guimarães et al. (2005) reported that commercialized squid muscle contained 17.1 mg of NH_3-N/100 g, which represented 88.6% of VBN. Similarly, Licciardello et al. (1985) and Paarup et al. (2002) observed a final exponential increase of NH_3 in whole iced squids (*Loligo pealei* and *Todaropsis eblanae*, respectively). Leblanc and Gill (1984) showed that NH_3 has been shown to be an excellent indicator of squid (*Illex illecebrosus*) quality.

Changes in pH. The pH values were slightly changed during storage in spite of a significant increase in TVB-N after 6 days of storage (Figure 2b). Ohashi et al. (1991) observed a pH change in common squid at a different temperature. A slight increase in pH observed at the time of the initial decomposition was recognized at 5 and 10°C storage, but no changes in pH were found when stored at 0°C. In addition, Sykes et al. (2009) revealed that pH of European cuttlefish (*Sepia officinalis*, L.) varied during iced storage. However, such variation was not significantly different. Squid muscle might contain buffering compounds such as TMAO-N (75–250 mgN/100 g; Sotelo and Rehbein, 2000).

Changes in the Protein Pattern During Storage. Protein patterns of whole squid and cuttlefish muscle are shown in Figures 3a and b, respectively. Myosin heavy chain (MHC; 200 kDa), paramyosin (110 kDa), and actin (45 kDa) bands were observed in the fresh mantle muscle at the beginning of storage (day 0). MHC bands intensity of both muscles were reduced after 8 days of storage. Changes in actin were less evident, whereas paramyosin and other bands remained unaltered. MHC was more susceptible to hydrolysis than was actin. The result corresponded with the increase in TCA-soluble peptide content of whole squid and cuttlefish (Figure 1). Konno and Fukazawa (1993) observed a gradual disappearance of the MHC band of squid mantle muscle incubated at 25°C within 4 h storage. The result is in agreement with studies of lizardfish (Benjakul et al., 2003) and black tiger and white shrimp (Sriket et al., 2007), in which MHC was more prone to proteolytic degradation than other muscle proteins including actin, troponin, and tropomyosin.

Microbiological Analysis

TVC in squid and cuttlefish was increased from 1.7×10^2 cfu/g and 1.1×10^2 cfu/g at the beginning of storage to 2.8×10^4 cfu/g and 1.8×10^4 cfu/g at day 16, respectively (Figure 4a). The psychrophilic counts increased from 7.0×10^1 and 4.5×10^1 cfu/g at

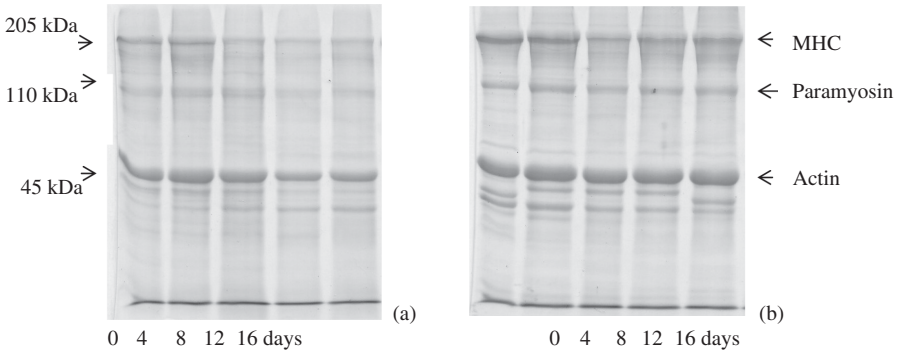

Figure 3. Changes in SDS-PAGE patterns of squid (A) and cuttlefish muscle (B) during iced storage.

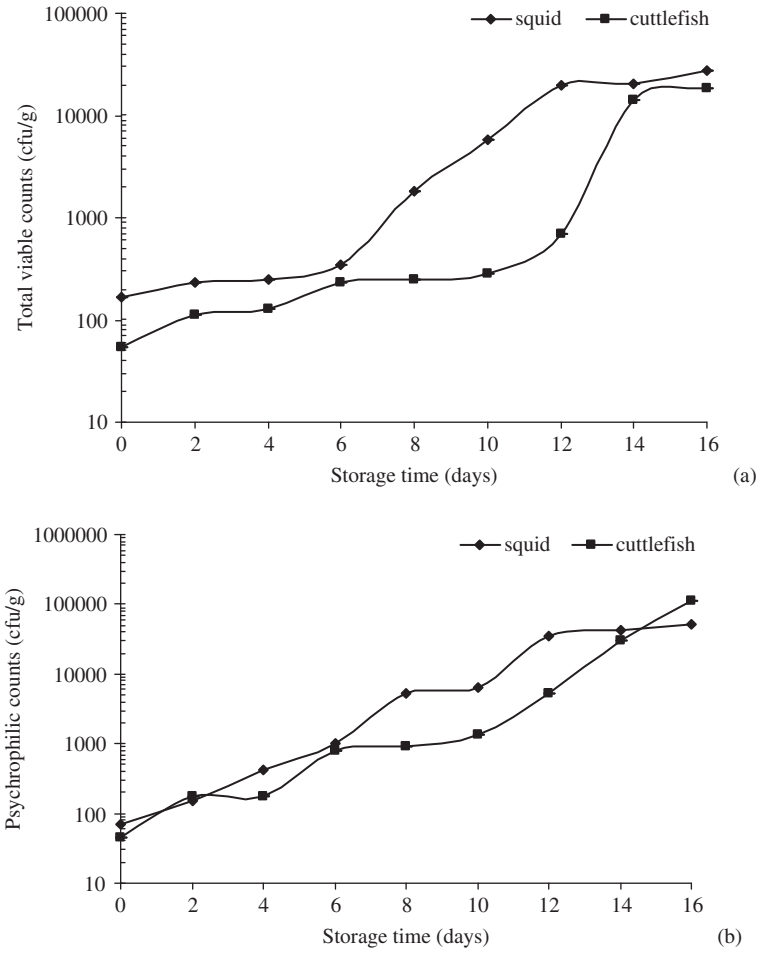

Figure 4. Changes in total viable count (A) and psychrophillic counts (B) of squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

the beginning of storage to 5.2×10^4 and 1.1×10^5 cfu/g after 16 days in squid and cuttlefish, respectively (Figure 4b). The common number of spoilage bacteria at the point of rejection of fish products was 10^7–10^9 cfu/g (Huss et al., 1997; Olafsdóttir et al., 1997; Vaz-Pires and Barbosa, 2004). The results demonstrated that TVC and psychrophilic count of both samples were below 10^7–10^9 cfu/g after 16 days of storage, thus remaining safe for consumption. Considering the rejection point from sensory evaluation (after day 12), TVC levels of 10^4 and 10^3 cfu/g were the limit for squid and cuttlefish, respectively. The data confirmed results of previous studies on other species. Lapa-Guimarães et al. (2005) reported that psychrotrophic count of squid (*Loligo plie*) increased to 5×10^5 cfu/g after 16 days of storage in contact with ice. Vaz-Pires and Barbosa (2004) also found that TVC on the nutrient and iron agar of common octopus (*Octopus vulgaris*) at rejection (day 8) was 10^5–10^6 cfu/cm^2, suggesting that the enzymatic action was more rapid and effective in this species than putrefaction. The spoilage was different among various fish species.

In fish whose skin is much thinner and fragile, decomposition or spoilage is more pronounced. Additionally, the number of bacteria might be varied depending on species and their environmental habitat.

Physical Analysis

Changes in Color. Table 1 shows the changes in L*, a*, b*, hue angle (h°), and chroma (C*) values in deskinned squid and cuttlefish mantle. In both samples, the L* value showing the brightness was slightly decreased during storage, while the a* value representing the redness increased gradually and increased shapely in a final storage period ($p < 0.05$). The b* value indicating yellowness of squid had a tendency to decrease with time ($p < 0.05$), while marked changes in b* value were found in cuttlefish kept longer than 14 days ($p < 0.05$). At the end of storage time, the a* value of cuttlefish was much lower than that of squid. In addition, the h° of squid decreased, while that of cuttlefish increased at the end of storage. These results indicated that different colors stained in sample muscles. The increase in redness stained in the squid muscle, while cuttlefish became yellowish during storage in ice. The C* (color saturation) of squid muscle tended to decrease during storage up to 10 days, followed by an increase until the end of storage. However, the marked increase in C* was observed in cuttlefish at day 16 ($p < 0.05$). Lapa-Guimarães et al. (2002) observed that the intensity of the red and yellow colors of squid (*Loligo plei*) increased during storage in ice. The decrease of whiteness and reddish color are regarded as a sign of squid quality deterioration (Ke et al., 1984; Lapa-Guimarães et al., 2002). This might be due to the different chromatophores presented in the mantle of both species. The three types of pigments found in cuttlefish (*Sepia officinalis*) are orange, yellow, and dark brown (Loi et al., 1996). In squid (*Loligo pealeii*), pigments consist of yellow, brown, and red (Mäthger and Hanlon, 2007).

Changes in Shear Force. The shear force of raw squid and cuttlefish stored in ice are shown in Figure 5. In both samples, the shear force gradually decreased throughout storage of 16 days ($p < 0.05$). At day 0, the shear forces of cuttlefish were higher than that of squid. This was most likely due to the thickness of cuttlefish muscle being higher than that of squid. Olaechea et al. (1993) and Ando et al. (1999) reported that firmness of seafood muscle had a close relationship with collagen, the major constituent of connective tissue. For cooked samples of both species, the shear force gradually decreased during storage ($p < 0.05$). However, the shear force of the cooked muscle was lower than the raw muscle. Three hypotheses on the mechanism of cell detachment in softened squid are possible. First, shrinkage of muscle cells which creates intercellular spaces may soften squid muscle. Second, cell detachment may occur as a result of decrease in the intercellular integrity due to loosening of collagen fibers. Third, it may occur as a result of decrease in the binding force between cell membrane and collagen (Ando et al., 1999). Our results are in agreement with Otwell and Hamann (1979) and Kugino and Kugino (1994) who found that cooked squid (*Loligo bleekert*) mantle muscle was softer than raw muscle. When the muscle tissue of squid is cooked in hot water, the network of connective tissue is severely damaged and disappears by solubilization and gelatinization (Kugino and Kugino, 1994). Strength reduction in raw and cooked samples during iced storage was coincidental with an increase in TCA-soluble peptides (Figure 1) and a decrease in MHC as shown in protein pattern (Figure 3). These indicated that squid and cuttlefish muscles became softer as a result of muscle protein degradation and changes in muscle fiber network (Rodger et al., 1984; Nagashima et al., 1992; Dublán-Garcia et al., 2005).

Table 1
Changes in L*, a*, b*, hue angle (h°), and chroma (C*) of deskinned squid and cuttlefish during iced storage

Days in ice	Squid					Cuttlefish				
	L*	a*	b*	h°	C*	L*	a*	b*	h°	C*
0	74.37 ± 5.30^{a}	0.40 ± 0.45^{d}	9.78 ± 0.39^{a}	1.53 ± 0.05^{a}	9.80 ± 0.37^{a}	78.86 ± 3.78^{bc}	-2.32 ± 0.53^{c}	7.32 ± 1.16^{b}	-1.27 ± 0.04^{bc}	7.69 ± 1.23^{b}
2	71.48 ± 1.05^{ab}	1.12 ± 0.62^{cd}	8.93 ± 2.18^{ab}	1.44 ± 0.05^{a}	9.01 ± 2.23^{a}	76.06 ± 1.41^{cd}	-1.75 ± 0.35^{bc}	3.67 ± 0.84^{d}	-1.11 ± 0.13^{b}	4.07 ± 0.72^{d}
4	66.65 ± 2.05^{cd}	4.09 ± 1.47^{b}	8.25 ± 4.48^{ab}	1.07 ± 0.12^{bc}	9.26 ± 4.60^{a}	81.75 ± 1.38^{ab}	-0.99 ± 0.27^{b}	7.21 ± 0.95^{b}	-1.43 ± 0.05^{c}	7.28 ± 0.93^{b}
6	69.25 ± 2.41^{bc}	3.28 ± 1.80^{bc}	6.74 ± 2.29^{abc}	1.14 ± 0.01^{b}	7.55 ± 2.80^{ab}	80.99 ± 1.60^{ab}	-1.44 ± 0.28^{bc}	7.67 ± 0.55^{ab}	-1.38 ± 0.04^{c}	7.81 ± 0.54^{b}
8	70.34 ± 5.44^{abc}	2.35 ± 0.62^{bcd}	3.53 ± 1.25^{c}	0.97 ± 0.11^{bc}	4.27 ± 1.29^{b}	83.80 ± 0.75^{a}	-1.86 ± 0.49^{bc}	7.24 ± 0.57^{b}	-1.32 ± 0.06^{c}	7.48 ± 0.60^{b}
10	64.15 ± 4.25^{d}	2.69 ± 1.46^{bc}	3.07 ± 1.14^{c}	0.87 ± 0.25^{c}	4.17 ± 1.57^{b}	70.99 ± 2.21^{e}	-1.49 ± 0.55^{bc}	3.13 ± 0.69^{d}	-1.12 ± 0.20^{b}	3.51 ± 0.60^{d}
12	72.12 ± 2.81^{ab}	3.71 ± 2.19^{b}	5.97 ± 3.15^{bc}	1.04 ± 0.23^{bc}	7.16 ± 3.50^{ab}	80.88 ± 2.40^{ab}	-1.45 ± 0.80^{bc}	5.62 ± 1.87^{c}	-1.26 ± 0.28^{bc}	5.93 ± 1.47^{c}
14	70.16 ± 1.84^{abc}	3.45 ± 1.19^{b}	5.51 ± 1.87^{bc}	1.01 ± 0.09^{bc}	6.52 ± 2.15^{ab}	81.84 ± 1.53^{ab}	-1.20 ± 0.57^{b}	5.30 ± 0.70^{c}	-1.34 ± 0.13^{c}	5.47 ± 0.57^{c}
16	65.61 ± 2.10^{bc}	8.46 ± 1.57^{a}	5.78 ± 2.37^{bc}	0.58 ± 0.11^{d}	10.29 ± 2.63^{a}	73.48 ± 3.59^{de}	1.89 ± 1.04^{a}	9.84 ± 0.09^{a}	1.41 ± 0.08^{a}	9.70 ± 1.10^{a}

Mean $\pm$ *SD* ($n = 2$). For each run, five determinations were conducted.
Different letters within the same column indicate significant differences ($p < 0.05$).

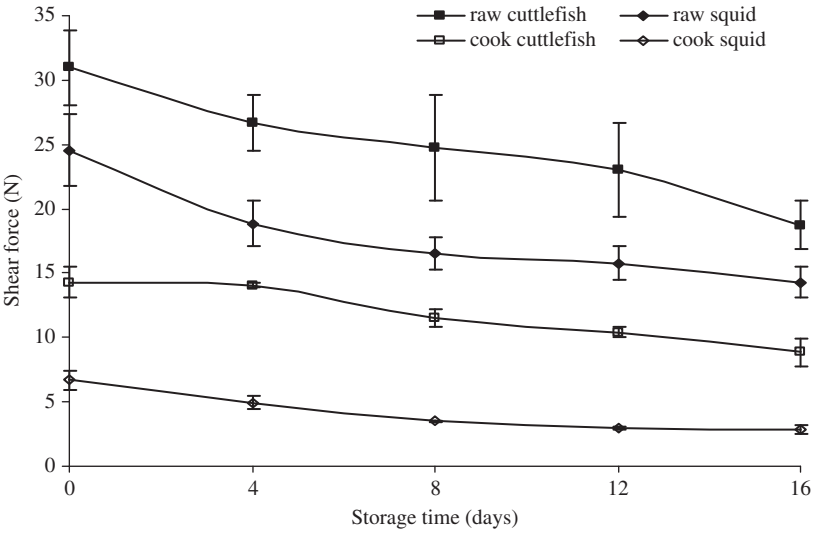

Figure 5. Changes in shear force of raw and cooked squid and cuttlefish during iced storage.Bars represent the standard deviation ($n = 2$). For each run, five determinations were conducted.

Changes in Expressible Drip. The expressible drip of squid and cuttlefish mantle during storage is depicted in Figure 6. The continuous increases in expressible drip of both species were observed throughout the storage ($p < 0.01$). The expressible drip might be due in part to the loss of water holding capacity (WHC) of muscle protein during storage. This could be caused by the destruction of proteins, in which the hydrophobic proteins were more exposed. Partial degradation of proteins caused by autolysis might favor the conformational change of proteins, resulting in the denaturation. This led to the decreased amount of water retained in the muscle structure (Sriket et al., 2007). WHC in muscle tissue is closely related to the myofibrillar network. Functional properties of proteins in fresh meat

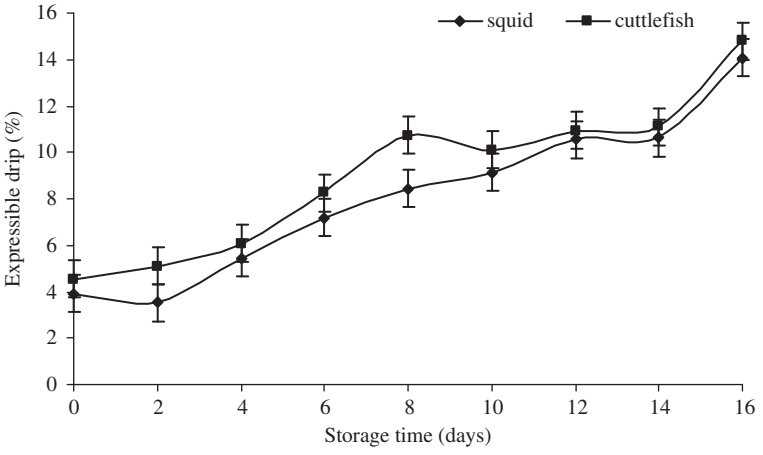

Figure 6. Changes in expressible drip of deskinned squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

are probably affected by proteolytic changes during storage (Zayas, 1997; Dublán-Garcia et al., 2005).

Sensory Analysis

Changes in QIM. Figure 7 shows the changes in quality index of squid and cuttlefish during iced storage, respectively. The exponential shape of QIM during iced storage was obtained with high correlation coefficients (R^2) for squid and cuttlefish (0.975 and 0.971, respectively). However, the graph of QIM overtime for octopus (*Octopus vulgaris*; Barbosa and Vaz-Pires, 2004), broadtail shortfin squid (*Illex coindetii*), and cuttlefish (*Sepia officinalis*; Vaz-Pires and Seixas, 2006) showed the linear correlation with R^2 of 0.99. These differences may be due to the morphological differences of cephalopod species. QIM scores of both samples increased with increasing storage time. Both general skin appearance and odor are considered as the more important parameters affecting selling and consumption in the world market (Vaz-Pires and Seixas, 2006). At the day 0, the smell was described as "seaweed." In the squid, the skin was normally iridescent, while the cuttlefish's normal dark pattern was soon lost, due to the post-mortem relaxation of the chromatophores,

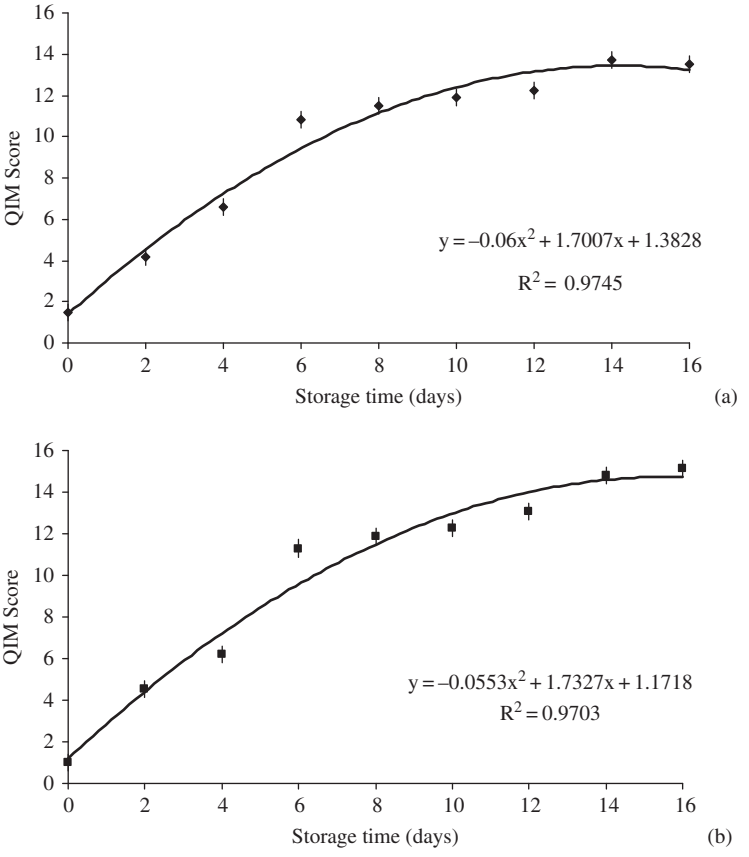

Figure 7. Changes in QIM score of squid (A) and cuttlefish (B) during iced storage. Bars represent the standard deviation ($n = 2$). For each run, 12 determinations were conducted.

which means that the zero demerit points are theoretically attainable. The differences found between the choice of attributes for cuttlefish and squid were due to biological and morphological factors (Vaz-Pires and Barbosa, 2004). After day 6 (unacceptable), there was a slight increase in fishy odor, development of pink color in the squid, and the central part of the cuttlefish mantle became brownish. In addition, soft flesh and eye opacity were observed, as compared to fresh squid and cuttlefish. The QIM scheme scores of squid and cuttlefish at this point were 10.8 and 11.3, respectively. Although the a^* value of squid and b^* value of cuttlefish displayed an increasing trend (Table 2), the QIM result of whole squid and cuttlefish was not well-correlated with L^*, a^*, b^* values of deskinned samples (Table 1). The color of stained squid muscle might differ from the visual color of whole squid or cuttlefish. A similar effect was reported by Lapa-Guimarães et al. (2002). They demonstrated that the coefficients of correlation between the sensory analysis and the a^* and b^* value of squid (*Loligo plei*) held in the contact ice measured in the whole squid with skin were high (0.813 and 0.947, respectively), whereas those measured in squid muscle showed low values (−0.688 and 0.383, respectively). Rejection of squid and cuttlefish (QIM = 13.7 and 14 demerit points, respectively) occurred after 12 days in ice with spreading/intensification of the pink color of the skin, the strong smell of spoilage, and flabby flesh of both species occurring. However, the QIM scores at rejection point of both samples did not reach the maximum scores (16 and 17 demerit points, respectively). It was observed that the changes of some characteristics—i.e., color of squid (*Photololigo duvaucelii*) and bone/head connection and color/appearance of cuttlefish (*Sepia aculeate*)—were not relevant to the QIM scheme of squid (*Illex coindetii*) and cuttlefish (*Sepia officinalis*) used in this study. Lapa-Guimarães et al. (2002) also reported that the squid (*Loligo plei*) quality markedly decreased after 7 days of storage. The color of squid skin increased during iced storage. Civera et al. (1999) and Lapa-Guimarães et al. (2002) found that sensory quality of common cuttlefish (*Sepia officinalis*), musky octopus (*Eledone moschata*), and broadtail squid (*Illex coindetii*) decreased after 7 days of storage at 1–2°C, and the sensory attributes reached the limit for human consumption after 10 days. In addition, Yamanaka et al. (1987) and Lapa-Guimarães et al. (2002) showed that the muscle of squid (*Todarodes pacificus*) held in a refrigerator at 0°C was classified as in the passable stage (no smell and firm flesh) after 6 days of storage, passing to a stage of advanced decomposition (putrid smell and very soft flesh) after 10 days of storage. Vaz-Pires and Seixas (2006) also reported that the cuttlefish and broadtail shortfin squid were rejected with a total of 17 demerit points and 16 demerit points, respectively. The shelf life of cephalopods is much shorter than for most fish species stored at similar temperature and conditions (Vaz-Pires and Seixas, 2006). This fact reflects the morphological and biological fragility of the cephalopods: more simple and fragile skin; lack of scales; higher softness and exposure of muscular tissue; and particularly, the general biochemical composition, much more easily degradable in this group, mainly due to rapid and effective autolysis (Vaz-Pires and Seixas, 2006).

Changes in Overall Quality. Figure 8 shows the changes of the overall quality for squid and cuttlefish during iced storage. The graph of the overall quality score of squid and cuttlefish overtime represents a linear correlation with R^2 of 0.97 and 0.96, respectively. The overall quality score of both samples decreased with increasing storage time ($p < 0.05$), indicating the lowered quality. Scores ranging from 3 to 5 and from 1 to 2 were considered as "unacceptability" and "rejection," respectively (Lawless and Heymann, 1998). Thus, our results showed that shelf life of 6 days was recommended for both squid and cuttlefish stored in ice, due to oxidized and fishy off-odor; appearance of an offensive, flabby, or

soft texture; and slight to intense pink spots. After 12 days of storage, the sensory quality reached the limit for human consumption (rejection point) when the samples had an ammonical off-odor or an intensity of putrid smell. According to the U.S. Department of Commerce, National Sensory Section, U.S. Food and Drug Administration (2005), descriptions of reject characteristics for fresh and frozen fish, scallops and shrimp were sour, yeasty, fermented, rancid, turnipy, ammonia, indole-like, putrid, sickly sweet, and fecal. For *Illex illecebrosus* (Ke et al., 1984) and *Loligo duvaceli* (Lakshmanan et al., 1993), high quality squid had skin showing a sheen and a red-brown color, and unacceptable squid had some or slight to intense pink spots. Paarup et al. (2002) reported that squid (*Todaropsis eblanae*) were rejected after 12 days of iced storage, due to ammonical off-odors. Our results are consistent with squid (*Todaropsis eblanae*; Paarup et al., 2002) and squid (*Loligo plei*; Lapa-Guimarães et al. 2005) whose ammonia content increased during storage until rejection. According to Fujii and Okuzumi (2000), TMA, dimethylamine (DMA), and ammonia are particularly involved in the smell of fishy odor.

Sensory Texture. Figure 9 shows the changes of texture score of raw squid and raw cuttlefish during iced storage. The texture score of samples decreased with increasing storage time ($p < 0.05$). Generally, squid and cuttlefish kept for longer time had a softened texture and led to unacceptability. These changes were in agreement with a reduction in shear force (Figure 5) as shown by a high correlation ($p < 0.05$) of 0.92 and 0.77 for raw squid and raw cuttlefish, respectively. As mentioned before, destruction of muscle fiber and the degradation of muscle proteins might be associated with the softened texture of squid and cuttlefish mantles.

Correlation between Objective and Subjective Qualities of Squid and Cuttlefish. Correlation between objective and subjective qualities of squid and cuttlefish is shown in Table 2. The changes in TCA-soluble peptide, expressible drip, and ammonia content were highly positively correlated with QIM and negatively correlated with overall quality of squid; whereas the changes in shear force, expressible drip, and

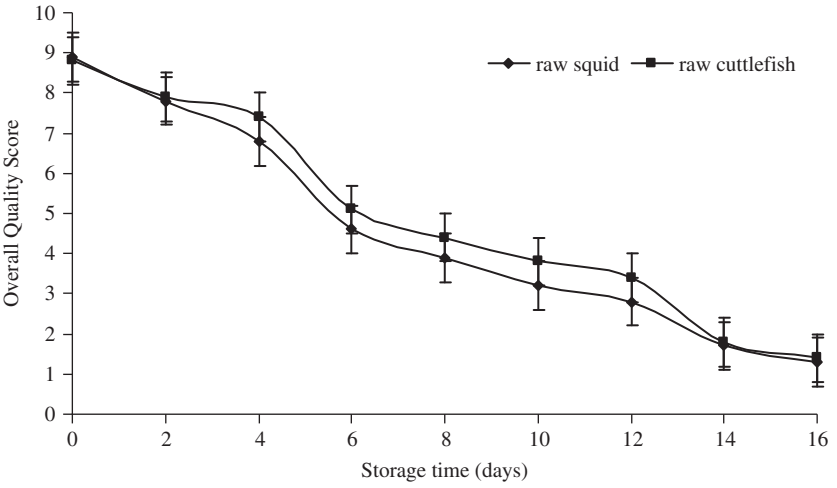

Figure 8. Changes in overall quality score of raw squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, 12 determinations were conducted.

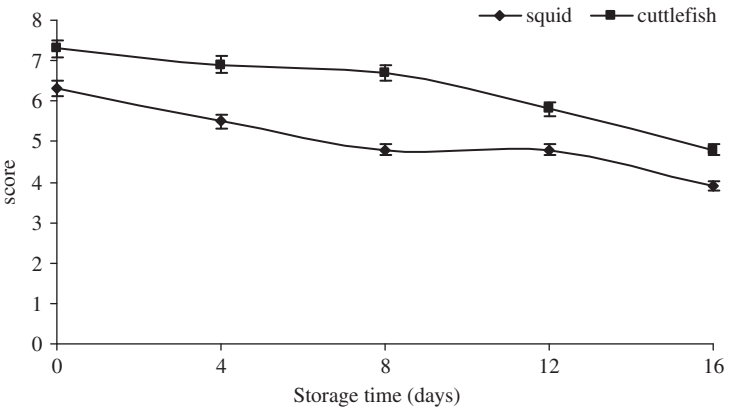

Figure 9. Changes in texture score of raw squid and cuttlefish during iced storage. Bars represent the standard deviation ($n = 2$). For each run, 12 determinations were conducted.

Table 2
Correlation coefficient between objective and subjective qualities of squid and cuttlefish during iced storage

	Squid		Cuttlefish	
Parameter	QIM	Overall acceptability	QIM	Overall acceptability
TCA-soluble peptide	0.95*	−0.98*	0.59*	−0.70*
Ammonia	0.69*	−0.79*	0.75*	−0.83*
TMA	0.46	−0.55*	0.55*	−0.63*
TVBN	0.57*	−0.70*	0.44	−0.56*
pH	−0.24	0.35	0.19	−0.08
L*	−0.35	0.34	0.02	0.10
a*	0.55*	−0.58*	0.43	−0.49*
b*	−0.57*	0.56*	0.07	−0.06
Shear force	0.63*	−0.63*	0.78*	−0.79*
Expressible drip	0.75*	−0.85*	0.81*	−0.87*
Psychrophilic count	0.38	−0.36	0.43	−0.58*
Total viable count	0.72*	−0.80*	0.51*	−0.65*

*Correlation is significant at the 0.01 level.

ammonia content were highly positively correlated with QIM and negatively correlated with overall quality of cuttlefish ($p < 0.01$) and would be appropriate indicators of freshness of squid and cuttlefish during iced storage. Among the parameters tested, expressible drip was shown as the most effective indicator related to sensory analysis. The increase in expressible drip of samples reflected the loss in water holding capacity associated with looser structure caused by degradation. The increased expressible drip was generally in agreement with the softer texture. As a result, the lower overall quality was observed in the sample kept for a longer time. Many promising chemical indices of

freshness indicators of different squid species during iced storage have been reported—including agmatin for squid (*Todaropsis eblanae*; Paarup et al., 2002), basic amino acids (arginine and ornithine) for common squid (*Todarodes pacificus*; Ohashi et al., 1991), the coefficient hypoxanthine (Hx)/adenosine 5'-monophosphate (AMP) for giant squid (*Dosidicus gigas*; Márquez-Ríos et al., 2007), free tryptophan and urea for squid (*Loligo plei*; Lapa-Guimarães et al., 2005), and ammonia for squid (*Illex illecebrosus*; LeBlanc and Gill, 1984).

Conclusion

Squid (*Photololigo duvaucelii*) and cuttlefish (*Sepia aculeate*) had psychrophilic counts and total viable counts below 10^6 cfu/g after 16 days of storage. However, sensory analysis indicated that the shelf life of squid and cuttlefish was 6 days. Destruction of muscle fiber and the degradation of muscle proteins might be associated with the softened texture of squid and cuttlefish mantles. Expressible drip and ammonia content could be useful freshness indices for squid and cuttlefish because their parameter increased in accordance with overall quality score and QIM. Those indices can be a promising protocol for the assessment of squid and cuttlefish quality.

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